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ABSTRACTS

Nineteenth Annual Meeting of the Association for Chemoreception Sciences (AChemS XIX) and the Twelfth International Symposium on Olfaction and Taste (ISOT XII)

Held in San Diego, July 1997

The Proceedings of the International Symposium on Olfaction and Taste XII will be published as a volume in the *Annals of the New York Academy of Sciences*. Included will be the invited symposium papers as well as extended abstracts of volunteer slide presentations. Space limitations, and not relative scientific merit, preclude publication of extended abstracts of the poster presentations in the Proceedings. The original abstracts of all presentations appear here in *Chemical Senses*.

1. New homologues of GUST27 as a possible taste receptor: molecular cloning and site-specific expression

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In our preceding study we cloned and characterized a G protein-coupled, seven-transmembrane receptor, GUST27, as a strong candidate of taste receptor in rat tongue epithelia. In the present paper we present some additional examples of homologous receptors.

RT-PCR primers were synthesized according to the amino acid sequences conserved in transmembrane domains II and VII of many G protein-coupled receptors known. RT-PCR was carried out using circumvallate papillary mRNA-derived cDNA as a template, with formation of four RT-PCR products. Corresponding genomic DNA clones were then obtained by screening from a genomic DNA library. These clones, GUST20A/20B, GUST22, GUST43 and GUST45, shared an amino acid sequence similarity of 50–60% to rat olfactory receptors.

Peptides corresponding to the C-terminal 15-amino acid portions of these receptors were synthesized to raise specific antibodies. Immunostaining showed that these receptors were expressed exactly at or near the taste bud sites of the circumvallate papillae, with different antibodies giving somewhat different patterns of the site-specific expression. These data suggest that the gustatory proteins we cloned, as well as GUST27, are probable taste receptors and also that their functions may be somewhat different from one another.

2. Draize eye scores and eye irritation thresholds can be combined into one QSAR

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Draize eye scores (DES) of 37 pure organic liquids have been converted into scores for the corresponding vapors, DES/P0, where P0 is the liquid vapor pressure in atmospheres at 298K. It is shown that there is a constant difference of 6.7 between values of $\log(DES/P_0)$ and log(1/ET), where ET is the eye irritation threshold in ppm of eight vapors for human subjects. The 37 $\log(DES/P_0)$ values can be combined with log(1/ET) values for 17 vapors into one QSAR for sensory potency (SP) using our general solvation equation,

$$\log SP = -7.979 + 1.016\pi 2H + 3.678\,\Sigma(\text{alpha})2H + 1.730\,\Sigma(\text{beta})2H + 0.838\,\log L16$$

$$n = 54, \quad r^2 = 0.924, \quad SD = 0.369, \quad F = 149.3,$$

where $\pi 2H$ is the compound polarizability-dipolarity, $\Sigma(\text{alpha})2H$ and $\Sigma(\text{beta})2H$ are the compound hydrogen-bond acidity and basicity, and logL16 is the gas–hexadecane partition coefficient. *n*

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is the number of data points, r the correlation coefficient, SD the standard deviation, and F the F-statistic. LogSP is then either $\log(DES/P_0 - 6.7)$, or $\log(1/ET)$, confirming the result for the eight common compounds. It is suggested that the equation can be used to predict eye irritancy of organic vapors and pure liquids. It is further suggested that for the compounds in the data set, the main process in eye irritation is transfer of the compound from the vapor or pure liquid to a biological phase, and a number of chemical properties of the biological phase have been mapped out through the equation. These properties are consistent with corresponding properties for a number of organic liquid phases.

Supported by a grant from the Center for Indoor Air Research.

3. Organizational complexity in lobster olfactory receptor cells

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Transduction in lobster olfactory receptor cells occurs via two parallel input pathways and a two-step amplification cascade. Components of two second messenger pathways occur in the transduction zone of lobster olfactory receptor cells. At least two odor-activated G-proteins, enzymes for the cyclic nucleotide and inositol phosphate signaling pathways, and cAMP and IP_3-activated ion channels occur on the outer dendrite of these primary sensory neurons. IP_3 activates a current that is nonselective for cations, while cAMP activates a K^+-selective current. These opposing conductances can mediate inputs of opposite polarity in the same cell. Calcium-dependent down regulation of both second messengers may allow additional, biochemical crosstalk between the two pathways. A third, Na^+-dependent nonselective cationic current also can be localized to the outer dendrite. The properties of the current and its underlying novel channel suggest that odor-induced increases in intracellular $[Na^+]$ secondarily trigger recurrent activation of the channel to generate much, if not most, of the total excitatory current. Overall, this organizational complexity allows lobster olfactory receptor cells to carefully regulate, and most likely integrate, the signal they transmit to the central nervous system.

Supported by grant # DC01655 from the National Institute on Deafness and Other Communication Disorders.

4. Long-range averaging of fluorescence in the light microscope and effect on fluorescence ratios

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The imaging of stimulated taste receptor cells with Ca^{2+} sensitive dyes has indicated increases in free Ca^{2+} concentrations which did not seem sufficient to induce synaptic release. Are these increases underestimates which resulted from the non-confocality of the microscopes used? Did such underestimates occur despite the use of ratiometric dyes?

The distribution of fluorescence in a biological cell was modeled by specifying small regions of high (or low) fluorescence embedded in larger regimes of low (or high) fluorescence. Three-dimensional numerical models representing such specimens were generated on a computer and subjected to 'blurring', by convolution with the theoretical point spread functions of conventional microscope objectives.

The fluorescence was found to spread beyond the range suggested by the spatial resolution of the wide-field microscope. Due to the blurring, averaging of fluorescence intensity occurred in the axial and lateral dimensions. When modeling the fluorescence of ratio-dyes like Fura-2 with the above procedure, it was found that the effects of blurring did not cancel when compartments of differing fluorescence were bordering at each other and the fluorescence ratios were taken (for each pixel of the blurred images). In a small space of increasing Ca^{2+} concentration, the Fura-2 ratios, and the concentrations of free Ca^{2+} ions calculated from it, were underestimated 2–20 fold, due to lateral and especially axial averaging. The error could not be decreased by changing to an objective of larger numerical aperture.

A mixing factor was defined which accounts quantitatively for the effect of blurring on the fluorescence ratio, and a semi-empirical correction procedure is given which, in certain, simple cases, allows to estimate the error due to blurring.

5. Imaging of olfactory bulb laminar development by means of *in vitro* magnetic resonance microimaging

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The rat olfactory system is minimally functional at birth and the olfactory bulb (OB) shows marked growth and development postnatally, including major structural and laminar differentiation during the first 10 days after birth followed by rapid growth in mass and volume until day 25 when mature features are largely established. We have shown that OB's laminar architecture can be visualized by noninvasive magnetic resonance imaging (MRI), utilizing a SISCO 4.7T/33 cm bore imaging spectrometer (Agahi *et al.*, 1994). More recently, we found that *in vitro microimaging* of a single detached rat OB (<5 mm in each dimension), at 500 MHz utilizing a Varian Research NMR spectrometer with a vertical system (11.7T/50 mm bore) and a Doty microimaging probe yielded remarkable images of OB layers at a high resolution of 35–50 μm, compared with 300 μm in the *in vivo* imaging at 200 MHz (Agahi *et al.*, 1996). Similarly the *in vitro* approach provided improved images of OB's laminar development. The mitral cell layer (MCL), one of the first layers to form, was clearly visible in *the in vitro* images of newborn while the glomerular layer (GL) was barely visible. By day 5, the GL could be distinguished in OB's lateral borders and interposition of EPL between MCL and GL was apparent. By day 12, all OB were readily visible in these images. Further development of glomeruli was observed at 18 days, mainly due to increase in their size and diameter. At 25 days, *in vitro* MR images displayed OB's adult cytoarchitecture and lamination in remarkable details. These results were in contrast to the *in vivo* images of the developing OB where laminar resolution was difficult before day 5 and the day 18 was the earliest when all OB layers could be visualized. Therefore, the *in vitro* MR imaging approach provides a valuable tool for analysis of the morphological development of olfactory and perhaps other neural systems and may have applications in neural degeneration and regeneration studies as well.

Supported by NSF DIR 89-20133COOP, NIH PHS 5 P41 RR05964, Servants United Foundation, UIUC Research Funds.

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6. Prognosis of olfactory disorder

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Forecasting the prognosis of olfactory disorder is important for the planning of treatment. We retrospectively investigated the relationship between certain characteristics of patients with olfactory disorder and their prognoses. From January 1982 to December 1995, we treated 797 patients at the Olfaction Clinic of Osaka City University Hospital. Their outcomes were determined on the basis of changes in subjective symptoms. The duration of follow-up differed among the patients but was always at least 2 weeks. The usual method of treatment was topical application of a corticosteroid. The length of time from the start of treatment to recovery or change in symptoms was also investigated. The following results were obtained.

1. The percentage of female patients with clinical improvement was significantly higher than that for males.
2. Patient age did not significantly affect outcome. However, longer-term olfactory disorder (>5 years) lowered the improvement ratio.
3. Improvement ratios were, by etiology, 56.6% for rhinosinusitis, 52.9% for nasal allergy, 58.4% for post common cold, 20.0% for head injury and 55.0% for drug-induced smell disorder. Recurrent hyposmia often occurred in cases of rhinosinusitis and nasal allergy.
4. The olfactory acuity before treatment, which is represented by the result of T&T olfactometry and Alinamin injection test, was correlated with outcome.
5. There was no relationship between improvement ratio and presence of subjectively abnormal sensations of smell.
6. In most cases of improvement, improvement appears within 6 months. However long-term follow up is advisable, since some patients exhibit recurrent hyposmia and a few exhibit improvement after long-term treatment.

7. *Comparing genetic algorithm and statistical methods* for selecting optimum aroma components to relate sensory and GC data

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Partial least squares (PLS) regression and principal component regression (PCR) analyses, both using orthogonal latent variables as predictors, have widely been propagating for correlating sensory scores to instrumental data. Due to great progress in personal computers, calibration using neural networks has also shown versatility for this purpose. However, MLR has still remained as the most popular method because of its advantages over other newer methodologies, availability of software and its comprehensive algorithm. Although requiring longer computing time due to the unique evolution process, genetic algorithms (GA) are a powerful optimization tool because of its attainability to global optima. The GA generally performs optimization through chromosome production, evaluation, reproduction, crossover and mutation processes. The GA-MLR starts from production of random combinations of components, i.e. chromosomes. Then chromosomes showing higher multiple regression coefficients (R) are preferentially reproduced so as to elevate the R level. However, in the GA-MLR, repeated analysis is needed to confirm whether the attained optimum is actually the best combination of components because a response surface in hyperspace is not necessarily smooth but often rather rugged. The GA-MLR and conventional stepwise MLR were comparatively applied to analyzing relationships between gas chromatographic profiles of tea volatiles and their aroma characteristics evaluated by sensory tests. The cross-validation was used to avoid over-fitting and multicollinearity, both frequently encountered problems in performing MLR. The Rs calculated in the GA-MLR were generally higher than those obtained by stepwise MLR. Reproducibility of optimum combinations seems to depend mainly on the response surface properties.

8. Olfactory information coding in *Drosophila* mediated by different transduction pathways at primary receptor neurons

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The presence of, at least, two transduction cascades, involving IP_3 and cAMP in olfactory receptor neurons, has been already shown for several species, from vertebrates to invertebrates, although the relative importance of both systems as information mediators has been discussed in some cases.

By combining electrophysiological measurements (electro-antennograms, EAG) with behavioral analysis in several *Drosophila* mutants, we have shown that (i) not only the IP_3 (as previously suggested) but also the cAMP system contribute to olfactory coding for this species. (ii) Opposite effects for both pathways can be deduced at the receptor cell level by EAG data. (iii) A single odorant is able to trigger both systems, although their relative importance depends on the odorant. (iv) Absence of any of both systems may have an effect in behavioral response. (v) For odorants triggering both pathways, the balance between them is related to concentration information.

Our data suggest that both transduction cascades are related to odorant quality as well as concentration information coding in *Drosophila*.

Supported by DGICYT PB94-1344.

9. Ultrastructural identification of apposed cell bodies in the mitral cell and granule cell layers in the adult salamander olfactory bulb

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Gap junctions have previously been observed between apposed cell bodies in the mitral cell and granule cell layers of mammalian olfactory bulbs (e.g. Reyher *et al.*, 1991). Optical recordings of Lucifer Yellow injections have also demonstrated cell coupling in

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these layers of live salamander olfactory bulb preparations (Hamilton and Maloney, 1996). In order to identify the coupled cell types in the salamander olfactory bulb and potential sites of gap junctions, clusters of cell bodies in the mitral and granule cell layers were examined at the ultrastructural level.

Semi-thin sections of adult *Ambystoma maculatum* and *Ambystoma tigrinum* olfactory bulbs were stained with toluidine blue and used to locate mitral and granule cell body clusters. Thin (silver and silver-gray) sections were then examined at up to 105 000× with a Philips CM-10 electron microscope. At the ultrastructural level, the salamander mitral and granule cell bodies closely resembled those of other species (Price and Powell, 1970; Byrd and Burd, 1991; Dryer and Graziadei, 1996). The granule cell bodies were smaller than the mitral cell bodies and they had an oval nucleus with dark chromatin located in clumps at the periphery. The nucleus was surrounded by a thin rim of cytoplasm containing dense bodies and a high density of free ribosomes (both single and rosettes). Mitral cell bodies had a well defined nucleolus and a thick rim of cytoplasm containing numerous mitochondria, microtubules, and large stacks of Golgi. Tight membrane appositions, suggestive of gap junctions, were observed between many of the granule cell bodies. Membrane appositions were also observed between mitral cell bodies, but they were less frequent and less tight.

Supported by the Whitehall Foundation and departmental funds.

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10. Topographic organization of the macroglomerular complex in different heliothine moth as revealed by 3D computer graphics

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The male antennae of the heliothine moths possess numerous olfactory sensilla of which the sensilla trichodea type 1 is male specific and houses pheromone responsive receptor neurons (RNs). Pheromone responsive neurons are also located in another type of sensilla, s. trichodea type 2 which has shorter hairs. Some of these type 2 sensilla contain plant odour responsive RNs. Where the axons of the RNs enter the antennal lobe (AL), a functional organization takes place; the pheromone RNs terminating in the macroglomerular complex (MGC) and the plant odour RNs in the ordinary glomeruli. A further functional subdivision of the MGC is shown in several moth species, including the heliothine moths (Lepidoptera: Noctuidae). For instance, in these species, pheromone and interspecific signal information seem largely to be conveyed to different MGC lobes. Several heliothine moths use the same compounds as pheromones and interspecific signals, however, the message mediated by each compound differ in the various species. For instance, one compound being a pheromone component in one species acts as an interspecific signal in another species and interrupts the attraction. In this study were presented MGC reconstructions from the ALs of male *Heliothis virescens*, *Helicoverpa zea*, *Helicoverpa assulta*, *Helicoverpa armigera* and *Heliothis peltigera*. Camera lucida drawings were digitized and data files containing information about individual sections of the insect brain were transferred to graphics workstations for alignment and three dimensional (3D) reconstruction. For this purpose, we used custom software running on Silicon Graphics Indigo2 and Indy workstations. The reconstructions showed MGC structures as surface covered units within the AL. The MGC of the five species is divided into three lobes except for *H. virescens* which have four lobes. In all species a large lobe, the cumulus, are positioned at the entrance of the antennal nerve. In *H. virescens* the other lobes are the dorso-medial, antero-medial and the ventro-medial lobes (as compared with the cumulus). In *H. zea* the additional lobes are dorso-medially positioned but in an posterior and anterior localization respectively. A striking similarity of the MGC appears in the three *Helicoverpa* species. However, the posterior dorso-medial lobe in *H. assulta* are more ventrally located. In *H. peltigerathe* two additional lobes are located medially with the lobe next to the cumulus showing a bended shape, a form and position similar to the toroid structure in *M. sexta*.

11. Rate of impulses at the labellar sugar receptor cell that induced proboscis extension of the blowfly

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Flies have contact chemosensilla at their legs (tarsi) and labella. When the chemosensilla contact with sugar solution of the concentration over the threshold, the proboscis extension reflex (PER) is bring about. Recently, we found a reversal relation between the PER-threshold value for glucose and that for fructose depending on aging after eclosion or physiological conditions of the blowfly, *Phormia regina* M. In our behavioral and electrophysiological experiments of the adult blowfly within 1 day after eclosion, few tarsal chemosensilla generated impulses, although most of the labellar chemosensilla could give rise to impulses resulting in proboscis extension. Using the labellar chemosensilla, we studied this reversal effect.We could obtain the PER-threshold values for both glucose and fructose on the single labellar chemosensilla. On the chemosensilla of the same labella, the concentration–response curves for glucose and fructose were also obtained by the tip-recording method. The curves for glucose and for fructose crossed at a point because the former had steeper gradient and higher maximum response than the latter. Comparing the two crossing curves with the PER-threshold values enabled us to determine the firing level of the neuron responsible for proboscis extension reflex at the central nervous system of the blowfly. By up-and-down movement of the firing levels before and after injection of blood sugar (trehalose) or physiologically active peptides into the fly haemocoele, we can evaluate the effect of chemicals to the nervous system of the fly.

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12. Portable olfactory threshold and identification batteries for clinical and medico-legal evaluations

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The objective is to develop a set of quantitative olfactory tests, that provide enough variety and information to yield a reliable evaluation of the subject's olfactory capabilities. The tests are based on the well-tried squeeze-bottle technique, with 2 oz polypropylene bottles containing the odorous solution, usually in mineral oil, absorbed in polypropylene felt to avoid spillage or spraying. Most of the odors offer a 12 month shelf life and permit the threshold testing of 100 subjects.

The first part of the test consists of a battery of nine odors, in quarter-log steps dilution series with paired blanks, covering the entire normal and hyposmia ranges of sensitivity, from –30 to +55 decismels (dS, by analogy with decibels, dB), or from 30 times mean normal sensitivity to 1/500 of normal. The nine odors cover a range from almost purely olfactory stimuli (phenylethyl methyl ethyl carbinol and tetrahydrothiophene) through graded degrees of olfactory and trigeminal stimuli (*n*-butanol, acetic acid, *s*-butanol, pyridine and menthol) to predominantly irritant chemicals (a-chloroacetophenone and methyl isothiocyanate). These tests allow a precise assessment of olfactory threshold deterioration, and of possible partial recovery after traumatic anosmia.

The second part of the test includes eight familiar odors, each at 45 dS (180 times mean threshold concentration), representing five food identifications (mushroom, apricot, pear, orange and lemon) and three hazards awareness (natural gas, smoke and mold). Used singly, in pairs, and as a group, these eight odors provide tests of identification (long-term odor memory), discrimination (short-term, 10 s odor memory) and a visual sorting test (instant association). These tests hold some promise for neurological evaluations, especially in head injury and early Alzheimer's disease.

The complete battery of tests is transportable in three insulated storage boxes (total weight 33 lb).

13. Evolution of olfactomedin: structural constraints and conservation of primary sequence motifs

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Olfactomedin is a glycosylated extracellular matrix protein originally identified at the mucociliary surface of the amphibian olfactory neuroepithelium and subsequently localized throughout the mammalian central nervous system. Although olfactomedin homologues have been identified in fish, frog, rat, mouse and human, its function is still unknown. As a first step toward elucidating the function of olfactomedin, sequences of teleost, amphibian and human homologues were compared to identify invariant, and hence, potential functionally important motifs. Previous studies revealed 33% amino acid sequence identity between rat and frog olfactomedin in their carboxyl terminal segments (Danielson *et al.*, 1994). Further analysis, however, reveals more extensive homologies throughout the molecule. Despite significant sequence divergence, cysteines essential for homo-polymer formation, such as the CXC motif near the amino terminus, are conserved as is the characteristic glycosylation pattern, suggesting that these posttranslational modifications are essential for function. Furthermore, alignment of a region of 53 amino acids of fish, frog, rat and human olfactomedin reveals seven invariant residues including a negatively charged cluster of aspartic and glutamic acid residues. Molecular evolutionary genetic analysis reveals an accelerated rate of nucleotide substitutions in the mammalian lineage. The evolutionary rate at the protein level, however, is constant, indicating that evolution of olfactomedin is constrained by structural limitations. Whereas considerable evolutionary divergence is evident between fish, frog and mammalian olfactomedins, olfactomedins of rat and human show 98% amino acid sequence identity. It appears that an ancestral olfactomedin gene arose before the evolution of terrestrial vertebrates and evolved independently in teleost, amphibian and mammalian lineages. The apparent evolutionary pressure toward conservation of primary structure supports the notion that olfactomedin has an important function in the mammalian nervous system.

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14. Central processing of aggregation pheromones in solitary and gregarious desert locusts, *Schistocerca gregaria*

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In the desert locust, *Schistocerca gregaria*, a number of behaviourally active aggregation pheromone components have been identified from adult male *S. gregaria*, and from third, fourth and fifth instar nymphs. Adult females seem not to produce aggregation pheromones. Adult males and females, but not nymphs respond to the adult male-produced mixture, whereas only nymphs respond to the binary nymph-produced mixture with aggregation behaviour (Obeng-Ofori *et al.*, 1994; Torto *et al.*, 1994).

We studied the structure and function of antennal lobe interneurons, responding to single aggregation pheromone components and adult- and nymph-specific mixtures. Neurons in adult males and females and in first instar nymphs of *S. gregaria* in the solitary and the gregarious phase were investigated. Projection neurons responded with different degrees of specificity to all behaviourally active aggregation pheromone components. Component-specific, blend-specific and generalist neurons were found in adults and first instar nymphs in both phases. Antennal lobe neurons were slightly more sensitive in solitary than in gregarious locusts. In gregarious adult males and females more neurons responded to the adult male mix than to any of the single components. In gregarious first instar nymphs most neurons responded to the adult male mix and the major component but also nymph mix specific neurons were found. The behaviour of adults and nymphs is thus partially reflected by the response characteristics of antennal lobe projection neurons.

Supported by a fellowship from the DFG, Germany to S.A. and by Swedish Research Councils (NFR, FRN and SAREC) to S.A. and B.S.H.

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15. Neural and hormonal correlates of olfactory imprinting to food odors in the ferret (*Mustela putorius f. furo*)

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As demonstrated previously, in the developing ferret food odor imprinting occurs during a sensitive phase lasting throughout the third month of postnatal life. Adult ferrets reliably search for food only when the odor of a known prey is offered. Subsequent experiments demonstrated, olfactory learning and odor discrimination are not limited to the sensitive phase. However, ferrets imprinted to a very limited array of odors show a different searching behavior pattern than animals imprinted to a wide range of odors.

In the altricial ferret postnatal changes in the olfactory system occur. The number of sensory neurons in the olfactory epithelium increases significantly from ~2.8×10^6 (neonate) to 24.6×10^6 (day 60–100) and decreases thereafter until adult values (22.6×10^6) are reached (>150 days). In the olfactory bulb the number of granule cell dendritic spines as well as the number of synapses in the external plexiform layer reach a maximum in subadult animals (60–100 days) followed by a significant decrease; adult values are reached around postnatal day 150. Quantification of postnatal thyroid hormone levels in the plasma reveals a clear peak for T4 around day 40 followed by a T3 plateau extending from day 60 to 100. This plateau coincides with the life span during which neural overshoot was established. Interestingly, since the number of mitral cells stays fairly stable during ontogeny the convergence ratio between receptor cells and mitral cells shows age dependent differences and changes from 35:1 to 308:1 and to 284:1. During the developmental stage when the young ferret becomes imprinted to prey odors the highest convergence ratio has been found. There is a remarkable correlation between neuronal, hormonal and behavioral development.

16. Human taste mechanisms for pyranose and furanose monosaccharides and for disaccharide sugars

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Response functions from human psychophysical and *Drosophila* behavioral and electrophysiological studies with furanose (D-fructose) and pyranose (D-glucose) monosaccharides, and a pyranose–furanose disaccharide (sucrose) suggest different receptor cell mechanisms for the furanose and pyranose monosaccharides. The data also suggest that the disaccharide (sucrose) and furanose monosaccharide (D-fructose) may stimulate the same mechanism. We tested these two hypotheses by human psychophysical studies using different furanose (L-sorbose) and pyranose (D-galactose) monosaccharides, and the pyranose–pyranose disaccharide maltose. The function for sorbose was predicted to be similar to that for fructose, as they are both furanose monosaccharides. Likewise, the pyranose monosaccharide (galactose) was expected to give a function similar to the glucose function. Because it lacked a furanose component, the response function for the pyranose-pyranose disaccharide (maltose) was expected to be similar to those of the pyranose monosaccharides (galactose and glucose), and to differ from that of the pyranose-furanose disaccharide (sucrose). Since the sugars exist in multiple tautomeric forms in solution, NMR spectroscopy was used to confirm the time required to reach tautomeric equilibrium and the proportions of the tautomers at equilibrium. Then subjects tasted various concentrations of the sugars, each sample paired with water, and identified the sweeter of each pair. Functions for Recognition Indices (proportions of subjects recognizing the sugar as sweeter) were constructed from the data, as in previous work. As predicted, the sorbose and galactose functions were similar to those for fructose and glucose respectively, in the previous work and support the hypothesis of separate furanose and pyranose mechanisms. The maltose function was similar to that for the furanose sugars in both the previous and present work and to the previous sucrose function; this result suggests that disaccharide species stimulate by the same mechanism as furanose monosaccharides, regardless of whether they contain a furanose component.

Supported by NIH DC/O02663. We thank M. Turnbull for assistance with the NMR and S. Eylam and J. Brink for discussion.

17. Excitatory synaptic responses and gabaergic inhibition in the glomeruli of the rat olfactory bulb

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In olfactory bulb (OB) slices from 14- to 22-day-old rats, the field potential (FP) evoked in the glomerular layer (GL) by olfactory nerve (ON) stimulation consisted of two negative components: N_1 (peak 9–13 ms) which was blocked by the kainate/AMPA receptor antagonist CNQX, and N_2 (peak 50–150 ms, duration >300 ms) which was enhanced by reduction of Mg^{2+} in the medium, and blocked by the NMDA receptor antagonist APV. Comparison of glomerular FPs before and after knife cuts isolating the GL from deeper layers indicated that both N_1 and N_2 were produced by currents generated, for the most part, within the GL. N_1 (in standard medium) and N_2 (in CNQX) reversed polarity in the external plexiform and mitral cell (MC) layers, suggesting that both components reflected synaptic responses in the distal, apical dendrites of mitral/tufted cells. Simultaneous FP recordings in the GL and intracellular recordings from MCs showed that (i) N_1 is associated with a short-latency EPSP, which triggers brief firing, and (ii) N_2 is associated with a prolonged EPSP and spiking activity, as N_2 and the late EPSP/cell firing had similar time courses, and were blocked by APV.

The $GABA_A$ antagonists bicuculline or picrotoxin enhanced selectively N_2. The enhanced N_2 was blocked by APV. Reduction of $GABA_A$ inhibition depolarized MCs and increased input resistance, suggesting that the preferential effect of the $GABA_A$ antagonists on the voltage-dependent, NMDA component of the glomerular response could be due to a reduction of tonic inhibition of MCs by granule cells. However, at least part of the $GABA_A$ receptor-mediated inhibition suppressing N_2 originates in the GL since bicuculline also enhanced this component in the isolated GL. Strychnine had similar actions as $GABA_A$

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antagonists, but only at high concentrations known to affect $GABA_A$ receptors.

The $GABA_B$ antagonist CGP-35348 caused a small but consistent enhancement of both N_1 and N_2, suggesting that $GABA_B$ receptors may be tonically active. The $GABA_B$ agonist baclofen nearly blocked both N_1 and N_2 in intact or in isolated GL slices. The effect of baclofen was antagonized by CGP-35348. Baclofen had no effect on a CNQX-sensitive, glomerular synaptic response evoked by stimulation in the MC layer. These observations suggest that $GABA_B$ receptors are not be present on mitral/tufted cells, but are located presynaptically on ON terminals. This possibility is currently under investigation with a number of approaches.

Supported by NIH DC00347, DC03195, NS29218 and NS29635.

18. Characteristics of the gustatory neurons in the epipharyngeal sensilla of *Antheraea yamamai* larva

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Epipharyngeal sensilla, one pair of coeloconic type gustatory sensilla, are present on the ventral side of the labrum in the wild silkmoth larva, *Antheraea yamamai*. Projection pathways and the spike response characteristics of the taste neurons were investigated.

Fifth instar larva, 24–72 h after molting, were fixed on wax plates with a parafilm and a dental wax, and their epipharynx was exposed. Backfilling with cobalt-lysine were conducted from each sensillum, and projection pathways in the central nervous system were observed in whole mount preparations. Spike responses were recorded using tip-recording method. Some sugars, generally known deterrent substances or their binary mixtures were filled in the stimulating/recording electrodes in which 50 mM KCl was included as an electrolyte. Recorded spike data were digitized and analyzed using SAPID Tools.

The axons of the taste neurons enter the brain via the labral nerve and have branches at the ipsilateral tritocerebrum. They proceed into the suboesophageal ganglion and branch to a ventral direction in the ipsilateral anterior medial region of the ganglion.

Three kinds of spikes could be classified on the basis of spike shapes. One cell elicited large-amplitude spikes in response to D-fructose. The dose-dependency and the adaptation rate for fructose was not so conspicuous as the other sugar sensitive cells in the maxillary styloconic sensilla. This cell responded to sucrose but was more sensitive to fructose. Another cell in the epipharyngeal sensillum showed tonic spike responses to some alkaloid solutions. This cell is supposed to be a deterrent cell and is sensitive to salts and alkaloids. Spikes of the third cell rarely appeared in response to the electrolyte solution. Activities of the two cells in response to 0.04 mM strychnine nitrate were suppressed by some sugars in the mixed stimuli, and the number of spikes decreased with increasing concentration of inositol. It is suggested that the effect of fructose and sucrose is doubly recognized by stimulating the fructose sensitive cell and suppressing the deterrent cell and that the deterrent response is more or less suppressed by total amount of sugars included in the diet.

19. Differences in responding to everyday odors: a Japanese–German cross-cultural study

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There is a growing appreciation that experience with odors—how frequently they are encountered and in what contexts—may strongly influence the way they are perceived. In particular, it is generally thought that familiar odors will be judged as more pleasant. To test this we compared the response of 40 Japanese and 44 German women to the odors of 18 everyday substances, some of which were edible and some not. Six substances were presumed to be familiar to Japanese, six to Germans, and six to both populations. They were presented in random order in opaque polyethylene squeeze bottles and subjects were asked to rate each odor for intensity, pleasantness and familiarity, say what it reminded them of, and whether it was from an edible substance or not.

Thirteen odors differed significantly on pleasantness ratings between the two groups, and of these, six were also rated significantly differently on familiarity. Fermented soybean (natto), dried fish (katsuobushi), Japanese tea and peanuts were judged significantly more familiar and pleasant by the Japanese, and anise and marzipan by the German subjects. Both groups rated the odors of coffee and chocolate as very familiar and very pleasant.

All these odorants are from foods or beverages and it is notable that the six odorants rated most differently were also those with the greatest differences in edibility judgments. Coffee and chocolate were judged edible by almost all subjects. These findings suggest that experience with odors, particularly in relation to food habits and possible associations of flavor with nutritional consequences, may indeed significantly affect perceived odor pleasantness.

20. Do difference procedures offer advantages to rating methods?

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Difference procedures have found extensive application in industry, especially in quality control and precede most descriptive tasks in the product evaluation sequence. Some advantages of these methods are that naive subjects can participate, comparisons can be made in the absence of an identifiable attribute, and results can be compared across experiments and across values of unrelated physical variables. Some disadvantages of difference procedures are that subjects need to sample larger numbers of stimuli and that these procedures provide less qualitative information than rating procedures.

Results will be presented of studies in which subjects used difference and rating procedures for a variety of stimuli. The subjects judged sensory attributes ranging from the perceived quality of cigarette smoke to the perceived whiteness of papers. In

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addition, the effects of methodological variables such as stimulus resampling were investigated.

21. Genetic locus on mouse chromosome 4 influencing taste responses to sweeteners

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Responses to sweet tasting compounds were studied in mice of C57BL/6ByJ (B6) and 129/J (129) inbred strains and their second generation (F_2) hybrids using two-bottle preference tests and electrophysiological recordings from the chorda tympani and glossopharyngeal gustatory nerves. Compared to the 129 mice, the B6 mice had higher preferences and intakes and larger neural responses to sweet-tasting carbohydrates and amino acids and some artificial sweeteners. Using linkage analysis in the F_2 generation with microsatellite markers, we identified a genetic locus responsible for most of this strain difference, and mapped it to a short (<4 cm long) segment of distal chromosome 4. This locus probably corresponds to the previously suggested *Sac* locus (Lush *et al.*, 1995). It accounts for more than half of the genetic variability among the F_2 hybrids in 0.12 M sucrose intake and it also has a strong effect on behavioral and neural responses to saccharin, D-phenylalanine, D-tryptophan and glycine, all of which taste sweet to humans. These data suggest that the perception of sweetness of several chemically different compounds by mice involves a common peripheral mechanism influenced by a gene on the distal part of chromosome 4.

Supported by NIH grants DC00882, DK44073 and DK48095.

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22. The role of apolipoprotein-E and olfactory functioning in people with alzheimer's disease

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Senile plaques and neurofibrillary tangles are the neuroanatomical hallmarks of Alzheimer's disease (AD). Recent studies have described the specificity of apolipoprotein-E (apoE) binding to bA4 peptide, the primary component of senile plaques. In addition, studies have shown increased prevalence of allele 4 (e4) in people with a positive family history of AD and in cases of sporadic AD. These two lines of evidence suggest that apoE, specifically the e4 allele, plays an important role in the development of AD; apoE allele status may provide useful information in diagnosis of AD. Given (i) that plaques and tangles form in those areas of brain that process olfactory information and (ii) the documented decline in olfactory functioning in patients with AD, the goal of this study was to examine differences in olfactory functioning in two at risk populations: (i) nondemented older adults at risk for AD by virtue of genetic status and (ii) older adults at risk by virtue of memory impairment but are nondemented. Within the memory impaired, but nondemeted population, those with e4 were significantly impaired on an odor threshold task compared with those without e4 ($P < 0.01$). Furthermore, retrospective analysis revealed that those individuals in the control group who later showed clinical symptoms and were ultimately diagnosed with AD showed poorer odor sensitivity prior to the dementia diagnosis ($P < 0.05$). These findings suggest that compromised olfactory processing may occur very early in the disease process and there may be differential rates of olfactory function decline driven by differences in apoE genotype.

Supported by NIH grant # AG08203 to C.M. and # AG12674 to M.B., and by the medical research service of the Department of Veterans Affairs. We gratefully acknowledge Drs R. Katzman and L. Thal and the staff and patients at the Alzheimer's Disease Research Center for their assistance with this study.

23. NaCl taste thresholds: relationship to tongue locus and area of stimulation

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Many sensory systems are capable of integrating stimulus energy over time and space to enhance signal detectability and perceived intensity. Unlike studies of vision and audition, however, psychophysical taste studies investigating such integration are scarce, primarily because of the difficulty in precisely controlling the stimulus. Using our recently developed Regional Automated Taste Testing System (RATTS) we measured detection thresholds on the tongue tip, 2.5 cm posterior to the tip, 1 cm posterior to the tip on the right lateral side, and 2.5 cm posterior to the tip on the right lateral side in eight subjects (18–29 years of age). Stimulus duration was 0.75 s; the sizes of the tongue areas stimulated were 12.5 mm^2 and 50 mm^2. The mean threshold values ranged in concentration from -3.02 $\log_{10}$ M to 0.16 $\log_{10}$ M. Subjects were found to be more sensitive to NaCl at the tip than on the more posterior regions of the tongue, and their sensitivity increased as the size of area stimulated increased, particularly on the tip of the tongue and 1 cm posterior to the tongue tip. These data indicate that NaCl taste thresholds are systematically related to the size and region of tongue stimulated.

Supported by NIDCD grant P01 00161, NIH.

24. Behavioral antagonism in the moth *Helicoverpa zea* in response to pheromone blends of three sympatric heliothine moth species is explained by one type of antennal neuron

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We have discovered a type of sensillum on the antennae of male corn earworm moths, *Helicoverpa zea*, that house two types of receptor neurons (RN) that explain both the upwind flight attraction of males to their own species' pheromone blend and

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arrestment to the quite similar blends emitted by females of three other sympatric North American heliothine species. The first RN type is a large-spiking neuron that is most sensitive to (*Z*)-9-hexadecenal (Z9–16:Ald), the secondary *H. zea* pheromone component that along with the major component, (*Z*)-11-hexadecenal, causes attraction to the female. This RN is also responsive to (*Z*)-9-tetradecenal (Z9–14:Ald; not a *H. zea* pheromone component) at higher dosages. The sensitivity of this RN thus explains the attraction that has been observed in other studies when small proportions of Z9–14:Ald are added to Z11–16:Ald to mimic the conspecific blend.The second type of RN in this sensillum is a small-spiking neuron that is again responsive to Z9–14:Ald (which in larger proportions acts as a strong antagonist to upwind flight) but this RN is actually more sensitive to two other strong behavioral antagonists, (*Z*)-11-hexadecenyl acetate and (*Z*)-11-hexadecenol. Thus, activation of this single broadly tuned 'antagonist' RN could explain why *H. zea* males will orient only to their conspecific females. These three compounds are emitted by females of three other North American species, *H. subflexa*, *H. phloxiphaga*, and *H. virescens*, as agonists in their blends, which also contain the *H. zea* components Z11–16:Ald and Z9–16:Ald. This antagonist RN may also explain why a blend of Z11–16:Ald and a small amount of Z9–14:Ald is never as attractive to *H. zea* males as the conspecific blend. Enhanced specificity for the conspecific blend arises because the antagonist RN is never stimulated to fire, even when large proportions of the pheromone component, Z9–16:Ald, are added to Z11–16:Ald. When Z9–14:Ald is used instead of Z9–16:Ald, however, and the proportion of Z9–14:Ald becomes too great, the threshold of the antagonist neuron as well as that of the agonist neuron is exceeded, and the upwind flight response begins to be suppressed.

Supported by USDA/NRICGP grant 95-37301-1805 to T.C.B. and T.A.C.

25. Enhancer trap lines mark subsets of neurons in the larval chemosensory organs of *Drosophila melanogaster*

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The antenno-maxillary complex of *Drosophila melanogaster* larva consists of three organs, each containing a small number of neurons. P element induced chemosensory mutants show reporter gene expression in cells of the complex. We have analysed the pattern of β-galactosidase expression at the level of single cells in the dorsal organ of several mutants. *chsA* and *chsB* are non-specific chemosensory mutants defective in olfaction and taste, *olf413* and *olf512* are specifically deficient in olfactory response to acetates and *olf186* is blocked to several esters. The mutants express β-galactosidase in a restricted number of neuronal and non-neuronal cells. The pattern of expression in heterozygotes allows us to ask whether the subsets of staining neurons are conjoint or disjoint and provides information about functional specificity of neurons.

26. Effects of concentration on sodium permeation and ion interactions in CNG channels of rat olfactory receptor neurons

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Cation permeation through cyclic nucleotide-gated (CNG) channels, directly activated by cAMP, initiates depolarization and receptor potential generation in olfactory receptor neurons (ORNs). We have investigated the effects of NaCl concentration on currents through these channels in order to understand the mechanism of cation permeation and ion-site interactions within them. The ionic dependence of macroscopic currents through such channels activated by 100 mM cAMP was studied in inside-out membrane patches from the dendritic knobs of ORNs enzymatically dissociated from adult female Wistar rats. In symmetrical NaCl solutions with no added divalent ions the current–voltage relations were almost linear, reversing close to 0 mV. With 150 mM $[NaCl]_o$, but varying $[NaCl]_i$ from 25–300 mM, the reversal potentials of the CNG currents followed the Na^+ equilibrium potential, indicating that $P_{Cl}/P_{Na} \approx 0$. However, at low external Na^+ (≤100 μM) there was some significant Cl^- permeability. Our results indicated that Na^+ currents through these CNG channels did not obey the independence principle but did display saturation kinetics with K_ms in the range of 100–150 mM inferring the presence of binding sites within the channel. In addition, the lack of voltage dependence of conductance in asymmetric solutions and a preliminary modelling with a two-site three-barrier rate theory model suggested that the ion-binding sites were close to the midway point along the channel. These characteristics seemed to indicate that the permeation properties of the olfactory CNG channels do differ from those of photoreceptor CNG channels.

Supported by the Australian Research Council.

27. The role of innervation in the development of taste buds: insights from studies of amphibian embryos

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Amphibian embryos have long been model organisms for studies of development because of their hardiness and large size, as well as the ease with which they can be experimentally manipulated. These particular advantages have allowed us recently to test the role of innervation in the development of vertebrate taste buds using embryos of an aquatic salamander, the axolotl. The predominant model of taste bud genesis has been one of neural induction, in which ingrowing sensory neurites induce taste bud differentiation in the epithelium that lines the mouth and pharynx. However, when we prevented embryonic sensory neurons from contacting the oropharyngeal epithelium by using transplantation or tissue culture techniques, we found that taste bud differentiation was independent of nerve contact. Additionally, using similar types of experimental manipulations, we have recently shown that taste bud

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differentiation is not a result of interactions of the oropharyngeal epithelium with craniofacial mesenchyme. Surprisingly, we found that although taste bud genesis occurs very late in embryonic development, it is an intrinsic feature of the presumptive oropharyngeal epithelium extremely early, in fact as early as the completion of gastrulation. These data have prompted us to propose a new model for the development of amphibian taste buds: (i) The presumptive oropharyngeal epithelium is specified by the time gastrulation is complete; (ii) Subsequently, a distributed population of taste bud progenitors is set up within this epithelium via local cell–cell interactions. These progenitor cells give rise to taste buds which are distributed throughout the mouth and pharynx. How widely applicable this model might be for the genesis of taste buds in other vertebrates remains to be seen. However, since it is likely that the taste system of axolotls more closely resembles the ancestral state from which both the amphibian and mammalian taste systems have evolved, it is possible that many of the same developmental mechanisms that give rise to amphibian taste buds are also used to generate these receptor organs in mammals.

Supported by NIDCD DC00114 to L.A.B. and DC01081 to R.G.N.

28. An analysis of odorant-induced currents in on-cell patches on mammalian olfactory receptor neurons

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Because of the difficulty of obtaining odorant-induced currents in mammalian olfactory receptor neurons using whole-cell recording, we have developed a mathematical model of the electrical circuit of the patch and rest-of-cell. This can be used to quantitatively analyse on-cell patch pipette currents in response to perfusion of the cell by solutions containing odorants or other compounds that can alter membrane conductance or cell potential. We have analysed pipette currents from on-cell patches of olfactory receptor neurons (ORNs) dissociated from adult rats. Initially, we perfused the ORNs with a high (100 mM; control 10 mM) KCl solution, which immediately induced a current flux from cell to pipette of a magnitude to imply a depolarization of ~52 mV, close to the value predicted from the Nernst equation (56 mV), and no change in the patch conductance. In contrast, perfusion by a cocktail of five cAMP-stimulating odorants (cineole, *n*-amyl acetate, methyl salicylate, limonene and α-pinene at a concentration of 1 mM), after a delay of 4–10 s, induced a current flux from pipette into the cell. Data in normal $[Ca^{2+}]$ and $[Mg^{2+}]$ implied an average patch conductance increase of ~36 pS, a cell depolarization of ~13 mV and an odorant-induced single channel conductance of ~16 pS. In low $[Ca^{2+}]$ and no Mg^{2+} ~40% of cells responded to odorants, with an induced current flow from cell to pipette implying a patch conductance increase of ~115 pS and cell depolarization of ~32 mV. The results were consistent with the odorants gating cAMP-gated cation channels. This analytical approach, which enables estimates of odorant-induced voltage and conductance changes to be made from changes in pipette current, should also be of general use for comparing cell responses to different perfusing solutions.

Supported by the Australian Research Council.

29. PROP (6-*n*-propylthiouracil) supertasters and the saltiness of NaCl

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Taste blindness to PTC (phenylthiocarbamide) and its chemical relative PROP was discovered in the1930s. Family studies showed that those who could not taste PTC/PROP (nontasters) carried two recessive alleles. In recent years, we have classified tasters into two groups: medium (PROP is moderately bitter) and supertasters (PROP is intensely bitter). With our classification, ~25% of Americans are nontasters, 50%, medium tasters, and 25%, supertasters. Studies showed that supertasters form a cohesive group. Anatomical studies showed that supertasters have the most fungiform papillae. Psychophysical studies showed that supertasters perceive the most intense bitterness and sweetness from a variety of compounds, the most intense burn from oral irritants, and the most intense tactile sensations from viscous solutions. Oral burn and touch are presumably perceived to be the most intense to supertasters because taste buds in fungiform papillae are innervated by the trigeminal nerve (pain, touch) as well as the chorda tympani nerve (taste).

The psychophysical scaling method used was magnitude matching with NaCl as the control modality. With this method, subjects rated the intensities of a series of NaCl and PROP solutions. The assumption that the taste of NaCl did not vary with PROP status allowed comparisons of the bitterness of PROP across subjects. Early magnitude matching studies, using sound as the control, had suggested that this assumption was reasonable. However, recent studies challenged that conclusion. Larger samples with more diverse populations, using sound as the control, showed that the taste of NaCl varied with PROP bitterness; supertasters perceived the strongest taste and nontasters, the weakest. Thus our earlier conclusions were conservative because differences between nontasters, medium tasters, and supertasters were concealed by using NaCl as a standard. Using magnitude matching with sound as the standard, or using the Green scale which employs intensity labels, the differences between PROP groups are larger. Note that the association between PROP status and salt taste is interesting in itself since variability in salt taste may have important nutritional consequences.

Supported in part by NIDCD 00283.

30. The mammalian bombesin-like neuropeptide neuromedin B is a candidate neuropeptide mediating output from mitral cells in the olfactory bulb

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The mammalian bombesin like neuropeptides neuromedin B (NMB and gastrin-releasing peptide (GRP) are known to mediate

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a wide spectrum of biological processes which include neuromodulation, hormone release, muscle contraction, and cell growth. To obtain a more complete understanding of the function of these peptides in physiologic and pathologic contexts, our laboratory and others have isolated cDNA clones encoding the prohormone for both NMB (preproNMB) and GRP (preproGRP) as well as clones for two high-affinity, seven transmembrane spanning, G-protein coupled receptors (NMB-R, or bb1; and GRP-R, or bb2) that bind NMB and GRP respectively. GRP-R and NMB-R share a similar signal transduction pathway, where ligand binding is followed by activation of heterotrimeric G-proteins that in turn activate phospholipase C, elevate inositol trisphosphate, and result in a transient elevation of intracellular calcium. We have surveyed the distribution of expression for these genes in the central nervous system using *in situ* hybridization to cRNA probes, and note an interesting pattern of expression in the olfactory bulb and cortex. PreproNMB mRNA is expressed at high levels in the mitral cell layer of the olfactory bulb, while NMB-R mRNA is expressed at high levels in regions that are major projection targets for mitral cells in olfactory cortex: the anterior olfactory nucleus, piriform cortex, taenia tecta and olfactory tubercle. These data suggest the hypothesis that NMB and NMB-R are important mediators of olfactory signaling at mitral cell synapses. With the ongoing and planned development of selective NMB-R antagonists as well as animal models with targeted NMB-R (or NMB) genes, this hypothesis can be tested rigorously in the future.

31. Genetics and development of glutamate taste preference

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The sodium salt of glutamic acid (monosodium glutamate—MSG) and certain other amino acids and ribonucleotides impart a unique taste sensation often called umami. We have been studying preference for umami substances in two systems: inbred mice and human infants. In 48 h tests, C57BL/6J (B6) mice exhibit a lower preference threshold for MSG than do 129/J mice. Moreover, B6 mice show a greater preference across a wide range of concentrations and, at high (e.g. 300–600 mM) concentrations, consume greater amounts of MSG. To examine whether the strain difference in MSG preference might be related to a similar strain difference in preference for sucrose and other sweeteners, as might be suggested from studies with rats, preferences for MSG and sucrose in the F_2 generation were examined. Preferences for sucrose and for MSG were not positively correlated in the F_2 indicating that these strain differences depend on different genes. For human adults, unlike mice, the taste of aqueous MSG is not palatable. Our studies of human infants also indicate that MSG alone is not preferred to plain water, but, when it is added to soup, the soup plus MSG if preferred to soup alone. Ongoing studies are designed to determine whether simple mixtures of MSG with other tastants, in particular NaCl, are preferred to water alone, NaCl alone, and MSG alone.

Supported by NIH DC 00882 and the International Glutamate Technical Committee.

32. Pharmacological characterization of biotin induced chemokinesis in *Paramecium*

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Paramecium tetraurelia utilize various bacterial metabolites to locate their bacterial food source via a cell hyperpolarization-mediated chemokinesis. Glutamate, folate, cAMP, acetate, and ammonia can act as attractants through at least three different transduction pathways. We previously identified biotin as an additional bacterial metabolite that is a strong attractant of *P. tetraurelia*. Structure/function analysis of several biotin analogs was carried out to identify structures necessary for stimulus activity. Diaminobiotin, an analog which lacks the ketone group in the imidazole ring, was shown to be a behavioral antagonist. Analogs with disruptions of the sulfur group or modifications of the valeric acid side chain, including modifications of the carboxyl group, proved to be agonists with little variation behaviorally from the parent compound. Initial binding studies have indicated the existence of at least two binding sites for biotin on the cell membrane. The first, a high affinity site with an approximate K_d of 1×10^{-8} M may be involved in transport. The second, a low affinity site with a K_d of $\sim 1 \times 10^{-4}$ M, correlates to concentrations of biotin which result in both measurable cell hyperpolarization and behavioral response. Binding studies with structural analogs will be conducted to correlate behavioral antagonism to receptor interaction or competition. In addition, affinity chromatography will be employed in attempts to isolate the putative biotin receptor.The transduction pathway activated by biotin is unknown. Studies indicate that cAMP and cGMP are not involved in biotin-mediated hyperpolarization.

Supported by NIH and VCC.

33. Receptors for odorants of similar and dissimilar qualities and molecular structure visualized by 2-DG maps of olfactory bulb glomeruli

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It was shown recently that specific odorant receptor gene probes hybridize *in situ* to small and distinct subsets of olfactory bulb glomeruli (Ressler *et al.*, 1994; Vassar *et al.*, 1994) and, more precisely, that neurons expressing a specific receptor project to only two topographically fixed glomeruli in each olfactory bulb (Mombaerts *et al.*, 1996). Maps were made of metabolic activity in rat glomeruli, using the radioactive 2-DG technique after controlled stimulation with odorants of similar and dissimilar quality and structure. With a series of acids of varying carbon chain length, aspects of the glomerular activity pattern were found to be conserved while others were not. This shows that specific receptors encode for common structural or epitopic features while others give the odorant its unique characteristic. A concentration series of one odorant (propionic acid) showed that the size of clusters of active glomeruli vary directly with odorant concentration. This indicates that different, lower affinity receptors are recruited into the encoding of the perceived intensity of an odor. Maps for odorants of different molecular structure and different quality are highly dissimilar, indicating the reliance of the system

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on a large number of receptors for encoding odor quality. Maps for simple and complex mixtures of odorants show that specific glomerular sites for particular mixture components are metabolically inhibited: the map does not become hypercomplex as component numbers increase, but rather becomes consolidated into a unique glomerular map, by elimination of some sites active for single compounds, either at the receptors or by intrabulbar inhibition. Multiple receptors for single compounds, as well, perhaps, as the capacity for some to be blocked, are therefore necessary for the encoding of a complex mixture into a 'smell fingerprint'.

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34. Effects of monosodium glutamate on human food palatability

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Monosodium glutamate (MSG) is a well-known flavor enhancer used in both Western and Eastern cuisines. Responsible for the UMAMI (delicious) taste, it is incorporated into a large number of solid and liquid savory foods. Experimental studies have established that the presence of added MSG in foods influences palatability, preference and selection. Sensory evaluation tests have shown that both traditional and novel foods get higher palatability ratings if MSG is added at an appropriate dose. In young adults, behavioral tests have shown that the acquisition of a liking for novel foods is facilitated by the addition of MSG to the recipe. In institutionalized elderly persons as well as hospitalized diabetic patients, the addition of MSG to target foods in a lunch meal induced an increased intake of those specific foods, with a subsequent decreased intake of foods presented later in the meal. In both populations, only prandial food selection was affected by MSG but meal size (kJ) remained the same. Experiencing the positive effects of MSG is thus possible without inducing hyperphagia. In conclusion, MSG can be used casually by the consumer in order to increase palatability, and it can also be used selectively by nutrition experts in order to orient food selection toward a healthy diet composition.

Supported by the International Glutamate Technical Committee, the Comité Français des Fabriquants d'Acide Glutamique and the Umami Manufacturers Association of Japan.

35. Olfactory threshold after nasal allergen challenge

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Olfactory threshold was investigated in patients with verified allergic rhinitis to birch pollen. In 20 patients the olfactory threshold was measured before and after challenge with topical applied birch pollen allergen during a non-symptomatic period. After provocation a statistically significant impaired detection sensitivity was found. The change in olfactory threshold was correlated with the measured amount of nasal secretions but not with subjectively or objectively registered nasal obstruction. A pronounced allergic reaction after allergen challenge is accompanied by an elevated olfactory threshold.

Supported by konsul Th C Berghs Stiftelse, the County Council of Skaraborg and # AG040, NIH.

36. Functional organization of the macroglomerular complex in *Heliothis virescens*. Projections of receptor neurons and dendritic arborizations of an antennal lobe neuron

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The functional organization of the macroglomerualar complex (MGC) of the antennal lobe (AL) in the moth species *Heliothis virescens* has been studied by staining different functional types of receptor neurons (RNs) for determining their projections in the MGC, and filling AL projection neurons during intracellular recordings for determining their dendritic arborizations. The results confirmed the previous finding that the RNs tuned to the major pheromone component project in the largest MGC lobe ('cumulus') at the entrance of the antennal nerve. However, the RNs tuned to the second pheromone component did not show projections in the cumulus, as previously reported. In the present study, the second type of pheromone RNs was always recorded together with a neuron tuned to the interspecific signal, which has also been found when recording from the base of the sensillum. Cobalt-lysine, applied to these sensilla, always showed two stained axons, each projecting in one of the smaller ventral MGC lobes. The results suggest that the previous conflicting results about the projections of the neurons tuned to the interspecific signal in the ventro lateral and the ventro medial lobe, respectively, is due to the fact that two neuron types are colocalized, making it difficult to stain only one particular type. No RNs have so far been found to project in the dorso medial lobe of the MGC. However, intracellular recordings from an AL projection neuron showed an extensive arborization exclusively in this lobe. The AL neuron responded to antennal stimulation with the pheromone components, both to the single compounds and to a mixture of them, eliciting a synergistic response. The results suggest that the input to this AL projection neuron is mediated via local interneurons. Whether the dorso-medial lobe of the MGC in H. virescens receives input directly from the RNs remain to be shown.

37. Reception of plant odours in *Pissodes castaneus* studied by linked gas chromatography–electrophysiology

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The weevil *Pissodes castaneus* DeG. (Coleoptera, Curculionidae) uses trees of the genus *Pinus* as host plants. Olfactory stimuli are thought to be important for the location of the host tree by *P. castaneus*. The aim of this study was to identify the volatile compounds released by host and non host trees that influence the activity of olfactory receptor neurons in *P. castaneus*.

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Responses of single receptor neurons to plant produced volatiles, were recorded using gas chromatography linked with electrophysiological recordings. Volatiles released from host and non-host tree species were collected by 'head space' procedure and then used to stimulate the receptor neurons via the GC. Chemical identification of compounds was made using gas chromatography linked with mass spectrometry (GC-MS).

Most neurons tested responded to a limited number of compounds and showed a best response to one or two of them. Some neurons responded best to major components of the host volatile mixture, such as α-pinene, β-pinene, 3-carene, β-myrcene, limonene and β-phellandrene, whereas others responded best to minor components as for example fenchone. The neurons were classified in groups, according to the compound that elicited the strongest response. No overlap of response spectra between neuron groups was observed

The results obtained so far suggest that plant odour information in *P. castaneus* is encoded by a restricted number of receptor neuron types, each responding to one or a few compounds.

38. Can solution chemistry explain synergy?

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The synergy which has been reported in binary mixtures of sweeteners by Schiffman and others has been attributed to 'multiple receptors and transduction mechanisms' or higher 'perceptual' effects. However, solution measurements may reveal differences in hydration behaviour which might in turn explain synergy. Mixtures of one bulk and one intense sweetener do not exhibit any notable differences from their components in apparent specific volumes. However, apparent isentropic compressibilities ($K\phi s$) do appear to be larger negative (i.e. *less* compressible) in the binary mixtures than in the single solute solutions, which shows that water-solute interactions are greater in the mixtures which might in turn facilitate transport to receptors. For example, a synergistic mixture of 4.55 sucrose plus 0.19g sodium cyclamate in 100 ml of water solution has a $K\phi s$ of -2.5×10^{-3} cm^3 mol^{-1} bar^{-1} whereas the $K\phi s$ of pure sucrose is -1.9×10^{-3} cm^3 mol^{-1} bar^{-1}. The apparent molar volume (ϕv) of the synergistic mixture is 204.5 cm^3 mol^{-1} whereas that of pure sucrose is 210.8 cm^{-3} mol^{-1} but the difference between these values accords with weight fractions of the solutes because the fv value of sodium cyclamate is 122.7 cm^{-1} mol^{-1} at the concentration tested. These measurements do not reveal whether both sweeteners or only one in the binary mixtures actually stimulate receptors. However, the vast molar excess of the bulk sweetener and the pure quality of sweet sensation are consistent with the involvement of the sucrose.

Supported by E.C. Grant No. PL94-2107.

39. Left and right nostril and odor intensity

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In a previous study, one experiment was performed to determine if an odor, phenylethyl alcohol, presented undiluted, could modify differently the right and the left nostril patency. The results showed an increased congestion on the left side in 3/4 of the subjects after inhaling phenylethyl. In the present study, phenylethyl was presented diluted to 1% in a mineral oil. The nasal air flow resistance was first measured bilaterally by rhinomanometry for 10 measures every 2 min, followed by ten 2 min measures with odorant. In the second, the subjects were asked to inhale phenylethyl before each measure. The results show a significant increase of right nostril resistance in 55% of the subjects ($P < 0.05$), an increase of the left nasal resistance in 9% of the subjects, an increase of left an right resistance in 9% of the subjects and no significant difference in 27% of the subjects. These findings suggest a left–right nostril difference, depending on odor intensity, the left nostril reacting more to the undiluted odor and the right nostril reacting more to the diluted odorant.

40. Double-label immunocytochemistry of taste cells in the rat: gustducin, NCAM, carbonic anhydrase and calbindin

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Lingual taste buds in rats contain ~100 taste receptor cells falling into three ultrastructural types (Types I–III). Previous immunochemical studies have shown that a variety of antisera, e.g. directed against gustducin, neural cell adhesion molecule (NCAM), carbonic anhydrase isoenzyme II (CAII), isoenzyme IV (CAIV) or calbindin, react with spindle shaped cells within each taste bud. Two of these, gustducin and NCAM, have been shown ultrastructurally to react with Type II (intermediate/light) taste cells. The present study was undertaken to determine whether the same or different populations of presumed Type II cells are immunoreactive (ir) to these various antisera.

Thirty micron cryostat sections were prepared from tongues immersion-fixed in 4% buffered paraformaldehyde. Free floating sections were treated with a mixture of primary antisera: gustducin & NCAM, gustducin & calbindin; gustducin & CAII, CAII and calbindin, etc. After exposure to primary antisera overnight at 4°C, the sections were rinsed and treated with a cocktail of secondary antisera carrying different fluorochromes. Control experiments showed no cross reactivity for the secondary antisera.

Antibodies to NCAM label spindle-shaped taste cells similar in appearance to those labeled by gustducin antiserum. Despite this similarity in appearance, no NCAM-ir cells are ir for gustducin. Thus Type II cells fall into two classes: those ir for gustducin and those ir for NCAM.

Amongst the gustducin-ir population, a subset reacts with antisera to CAII. The degree of reactivity for CAII and gustducin varied but the degree of reactivity of the two antibodies was not correlated within a single cell. Fewer gustducin/CAII-ir taste cells occur in fungiform taste buds than in the other papillae. Within the gustducin/CAII-ir population, a subset is also calbindin-ir. Fungiform taste buds rarely contain calbindin-ir cells. Within the NCAM-ir population, a subset of cells are also immunoreactive for CAIV.

These results indicate that despite similarities in ultrastructural type, cytochemical differences may exist within a single morphological type of taste cell. Whether these reflect different relative ages of taste cells or different functional types is yet to be determined.

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Supported by NIH Grant PO1 DC00244 to T.E.F.

41. Ultrastructure of the taste buds in the blind cave fish *Astyanax jordani* (*'Anoptichthys'*) and its sighted relative *Astyanax mexicanus* (Teleostei)

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The ultrastructure of the teleostean taste bud (TB) is well known. Nevertheless, here we describe the TBs of two Mexican characids systematically closely related, but highly adapted to different habitats, the sighted river fish *Astyanax mexicanus* (*'Astyanax'*) and its cavernicole blind relative, *Astyanax jordani* (*'Anoptichthys'*). We investigated the TBs situated on the lips and in the oral cavity. After sacrificing with MS 222, the fish were fixed in a 2% glutaraldehyde–2% paraformaldehyde mixture and processed for transmission electron microscopy. The TBs of both, *Anoptichthys* and *Astyanax*, measure only ~55 μm in height and 25 μm in width and sit on top of a dermal papilla. Labial TBs located closely to the oral cavity lie in hillock-like epidermal papillae. The more distal parts of the lips and the posterior epithelia of the oral cavity are rich in non-elevated TBs. The TB's sensory epithelium consists of ~100–120 cells which belong to five cell types: light cells, dark cells, dense-cored-vesicles (dcv) cells, basal cells and marginal cells. Light-, dark- and dcv-cells are slender and build the main sensory epithelium of the TB. The basal cells lie directly on the basal lamina; they do not reach the TB's receptor area. Marginal cells are 'normal' epidermal cells lining the organs' sensory epithelium against the epidermis. Light cells apically end in one large receptor villus and are rich in smooth endoplasmic reticulum, Golgi systems and microtubules. Dark cells apically bear several small undivided microvilli and are rich in intermediate filaments. They have thin lobar processes ensheathing the roundish light cells. Dcv-cells occur in both, *Anoptichthys*- and *Astyanax*-TBs, and have not been found before in other teleosts. Apically, they contribute to the receptor area with several intermediate microvilli. They possess many organelles including numerous large electron dense vesicles (150–200 nm) and abundant clear (30nm) and dense-cored (60 nm) vesicles. Characteristically, only the basal cells of elevated TBs possess spine-like processes. Afferent synapses lie between light-, dark-, dcv-, basal cells (presynaptic sites) and nerve fibers (postsynaptic sites). Efferent synapses were not found. It should be pointed out that only elevated TBs have basal cells rich in spines. Possibly these TBs have an additional mechanosensory function in food search, when food particles touch and mechanically stimulate TB-bearing papillae.

42. Amiloride suppresses the responses to acids in NaCl-best neurons of the hamster solitary nucleus

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Hamster taste receptor cells with amiloride-blockable Na^+ responses have also been shown to possess an amiloride-sensitive H^+ current. In the present study, we investigated the effects of amiloride on responses to acids in neurons of the hamster solitary nucleus (NST). Cells were recorded extracellularly while the anterior tongue was stimulated with solutions representing the four taste qualities (0.03 M NaCl, 0.1 M sucrose, 0.003 M HCl, 0.001 M quinine–HCl), which were used to characterize each cell on the basis of its best stimulus. The effects of amiloride on responses to 0.01 M HCl, 0.01 M citric acid, 0.1 M NaCl and 0.1 M sucrose were then investigated. Stimuli were either presented alone for 30 s, or presented for 10 s, followed by a mixture of the stimulus with 10 mM amiloride for 10 s, followed by the stimulus alone again for 10 s. The effects of amiloride addition were then assessed by comparing the responses of cells to the stimulus + amiloride to that of the stimulus alone. In neurons classified as NaCl-best, amiloride reversibly blocked responses to NaCl, HCl and citric acid, but not responses to sucrose. In HCl-best neurons, amiloride had no effect on responses to NaCl, HCl or citric acid. In sucrose-best neurons, amiloride blocked the response to NaCl, but not those to sucrose or to either HCl or citric acid. These results support the hypothesis that acid taste responses in the hamster are transduced by at least two different mechanisms: amiloride-sensitive and amiloride-insensitive. At the NST, these inputs are maintained in two different populations of neurons. Sucrose-best neurons, in which amiloride reduces the response to NaCl but not to acids, may receive converging inputs from both amiloride-sensitive and amiloride-insensitive receptor cells. These data suggest specific patterns of convergence of chorda tympani afferent fibers onto second-order NST neurons.

Supported in part by NIDCD DC00353 (D.V.S.).

43. Taste bud distributions of α-gustducin and the Lewis^b blood group antigen correlate with gustatory sensitivity

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Gustducin is a taste-cell specific G-protein that has been implicated in the transduction of responses to bitter- and sweet-tasting substances. The human blood group antigens A, B, H and Lewis^b, which are differentiation antigens in mammalian epithelia, are differentially expressed in rat taste cells. We prepared cryostat sections from several taste bud regions of rats. Sections were double-labeled for indirect immunofluorescence with a monoclonal antibody to the Lewis^b or A carbohydrate antigens and a polyclonal antibody to α-gustducin, then examined by confocal microscopy. Gustducin and the Lewis^b and A antigens were present only on spindle-shaped cells whose apical processes reached the taste pore. A subset of gustducin-positive cells expressed the Lewis^b antigen, but all Lewis^b-positive cells expressed gustducin. The A blood group antigen and Lewis^b were rarely expressed by the same cells. About one-third of the cells expressing α-gustducin and blood group A were double-labeled for these two molecules, whereas two-thirds expressed one or the other.Taste buds in fungiform papillae, a region primarily sensitive to salt and acid stimuli, contained relatively few cells expressing gustducin (3.1 cells/bud). Taste buds in the vallate and foliate papillae, regions especially sensitive to bitter- and somewhat responsive to sweet-tasting stimuli, contained many cells expressing gustducin (9.8 and 8.9, respectively). Palatal taste buds found within the geschmacksstreifen or lining the nasoincisor duct, areas especially responsive to sweet-tasting but not

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bitter-tasting stimuli, also contained many cells expressing gustducin (9.6 and 9.3, respectively). Thus, the distribution of gustducin expression is consistent with its proposed role in the transduction of responses to both sweet- and bitter-tasting substancesthe distributions of these three antigens suggest considerable molecular diversity among Type II (light) taste cells.

Supported in part by NIDCD DC00347 (D.V.S.).

44. Effects of bitter stimuli on hamster behavioral and chorda tympani responses

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The chorda tympani (CT) nerve responds to aversive stimuli, although it responds more strongly to appetitive stimiuli. For example, the hamster (*Mesocricetus auratus*) CT responds to quinine.HCl (QHCl), a prototypic bitter stimulus. Hamsters cross-generalize aversions learned to 1 mM QHCl and 100 mM $MgSO_4$, which suggests they are both bitter. Other stimuli that are bitter to humans have unknown effects in hamsters. These bitters show a range of chemical features. $MgSO_4$, QHCl and denatonium benzoate are ionic; denatonium and QHCl are large hydrophobic cations. Sucrose octaacetate (SOA) and caffeine are non-ionic, but SOA is considerably more hydrophobic than caffeine. We used behavioral tests of preference and generalization of conditioned taste aversion (α level = 0.01), as well as electrophysiologic recordings from the CT nerve to differentiate the characteristics of the five bitters. Hamsters find 180 mM $MgSO_4$, 3 mM denatonium, 30 mM caffeine and 1 mM SOA aversive. Learned aversions to the ionic stimuli $MgSO_4$ and denatonium generalized to 1 mM QHCl and elicited concentration-dependent CT responses. The non-ionic stimuli did not stimulate the CT. We were not able to condition aversions to 30 mM caffeine or 1 mM SOA. Aversions learned to the saturated solutions: 100 mM caffeine and 1.5 mM SOA did not generalize to quinine, $MgSO_4$ or denatonium; the strongest generalization was from SOA to caffeine. The ionic bitters are quinine-like. However, caffeine and SOA are not quinine-like and must have another aversive quality. the ionic bitters (QHCl, $MgSO_4$, denatonium benzoate) and non-ionic aversive stimuli (caffeine, SOA) have distinct stimulus qualities in hamsters, and information about them is conveyed to the brain by more than one nerve.

Supported by NIH grant DC-00058.

45. Odorant responses from mouse olfactory receptor neurons that project to discrete regions of the dorsal olfactory bulb

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Previous electrophysiological, optical recording and molecular studies have suggested that there may be odorant-related projection patterns from the olfactory epithelium (OE) to the olfactory bulb (OB). There are no data available, however, that directly relate sensitivity differences of olfactory receptor neurons (ORNs) to the their topographic projection patterns onto the OB surface. We have developed a method for applying focal injections of fluorescent latex microspheres into groups of visualized glomeruli in living mice. Retrogradely traced ORNs are then acutely isolated for Fura-2 calcium imaging and tested with six mixtures of odorants in order to define ORN response profiles. This technique has allowed us for the first time to investigate the differential responsiveness of ORNs that project to defined regions of the mammalian OB. As a first step, we are asking (i) whether response profiles of ORNs that project to small clusters of glomeruli are more similar to each other in comparison to the response properties of ORNs that project more broadly across the OB; and (ii) whether ORNs that project to two sites in the dorsal OB exhibit distinguishably different odorant response profiles. We are presently comparing the response properties of ORNs that project to two discrete groups of glomeruli in the dorso-medial and dorso-lateral OB to those of ORNs that project to the entire dorsal surface of the OB. ORNs that project to the dorso-medial site exhibit a more restricted set of response profiles compared with dorsally projecting ORNs. We also show that ORNs labeled from dorso-medial or dorso-lateral sites (which are separated by <1 mm) exhibit different response profiles. Further studies should allow us to relate the sensitivity of ORNs to molecules with known chemical structures with the location of ORN synapses in the OB.

Supported by research grants from the NIDCD and the ONR.

46. Exciting and depressing news about NTS circuits

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The rostral nucleus of the solitary tract (rNST) plays a key role in modulating, organizing and distributing the sensory information arriving at the CNS from gustatory receptors. However, except for some anatomical studies of rNST synapses, the neural ci rcuits responsible for this first stage in synaptic processing of taste information are largely unknown. Over the past few years we have used an *in vitro* brain slice preparation of the rNST to study synaptic processing and it has become apparent that the rNST is a very complex neural relay.

Synaptic potentials recorded in rNST neurons resulting from stimulation of afferent taste fibers are a composite of excitatory and inhibitory post synaptic potentials. Pure excitatory synaptic potentials (EPSP) can be isolated by using $GABA_A$ receptor blockers to eliminate the inhibitory postsynaptic potentials (IPSP). Application of glutamate ionotropic receptor blockers effectively eliminates all postsynaptic activity indicating that glutamate is the transmitter at the first central synapse in the taste pathway. Stimulation of the afferent taste fibers originating from the anterior (chorda tympani) and posterior (glossopharyngeal) tongue results in a postsynaptic potential that is a complex sum of the two individual potentials. Thus, rNST neurons receive convergent synaptic input from the anterior and posterior tongue.

The IPSP component of the synaptic potentials in rNST results from stimulation of interneurons. If these IPSPs are initiated by tetanic stimulation they undergo both short term and long term changes. Short term changes result in the development of biphasic depolarizing IPSPs and long term changes result in potentiation of the IPSPs that can last over an h in some neurons. This remarkable synaptic plasticity may be involved in the mechanism of learned taste behaviors.

Synaptic transmission in rNST consists of excitation combined with inhibition. The inhibition does not simply depress excitation

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but probably serves many roles such as shaping and limiting excitation, coordinating the timing of synaptic events and participating in synaptic plasticity. Knowledge of these synaptic mechanisms is essential to understanding how the rNST processes taste information.

Supported by NIDCD, NIH Grant DC 00288.

47. Bitterness in flavor profiling of soft cheeses

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Quantitative descriptive sensory profiling is a method that uses human assessors to quantify the sensory characteristics of a set of products. In this study the flavor of five Danbo-type cheeses fermented with different starter cultures was profiled using 22 attributes. The profiling data were analyzed by Analysis of Variance (ANOVA), Principal Component Analysis (PCA) and PLS Discriminant Analysis (PDA), to reveal the (co)variation between products, replicates and assessors. Seven attributes significantly ($P < 0.05$) discriminated the five cheeses. PCA showed the differences between the samples spanned by the attributes meaty, salty and harmonious in PC1 as well as bitterness and tingling aftertaste in PC2.

Differences in the proteolytic activity of the starter cultures may explain the variation of bitterness in these cheeses. The role of bitter taste perception in soft cheeses was further investigated using a model cheese with added quinine. The bitter taste of the model cheeses was shown to interact with the perception of other basic tastes. Increased bitterness significantly ($P < 0.05$) reduced the perceived intensity of salty and sweet taste. This model appeared to be useful to study sensory interactions between flavour components in soft cheeses.

48. Expression and functional analysis of olfactory receptors

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The olfactory system recognizes and discriminates myriads of odorants of diverse molecular structure. This task is supposed to be accomplished by a large array of seven-transmembrane domain receptors encoded by a multigene family. Although circumstantial evidence suggests that the identified genes encode odorant receptors, unequivocal proof requires demonstration that the resulting proteins should be able to interact with odorous molecules and couple via G-proteins onto second messenger cascades. This goal can be achieved by heterologous expression of receptor proteins in surrogate eucaryotic cells, although the task is complicated by the diversity of putative odorous ligands and the large size of the receptor family. Employing the baculovirus/Sf9 cell system it was found that receptor proteins can be expressed at high levels. Stimulating receptor-expressing Sf9 cells with a mixture of numerous odorous compounds elicited a significant and dose dependent second messenger response, which was never oberserved in control cells. Assaying a large panel of odorous compounds, including representatives of different odor classes and compounds of different chemical classes revealed that distinct receptor subtypes respond to certain odorants but not to others. Graded responses to only a subset of odorants indicate that the heterologously expressed receptor types have a selective but relatively broad ligand specificity.The easily manipulated bacterial system was employed to produce olfactory receptor proteins in large quantities. It was solubilized from inclusion bodies and upon reconstitution in liposomes displayed specific interaction with odor ligands.

Supported by the Deutsche Forschungsgemeinschaft.

49. Quality of life for patients with chemical senses disorders

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Although disorders of the chemical senses reportedly have a profound negative impact upon quality of life (QOL), few studies have addressed QOL and the chemical senses directly. The Monell–Jefferson Quality-of-Life Questionnaire was mailed to 100 individuals seen consecutively at the Monell–Jefferson Chemosensory Clinical Research Center. The goal was to investigate the self-reported importance of the chemical senses to these patients and the impact of the disorder on their lives. The questionnaire consists of two sections: the first asks subjects to engage in various hypothetical trade-offs for the return of their chemical senses to normal; the second asks specific questions about how respondents' disorders affected their lives. Responses were received from 34 patients, most of whom were diagnosed as suffering from olfactory dysfunctions. Results are presented as geometric means (with arithmetic means inside parentheses to show the high positive skew in responses). In exchange for a complete return to normal smell and taste function, these patients were willing on average to: (i) endure a night of insomnia every 34 (25) days or a 24-h-long bout of diarrhea every 55(14) days; (ii) be without their skin senses for 7 (799) days; (iii) be deaf for 1.7 (203) days; (iv) be blind for 0.7 (436) days; (v) shorten their life span by 3.6 (1126) days; or (vi) pay $2750 ($27 000) for a cure. Patients reported decreased pleasure in eating fatty, acidic, and chewy foods, and alcoholic beverages, and increased enjoyment of eating hot, spicy foods. Patients' concern for detecting smoke, cleanliness of public areas, personal body odor, spoiled and rotten food, and gas leaks all increased greatly. Patients' enjoyment of life in general, specifically, of social outings (such a seating in restaurants) decreased because of their disorder. Therefore, the chemical senses are of great importance to these patients and critical to their enjoyment of food, as well as of life in general.

Paul Breslin is a Morley R. Kare Fellow. This work was also funded by NIH grants P50 DC00214 and R29 DC02995 (P.A.S.B.).

50. Psychophysics of taste lateralization on anterior tongue

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There have been few investigations of the spatial sensory

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properties of taste stimuli presented to a specific area of the oral cavity. This is surprising since the localizability of taste sensations may be as integral a component of the overall taste percept as are quality, intensity and temporal characteristics. One reason there may be few taste localization studies is the difficulty in eliminating the confounding factor of a tactile sensation, since a gustatory stimulus cannot be easily presented without a tactile component. As a step toward understanding the localizability of gustatory sensations, we designed a yoked-stimulator and an experimental procedure to control for tactile cues by presenting a taste and a blank (diluent) solution simultaneously at two locations on the tongue. We evaluated lateral discrimination at the tip of the tongue with four taste stimuli (Na–saccharin, NaCl, citric acid and quinine–HCl) and found that subjects could lateralize all four taste stimuli in the absence of any discriminative mechano-tactile cue. Subjects' ability to lateralize varied as a psychometric function of the stimulus concentration. Lateralization threshold values derived from the function were about an order of magnitude higher than existing whole mouth reports of detection thresholds. Furthermore, detection thresholds, measured in a two-interval staircase procedure with the same yoked-stimulator that was used in the lateralization task, were comparable with whole mouth detection thresholds, and both lateralization and detection threshold were correlated with each other within subjects. Subjects were unable to lateralize taste cues on a non-gustatory surface (under the upper lip) at the highest tested concentrations [10, 100, 13, 4 mM respectively], at which performance was 100% on a gustatory surface. These results demonstrate that taste compounds can be lateralized in the absence of any discriminative mechanical cue and that they need to be detected to be lateralized.

P.B. is a Morley R. Kare Fellow. This work was also funded by NIH grant # R29 DC02995 (P.A.S.B.).

51. Nitric oxide and cyclic nucleotide-gated channels in olfaction

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Cyclic nucleotide-gated channels are important in olfactory transduction, and possibly in other neuronal functions. These channels have a complex permeability to Ca ions that may be important in their cellular functions. The channels are composed of two subunits, α and β, that have been cloned and expressed, but the β subunit alone cannot be activated by cyclic nucleotides, confounding the analysis of its characteristics. In heterologous systems, the α subunit is capable of forming a homomeric functional channel, although its properties differ from the native channel. The β subunit, when expressed alone and tested with cyclic nucleotides, shows no activity, but, when expressed together, the two subunits form a channel that very nearly approximates the functional characteristics of the native channel. We have recently demonstrated that, in addition to activation by cyclic nucleotides, the native CNG channels can be independently activated by NO generating compounds, apparently through a redox reaction at free SH groups on the intracellular face of the channel protein, providing a new tool for exploring channel function. We utilized this to test the function of heterologously expressed homomeric β channels, which do not respond to cAMP or cGMP, and found functional conducting channels that have properties significantly different from both homomeric a channels and native channels from rat olfactory neurons. Recently the β subunit has been detected without the accompanying α subunit in neural tissue, in particular in the neurons of the rat vomeronasal organ (VNO), indicating a possible physiological role for a β channel. Indeed we have determined that β channels in VNO neurons can be activated by NO providing a possible transduction pathway.

This research was supported by NIH, HFSP and Fonds National Suisse.

52. Conditioned aversion to aqueous odours

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Three experiments were conducted to assess one-trial conditioning of aversions to the odour of aqueous amyl acetate (0.05%), known to be below gustatory threshold. In a one bottle test, aversions were reliably obtained when the US (128 mg/kg of 0.15 M LiCl) was administered 0.25 or 1 h after consumption of amyl acetate (CS). CS–US delays of 2–8 h produced progressively weaker aversions. Experiment 2 used a two-bottle test to show that when the odour presented during extinction (almond) differed from that presented during conditioning (amyl acetate) drinking was not suppressed, indicating that conditioning was specific to the odour used on the conditioning day. When access to the odorous liquid was prevented on the conditioning day by a wire-mesh barrier, an odour aversion was not learned. Experiment 3 found that with a short CS–US delay and weekly tests, strong aversions to amyl acetate odour were still present 12 weeks after the conditioning trial, indicating a long-term memory for the odour. These results, together with those of Slotnick *et al.* (1995), demonstrate that odour aversion learning is comparable to taste aversion learning with regard to one-trial learning, conditioning with long CS–US delays, specificity of the conditioned response to the CS, and long-term retention of the aversion.

Supported by NSERC of Canada grant A7441 and Wyeth Research.

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53. Glomerular cytoarchitectures within topographically organized chemosensory pathways of arachnids

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In most animals the central pathways of olfactory systems are associated with glomerular neuropil and lack topographic mapping of sensory inputs. Among arthropods, the insect and crustacean olfactory (antennal) pathways are typical examples. Two orders of chelicerate arthropods, the scorpions and solpugids (Cl. Arachnida), present striking exceptions to this generalization. The major chemosensory organs of scorpions are the pectines, two ventral appendages that contact the substrate intermittently as the animal searches for food or mates. In solpugids chemosensory input is from the antennalized pedipalps and first leg pairs, and

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from ten fan-shaped malleoli extending ventrally to the substrate from the 4th leg pair. The pectinal and malleolar sensory systems have highly ordered arrangements of 10^5 to 10^6 primary chemoreceptors, with one (pectines) forming a two-dimensional array and the other (malleoli) assembled in a linear array. The spatial frequencies of these chemoreceptive inputs exceed 100/mm and 1000/mm, respectively, indicating a capacity for resolving structure of chemical deposits on substrates. Using several histological and axonal tracing techniques, the organization of pectinal and malleolar central projections has been resolved. The pectinal projection terminates posteriorly in the cephalothoracic mass and shows a high degree of topographic precision, perhaps to the level of individual receptors in the sensory field. This chemosensory 'map' is imposed on laminar cytoarchitecture posteriorly in the brain but merges anteriorly into glomerular substructures. The sensory projection from the malleoli shows less topographic order with fewer and larger glomeruli reminiscent of insect olfactory system. These comparison between arthropod taxa suggest that olfactory projections are, to varying degrees, typically glomerular but may evolve topographic and laminar organization when the stimulus field is of fixed form.

Supported by NSF (IBN-9320362).

54. Regulators of G-protein signaling (RGS) genes in olfactory receptor neurons

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Heterotrimeric GTP-binding proteins (G-proteins) coule odorant receptors to second messenger signaling systems. Members of the multigene RGS family interact with G-protein α-subunits and accelerate the rate of GTP hydrolysis by the intrinsic Ga GTPase and thereby rapidly return Ga to the inactive GDP-bound state. RGS proteins thus act to inhibit G-protein signaling. We recently showed that the RGS3 gene was expressed in olfactory receptor neurons from channel catfish (*Ictalurus punctatus*) (Bruch and Medler, 1996). Sequence analysis of 16 cloned TGS PCR products from olfactory neurons showed that four additional RGS genes were expressed in these cells. Three genes were similar to the mammalian RGS2, 4 and 5 genes while another RGS (RGSo) was unlike any in the databanks. Northern blotting showed that RGSo was expressed in several tissues. The RGS PCR products encoded 67 of the 130 amino acids of the RGS domain and contained the Ga binding site. However, hexahistidine epitope-tagged RGS polypeptides consisting of 67 amino acids did not interact with Ga with high affinity. Thus, full-length RGS domains are being cloned to identify specific Ga subunits that interact with individual RGS proteins.

Supported by NIH DC01500 (to R.C.B.)

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55. Symposium: Development of the olfactory system

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There are many reasons to study development. Some are purely pragmatic: developmental work might help to allow organizational principles to be derived before the obscuring complexities of the adult state emerge, and they can help to decipher errors that lead to functional problems. However, studies of maturing animals are interesting in their own right, as early life is a period of fascinating synthesis and interaction. The olfactory system is especially important to examine from a developmental point of view because, unlike nearly every other area of the nervous system, it exhibits lifelong cell proliferation, migration, synaptogenesis, differentiation and cell death. Indeed, its protracted development suggests that to completely understand it will depend on our being cognizant of the patterns and principles of its ontogenesis. The speakers in this symposium will examine olfactory system maturation from several general perspectives: aspects of chemical, neuronal, glial, and functional development as well as patterns of invertebrate maturation will be discussed. The presentations will allow us to gauge how much is presently known and to identify areas that demand the most attention in the future.

56. Experience-induced changes in chemosensory responsiveness: mechanistic basis and functional significance—the lingual trigeminal system

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The lingual trigeminal branch of the oral somatosensory system mediates the reception of both noxious and innocuous thermal, mechanical and chemical stimuli. Since one of its functions is to monitor the condition of peripheral tissue, it is not surprising to find a complex of interactions with the surrounding tissue that affect neural sensitivity. Early exposure of somatosensory neurons to nerve growth factor is necessary for the expression but not the maintenance of sensitivity to irritants such as capsaicin. Tissue injury and inflammation cause the release of cytokines and inflammatory mediators as well as decreases in tissue pH, that activate peripheral nerve endings. These endogenous compounds also sensitize nerve endings, lowering thresholds and inducing sensitivity to stimulus modalities to which the neurons were previously insensitive. Long lasting chemical sensitization of somatosensory nerves *in vivo* has been best characterized as the hyperalgesias produced by irritants such capsaicin and mustard oil. Protons have also been shown to alter the sensitivity of trigeminal nerves. However, studies with isolated somatosensory neurons generally have found desensitizing responses to repeated chemical stimulation. This suggests that peripheral sensitization is largely a function of interactions with the tissue matrix surrounding nerve endings rather than being solely an intraneuronal process. Supporting this interpretation are findings of sensitizing interactions between endogenous pain and inflammatory mediators, protons and irritants such as capsaicin and piperine. Long lasting desensitization of isolated neurons to capsaicin stimulation has been demonstrated to be dependent upon calcium-activated processes, suggesting a peripheral basis for capsaicin desensitization that has been demonstrated psychophysically.

Supported in part by NIDCD 01921.

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57. Information coding in the olfactory system

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The mammalian olfactory system is able to discriminate an enormous variety of volatile molecules. This ability is thought to derive from a series of information processing events that occur at different sites through which olfactory signals flow in the nervous system. To gain insight into how perceptual acuity is achieved in the olfactory system, we have asked how odors are initially detected in the nose and how information about different odors is organized in the nose and the brain. Our studies indicate that the detection of odors is mediated by as many as 1000 different types of odor receptors, which are encoded by a multigene family. Of the millions of olfactory sensory neurons in the mouse nose, we found that only a small fraction expresses each type of receptor, suggesting that each neuron transmits to the brain information derived from a single receptor type, rather than a combination of receptors. Our experiments also revealed that there are four different spatial zones in the nose in which different sets of receptors are expressed. Each zone is a mosaic in which neurons expressing a given receptor are interspersed with neurons expressing a variety of other receptors. When we examined how information provided by different receptors is organized in the olfactory bulb of the brain, the first relay in the olfactory pathway, we found something quite different. Here, information provided by each receptor type is focused onto two fixed sites, one on either side of the bulb. The bulbar spatial maps of receptor input are virtually identical in different individuals. Given the functional characteristics of individual olfactory sensory neurons, our findings suggest a model in which different combinations of receptors serve as codes for different odorants, but each receptor is part of the codes for many different odorants. In the nose the identity of an odorant is likely to be encoded by a unique combination of randomly dispersed neurons. In the olfactory bulb, the code is again combinatorial, but here it has a specific spatial pattern, which is the same in every individual.

58. Neurogenesis in the *Xenopus* olfactory bulb during development

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Previous research on development of the *Xenopus* olfactory bulb from our laboratory has shown that mitral cells begin to differentiate after olfactory axons make contact with the olfactory bulb, and the number of olfactory axons is correlated with the number of mitral cells throughout development. In embryos, removal of all afferent innervation before the mitral cells begin to differentiate results in a failure of the bulb to form; removal of half of the olfactory axons, results in development of half the normal number of mitral cells. At larval stages, transection of the olfactory nerve results in a decrease in the number of neurons in the olfactory bulb. Thus, the olfactory axons play a major role in stimulating or maintaining development of the olfactory bulb neurons.

Since we have found that neurogenesis in the bulb continues through metamorphosis, the goal of the current study was to determine whether olfactory axons influence cell genesis and/or neuronal maturation in the larval olfactory bulb. To study cell genesis, we cut the olfactory nerves, and 6 days later, injected the animals with [^{3}H]thymidine. After 6 h, the animals were killed and the tissue was processed for autoradiography. The number of labeled cells in the ventricular zone of the olfactory bulb was determined in every fifth section through the bulb in control and experimental animals. There was no statistical difference (Mann–Whitney rank sum test) in the number of labeled ventricular cells between controls and experimentals. Thus, olfactory axon innervation does not appear to play a role in precursor cell division during larval development. To study the influence of olfactory axon innervation on the ability of newly generated neurons to mature, we followed the same procedures, however, the animals were killed 21 days after the [^{3}H]thymidine injection. The results from this experiment showed that there are many fewer labeled mitral cells in the experimental animals at 21 days. Together these results suggest that sensory deafferentation influences mitral cell differentiation or survival even during late stages of larval development.

Supported by NIDCD # DC00446.

59. A new computer-controlled olfactometer for the recording of evoked potentials

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A new olfactometer was developed by the aid of which it is possible to chemically stimulate the nasal mucosa in man. In order to obtain bioresponses repeatable stimuli are required, i.e. concentrations and time courses must be exactly reproducible. Moreover, stimulus onset has to be exceedingly steep (<20 ms) in order to achieve an acceptable signal-to-noise ratio. Likewise, a synchronous excitation of other sensors, especially of mechano- or thermosensors, has to be avoided at all events. The new olfactometer meets all of these demands. Pulses of the stimulants are mixed into a constantly flowing air stream with controlled temperature (36.5°C) and humidity (80% relative humidity) by switching between two gaseous streams. The switching is induced by the applying of vacuums so that no moving parts have to be installed in front of the subject. This has several advantages: there are (i) no electro-magnetic disturbances of the recordings, (ii) no noise of the switching process and (iii) long distances between the main part of the olfactometer and the outlets are possible (e.g. for fMRI), and (iv) non-magnetic material can be used. By this technique it is guaranteed that the subjects have no additional cues, such as tactile, thermal or acoustic sensations, which would provide extraneous information about the timing of the stimulus presentation. The total flow rate can be varied in a wide range, but generally amounts to 140 ml/s. Three odorants can be randomly applied in a wide range of concentrations. Every pair of stimuli may have an individual interstimulus interval and individual stimulus durations. All parameters of the stimulation and additionally the recording of EEG or MEG is controlled by a PC. The recorded data are evaluated on-line.

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60. Addition of new cells to the olfactory bulb of adult zebrafish

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We have been examining patterns of cell proliferation in the brain of adult zebrafish. Understanding this process in fish may lead to important insights due to the tremendous regenerative capabilities of these animals. Fish were exposed to a 1% solution of the thymidine analog 5-bromo–2′-deoxyuridine (BrdU) for 1 h before being returned to small aquaria that received frequent water changes. Animals were over-anesthetized and perfused with Bouin's fixative solution after two survival periods (4 h or 4 weeks). Paraffin immunohistochemistry using a monoclonal antibody against BrdU was used to visualize the newly generated cells.

Quantitative analyses were performed on serial, 10 μm sections from four animals for each survival group. Statistical determinations were based on the non-parametric Mann-Whitney U test. The average number of BrdU-labeled nuclear profiles in the bulbs, from analysis of every 5th section, was significantly different between the two groups [22.0 ± 6.8 (SEM) for the 4 h group and 136.7 ± 11.3 for the 4 week group; $P < 0.05$). The volumes of the bulbs, however, were not different between the two groups ($P > 0.5$). These data indicate that either cells divided repeatedly during the longer survival period or cells migrated into the bulb from other brain regions.

To examine this phenomenon further, the location of the new cells was analyzed in three mid-bulb sections (20 μm apart) from each animal. Both the area and number of labeled nuclei in each lamina were measured to obtain an average profile density. Comparison of the 4 h and the 4 week groups showed that density was significantly greater in all bulb layers in the long survival group ($P < 0.05$ for all). In the 4 h survival group, cells were found mainly in the olfactory nerve layer. When examined after 4 weeks, proportionately more labeled cells were found in the internal cell layer. This addition of new cells could be a result of neurogenesis, gliogenesis, and/or angiogenesis. We are currently performing double-labeling experiments to determine the types of cells that are added to the adult bulb. In addition, our future plans include investigating the origin of these cells and the signals that direct their formation.

Supported by WMU (C.A.B.) and NIDCD grant DC-00338 (P.C.B.).

61. Recent results around the Gamma model on olfactory and gustatory mixtures

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The G (Gamma) model for olfactory and gustatory mixtures was recently improved in its definition, which makes the scattering of the intrinsic indices of interaction slightly smaller than previously. On the basis of several published data in olfaction, taste and pharmacology, this improved model, called now G′, appears rather satisfactory. By contrast, an alternative model, called G-vectorial, has been disappointing in all cases (Sérée and Laffort, 1996, ECRO XII, Zürich).

The first goal of the present study is to justify the calculations cascade which arises in the definition of this mixtures model. Untill now, it only appeared as a generalization of several partial models (of competitive interaction from Beidler, 1971; MDI or substitution model from Hyman and Franck, 1980; g model from Laffort and Dravnieks, 1982), but without any strict demonstration.

Secondly, by using the available experimental data, G′ values were established for 171 pairs of odorants. A first question arises from these data: if the intrinsic interactions AB and BC between odorants A, B and C are known, is it able to derive the interaction AC? The answer is *yes* statistically speaking, but *no*, by the moment, in a predictive perspective ($r = 0.31$; $P < 0.001$).

Another question is to know if there is some link between indices of mixtures interactions and indices of qualitative similarity. The answer is apparently negative ($r = 0.09$; $n = 150$; n.s.).

Supported in part by RATP (Régie Autonome des Transports Parisiens) and Conseil Régional de Bourgogne.

62. Factors regulating neurogenesis and programmed cell death in mouse olfactory epithelium

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To identify factors regulating neurogenesis and programmed cell death in mouse olfactory epithelium (OE), and to determine the mechanisms by which these factors act, we have studied mouse OE using two major experimental paradigms: tissue culture of embryonic OE and cell types isolated from it; and ablation of the olfactory bulb ('bulbectomy') of adult mice, a procedure which induces programmed cell death of ORNs and a subsequent surge of neurogenesis in the OE *in vivo*. Such experiments have been used to characterize the cellular stages in the olfactory receptor neuron (ORN) lineage, leading to the realization that there are at least two distinct stages of proliferating neuronal progenitor cells interposed between the ORN and the stem cell that ultimately gives rise to it. The identification of a number of different factors that act to regulate proliferation and survival of ORNs and progenitor cells suggests that these multiple cell stages may each serve as a control point at which neuron number in the OE is regulated. Our recent studies of neuronal colony-forming progenitors (putative stem cells) of the OE suggest that even these cells, at the earliest stage in the ORN lineage so far identified, are subject to such regulation: if colony-forming progenitors are cultured in the presence of a large excess of differentiated ORNs, then the production of new neurons by progenitors is dramatically inhibited (Mumm *et al.*, 1996). This result suggests that differentiated ORNs produce a signal that feeds back to inhibit neurogenesis by their own progenitors, and provides a possible explanation for the observation that ORN death, consequent to bulbectomy, results in increased neurogenesis in the OE *in vivo*: death of ORNs may release neuronal progenitor cells from this inhibitory signal, produced by the differentiated ORNs which lie near them in the OE. Our current experiments are directed toward identifying the

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molecular basis of this inhibitory signal, and the cellular mechanism(s) by which it acts.

Supported by NIH grants DC02180 and NS32174 to A.L. Calof.

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63. Total intensity, saltiness, and pungency of NaCl and KCl presented singly and in mixtures

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Metallic salts such as NaCl and KCl stimulate both the gustatory and the oral trigeminal systems to an extent that depends on the concentration and the duration of the salty stimulus. We explored the rules of gustatory and oral chemesthetic additivity for mixtures of NaCl and KCl via magnitude estimation. All combinations of six equimolar concentrations of NaCl and KCl were evaluated (0, 0.01, 0.032, 0.1, 0.32 and 1 M). All 10 subjects performed two experimental tasks. The first task involved ratings of total perceived intensity after 15 s of stimulus presentation. The second task involved ratings of saltiness at 5 s, followed by ratings of oral pungency at 15 s. Each task was performed in a different session. Half the subjects rated total intensity in the first session, and half rated saltiness and pungency in the first session. The outcome revealed that for all three attributes (total intensity, saltiness and pungency) the sum of the perceived intensity of the single salts surpassed the perceived intensity of their mixture at the same concentration levels (i.e. hypoadditivity). Power functions characterized the relationship between total cation moles (Na^+ + K^+) and sensory responses. Exponents averaged 0.73 ± 0.04 (SD), consistent with hypoadditivity, and were similar across salts, mixtures and perceptual attributes. Nevertheless, exponents for oral pungency of the *mixtures* were close to 1.00 and significantly different from the other exponents. These results indicate that oral pungency, compared with saltiness, shows a higher degree of sensory integration in response to mixtures of NaCl and KCl.

64. Use of graphical representation of assessor performances (GRAPES) to evaluate performance of judges on four sensory descriptive panels

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Various methods are available for studying performance of judges on sensory panels. The purpose of GRAPES (Schlich, 1994) is to shorten and simplify the analyst's job in evaluating judge performance. GRAPES is a method which computes analyses of variance and produces six scatterplots by which judge performance can be evaluated quickly and easily. The scatterplots allow comparison of scale use, within and between session error, ability of panelists to discriminate objects, and agreement within the panel for each attribute. GRAPES also produces graphs of the mean statistics of judges' scores over all attributes, and panel means for each attribute.

The objective of this study was to use GRAPES to evaluate performance of judges from four panels which had completed descriptive sensory analysis on five varieties of corn and potato chips in two sessions. Panels comprised experienced males (EM), experienced females (EF), inexperienced males (IM), and inexperienced females (IF) (n = 9, 10, 11 and 11, respectively). Twenty-eight attributes which were common to the four panels were used for this study.

The analysis showed that inexperienced judges used less of the scale range when scoring attributes. Experienced panelists were responsible for the highest levels of disagreement. Results indicate that, as a group, EF judges were the most consistent of the four panels. The greatest number of outliers was found in disagreement and discrimination, with EF generating 75% of the total disagreement outliers. Judges made more within and between session errors when scoring visual and flavor/taste attributes than when scoring texture attributes. Texture attributes were scored with higher intensity than other attributes.

GRAPES can be used to evaluate judge performance, and is especially valuable in determining possible outliers within a panel.

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65. Intracerebro-ventricular (icv) LHRH injection increases FOS expression in medial preoptic area of mating male hamsters

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Mating, and stimulation by female pheromones has been shown to elicit characteristic patterns of FOS expression in the central vomeronasal pathways of male golden hamsters, including the anterior medial (MeA) and posterior medial (MeP) amygdala, as well as in the medial preoptic area (MPOA). LHRH facilitates mating in several species including inexperienced male hamsters whose behavior has been impaired by vomeronasal organ lesions, and LHRH is thought to be released in male mice following vomeronasal stimulation by female urinary pheromones. We investigated the effect of icv injection of LHRH on FOS expression in the central vomeronasal pathways of inexperienced male golden hamsters, either intact or with vomeronasal lesions. Animals were given an icv injection of LHRH (50 ng in 2 µl) or saline (2 µl) through a previously implanted guide-tube, 30 min prior to mating. They were allowed to mate with three successive females for a total of 45 min, or were placed in a similar clean cage for 45 min. Following a further 45 min, animals were deeply anesthetized and perfused with 4% paraformaldehyde. Immunocytochemistry was performed on 50 µm Vibratome sections using anti-FOS antibody (Genosys), avidin-biotin-HRP complex (Vector) and diaminobenzidine. A two-way ANOVA analysis of the density of FOS positive nuclei in mating animals showed a significant increase of FOS expression in the medial preoptic area of LHRH injected intact animals compared with saline injected intact animals. Animals with vomeronasal lesions and animals not exposed to females had lower levels of FOS expression in all areas

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analyzed than did intact female-exposed animals, but there were no other significant effects of LHRH injection.

Supported by NIDCD-00906.

66. Zonal organization of the developing mouse accessory olfactory bulb revealed by OCAM immunohistochemistry

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Sensory Neurons of the vomeronasal organ are functionally and spatially subdivided into two populations. This subdivision is preserved in the accessory olfactory bulb (AOB) where the axon terminal fields of the two subtypes of vomeronasal sensory cells segregate into a rostral and a caudal zone. We show evidence that this zonal organization is maintained at the level of mitral/tufted cells of AOB and emerges early in development. The expression of a novel cell adhesion molecule OCAM (R4B12 antigen) was studied immunohistochemically in the developing mouse AOB.

As early as E14 a clear subdivision of the emerging AOB into OCAM positive and OCAM negative zone can be observed. Later, at early postnatal days, OCAM is strongly expressed by mitral/tufted cells in the caudal zone where OCAM negative vomeronasal axons terminate. In contrast, in the rostral zone of the AOB receiving input from OCAM positive vomeronasal axon terminals, OCAM is only weakly expressed. Until P15 this transient, checkerboard like pattern of OCAM expression is transformed in to the adult pattern: while the vomeronasal afferents still preserve their zonal organization, all mitral/tufted cells are heavily labeled by the OCAM antibodies.

We suggest that this transient zonal organization of mitral/tufted cells during AOB circuitry formation reveals an early organization of the AOB into functional compartments and suggests that OCAM may be involved in the development of this zonal organization.

Supported by RIKEN, Japan and DAAD, Germany.

67. Mutations in I-POU, a POU-domain neuronal transcription factor, cause odorant-specific olfactory defects

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acj6 (abnormal chemosensory jump 6) was isolated as a mutant of *Drosophila* defective in olfactory behavior. Interestingly, *acj6* causes a severe reduction in physiological response to most odorants, but not to benzaldehyde (odor of almond), as if response to benzaldehyde is mediated through a different pathway, or a different class of neurons, from other odorants. *acj6* was mapped to cytological region 13C1–2 of the X chromosome. We have used a new screen to isolate three new X-ray-induced alleles and one new EMS-induced allele of *acj6*. All three X-ray-induced alleles contain rearrangement breakpoints in the I-POU gene, which encodes a neuronal transcription factor. In the original $acj6^1$ allele, a highly conserved alanine residue is changed to a valine in the I-POU DNA-binding homeodomain. In $acj6^6$, a mutation in a splice acceptor site changes the position of a splice, which leads to a translational frameshift. POU-domain transcription factors have previously been associated with defects in mechanosensory defects (*C. elegans: unc-86*), auditory defects (mouse *Brn–3.1*), or visual defects (mouse *Brn-3.2*), either because subsets of cells are missing or because of alterations in cell fate in these sensory systems. We are examining the olfactory system of the *Drosophila acj6* mutants to see if their odorant-specific defects are due to loss of a subset of primary olfactory neurons, or changes in their cell fates; the goal is to determine whether I-POU plays a critical role in the acquisition of olfactory neuron identity.

Supported by NIDCD R01 DC02174.

68. Intracellular calcium stores in olfactory bulb neurons

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The expression and activation of intracellular Ca^{2+} release pathways in rat olfactory bulb neurons were studied with the use of Ca^{2+} imaging and immunocytochemistry. We focused on Ca^{2+} release regulated by the inositol-1,4,5-triphosphate receptor (IP_3R), and by the ryanodine receptor which regulates Ca^{2+}-induced Ca^{2+} release. The expression of IP_3R changes dramatically during development. In adults, IP_3R is expressed preferentially in GABA-immunoreactive neurons in the glomerular layer, in a small number of cells in the external plexiform layer, and in neurons in the mitral cell body and granule cell layers that are *not* GABA immunoreactive. Projection neurons, labeled by retrograde transport of dextran from the lateral olfactory tract, rarely express the IP_3R. In contrast, during the first postnatal week the receptor is detected almost exclusively in mitral cells. Expression of IP_3R in intrinsic neurons begins during the second and third weeks, concomitant with a decrease in immunostaining of mitral cells. In dissociated cell cultures, morphometric analyses and immunocytochemistry for glutamic acid-decarboxylase (GAD) revealed a heterogeneous population of cells, with phenotypes corresponding to both projection and intrinsic neurons of the *in vivo* olfactory bulb. Immunocytochemistry for the IP_3R, and labeling with fluorescent-tagged ryanodine, revealed that, irrespective of cell type, almost all cultured neurons express IP_3R and ryanodine binding sites in both somata and dendrites. Functional imaging revealed that intracellular Ca^{2+} fluxes can be generated in the absence of external Ca^{2+} (0 Ca^{2+}), using agonists specific to each of the intracellular release pathways. Local application of glutamate or quisqualate evoked Ca^{2+} fluxes in both somata and dendrites in 0 Ca^{2+}, suggesting the presence of IP_3-dependent Ca^{2+} release. These fluxes were blocked by preincubation with thapsigargin, and persisted in the presence of the glutamate receptor antagonist CNQX. Local application of caffeine, a ryanodine receptor agonist, also evoked intracellular Ca^{2+} fluxes in 0 Ca^{2+}. These Ca^{2+} fluxes were suppressed by preincubation with ryanodine. The developmental shifts in IP_3R expression, and the expression of this receptor by all classes of cultured neurons, raise the possibility that the expression of IP_3R in specific classes of neurons is activity-dependent.

Supported in part by PHS:NINDS 31078 & 35360.

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69. Initial development of a small subclass of rat olfactory receptor neurons characterized by antigenicity to HSP70

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We have described a subclass of rat olfactory receptor neurons (ORNs) that constitutively shows immunoreactivity with a monoclonal antibody (2A4) directed to the 70 kDa heat shock protein (Carr and Farbman, 1994). These ORNs are scattered nonuniformly in olfactory epithelium (OE) Zones II–IV (Ressler *et al.*, 1993) and project to just 2–3 glomeruli at consistent locations in the ventrolateral and ventromedial olfactory bulb (OB) via consistent pathways. To examine early neurogenesis of this subpopulation, paraffin sections from embryonic day E14 to postnatal day P63 rats were examined using immunoperoxidase techniques. Results show: (i) a few faintly reactive 2A4(+) ORNs first appear between P7 and P10. Their numbers (and IR intensity) increase to adult levels by P21, reach a peak density approximately twice that of adults by P49, and then decline to adult values by P56. (ii) [^{3}H]TdR autoradiographic birthdating studies show that the vast majority of 2A4(+) ORNs present at P21, when adult 2A4(+) ORN densities are first observed, were 'born' postnatally, between P3 and P10. (iii) The initial 2A4(+) ORN OE zonal distribution is the same as in adults. (iv) Through P21 2A4(+) ORN cell bodies are situated quite apically within the OE, but then assume more basal locations as well. (v) In the OB, glomeruli showing 2A4(+) axons appear in some animals as early as P14 and in all animals by P21. Initial location of the (+) glomeruli is similar to that of adults, despite extensive growth and development postnatally. The postnatal neurogenesis of the 2A4(+) ORNs, in contrast to the very early (E13; Sullivan *et al.*, 1995) initial appearance of ORN subclasses characterized on the basis of their putative olfactory receptor mRNAs, indicates that different ORN subclasses may vary in the time of their initial neurogenesis.

Supported by NIH grants DC01593 and DC02774 to V.McM.C.

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70. Activation of neurons in trigeminal nucleus caudalis (Vc) by application of a variety of irritant chemicals to the tongue: evidence for a 'common chemical sense'

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To study trigeminal mechanisms of oral irritation, we recorded responses of neurons in brainstem Vc to application of a wide spectrum of irritant chemicals to the tongue in thiopental-anesthetized rats. Units in superficial layers of dorsomedial Vc responding to noxious heating of the ipsilateral tongue were tested with the following chemicals delivered epilingually: histamine (his), nicotine (nic), capsaicin (cap), piperine (pip), mustard oil (mo), serotonin (5HT), acid (phosphate buffer pH 4 to 1 = pH), NaCl and ethanol (EtOH). Dose-response relationship, and tachyphylaxis were tested for each chemical.

Out of 32 units, 31 responded to at least one, and several to all nine, chemicals. The population responded to 129/171 (75.4%) of tested chemicals. The incidence of responses was independent of the order of chemicals tested, except that cap reduced subsequent responses (desensitization). Onset and duration of averaged responses were longest for cap, pip and mo. Responses significantly increased in a dose-related manner for his (9×10^{-1}–10^{-4} M), nic (6×10^{-1}–10^{-4} M), NaCl (0.5–5 M), EtOH (15–50%) and pH 4–1. Successive responses to repeated application decreased significantly only for nic, NaCl, 5HT, cap and pip. Spontaneous firing increased significantly 5–10 min after cap. Responses to his and nic were significantly reduced by ceterizine (H1 antagonist) and mecamylamine, respectively.

These data support the idea of a common chemical sense ('chemesthesis'), whereby different irritant chemicals activate a common population of sensory neurons in Vc, presumably via independent peripheral transduction mechanisms.

Supported by a Fulbright Award (to EC), Calif. Tobacco-Related Disease Research Program, and the Deutsche Forschungsgemeinschaft, SFB 353.

71. The effects of odour congruency and odour hedonics on visual event-related potentials

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Two studies were undertaken, examining the way in which people process sensory information and use this mode of processing in making perceptual judgements regarding the relatedness between olfactory and visual stimuli.

In order to investigate this, firstly a measure of field dependence–independence was obtained (using the Rod & Frame Test), reflecting the extent to which context plays a role in the processing of information, ie, in subjects' cognitive style. Secondly, an olfactory 'oddball'/priming paradigm was implemented (the latter technique having been successfully employed in examining the N400 component of the Event-Related Potential in semantic priming studies).

Subjects (n = 40) were right-handed, non-smokers, who had successfully completed a triangle test for olfactory discrimination. All subjects participated in the Rod & Frame Test prior to the experiment.

Subjects performed a modified version of a standard 'oddball' procedure, in which they were required to view a series of images presented on a computer screen and make a decision as to whether each image was congruent or incongruent with a preceding olfactory stimulus.

A computer-controlled odour delivery system was used to present four hedonically similar synthetic odorants. In the interests of odour congruency, the odorants were selected for their similarity to each other, i.e. they were all derivatives of 'washing powder' fragrances, related to different International markets. Odorant concentrations were at 'natural product' suprathreshold levels and odorants were adjudged to be isointense.

Visual event-related potentials were recorded using a NeuroScience Series III Brain Imager. Filters were set at 1.05 and

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80 Hz. Measurement was taken from electrodes at 28 scalp loci, in accordance with the International 10/20 System.

Findings will be discussed in terms of changes in the N400 component of the event-related potential between congruent and incongruent stimuli. In addition, the relationship between congruency and field dependence-independence will be discussed.

72. Spatio-temporal mapping of the olfactory bulb activity induced by electrical stimulation of the olfactory epithelium studied with a voltage-sensitive dye in the rat

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The hypothesis of a spatial coding, i.e. an odor would be coded by a specific pattern of activity, is supported by several studies. Indeed, even if the neuroreceptors of the olfactory epithelium (OE) present extended fields of sensitivity they are non-homogeneously activated by different odors. In order to determine if this heterogeneous distribution of peripheral activity is preserved at the olfactory bulb (OB) level it is necessary to take into account the complexity of the projections from the OE to the OB. In the current study, we analyzed the distribution of the peripheral inputs onto the OB in the Rat by using a functional approach. Experiments were performed on anaesthetized and curarized animals. Activity induced on the lateral OB was recorded in response to electrical stimulations (single shock) delivered to either a distal point or a proximal one of the five external turbinates. Optical monotoring, using a voltage-sensitive dye (RH795) and a photodiode array, allowed us to map the activity of the whole OB lateral area. The OB responses recorded were slow depolarizations (60–70 ms) possibly preceded by a fast component (1–4 ms latency) which could be an image of a synchronized discharge in the olfactory nerves. Responses were induced only in the ventro-posterior part of the lateral OB but were never observed in its antero-dorsal part. Stimulation of different turbinates indicated a large overlapping of their respective projections to the OB. When the stimulus was delivered near the cribriform plate, the extent of the responsive area was enhanced in comparison with the one noted for a distal stimulation. The number of responses and their spatial distribution increased and reached the dorso-postero-lateral OB when the intensity or the duration of the stimulus was enhanced. Some olfactory axons located more internally in the OE might be also recruited in this case. These anatomo-functional data will help to understand the location of the neuroreceptors activated by different odors in the OE.

73. Functional lateralization of human gustatory cortex related to handedness disclosed by fMRI study

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Ten healthy subjects aged 20–25 including five right-handed and five left-handed according to Dellatolas test (Dellatolas *et al.*, 1988) participated in this fMRI study. A 3 Tesla whole-body MR scanner allowed EPI functional imaging (64 × 64 pixels, TR = 6s, FOV = 20 × 20 cm²) associated to acute anatomical localization of activated foci (256 × 256 pixels). Subjects were bilaterally stimulated with NaCl 85 mM, aspartame 2 mM, quinine hydrochloride 1 mM, glycyrrhizic acid 0.5 mM, guanosine monophosphate 1 mM and D-threonine 250 mM alternating with water. Stimuli and rinse were continuously pushed as bolus of 50 µl every 3 s to the subject's mouth through microsyringes. We detected brain activated areas by correlation of the MR signal to an on-line perception profile recorded for each experiment and each subject with the finger-span method (B. Cerf *et al.*, submitted for publication). We found most activations in the insula and the perisylvian region in agreement with previous electrophysiological studies on monkeys (Patton and Ruch, 1946; Benjamin *et al.*, 1968; Scott *et al.*, 1986) and clinical reports in humans (Bornstein, 1940; Penfield and Faulk, 1955). The superior part of the insula was bilaterally activated, in accordance with a whole mouth stimulation. A striking lateralization related to handedness was found in a lower part of insula. This projection in the dominant hemisphere, located in the same coronal plane as the upper insular activation, is the first evidence of a functional lateralization of brain processing involved in taste perception.

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74. Breakdown of semantic memory in AD: evidence from verbal, perceptual and olfactory domains

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A series of studies were initiated to model the organization of semantic memory in Alzheimer's disease (AD) patients using various techniques, including multi-dimensional scaling (MDS) and Pathfinder analyses. The resulting models (cognitive maps or semantic networks) embed studied stimuli in a coordinate space or network where distances between points are assumed to reflect psychological proximity between items. The organization of semantic networks in AD patients were modeled based upon the sequence of their responses in the animal fluency task and the frequence of their choice of two concepts as most alike on the triadic comparison task, the latter in several experiments with stimuli from different domains (i.e. olfactory and perceptual domains). Results suggested that while the organization of concepts in the semantic networks of AD patients was primarily based upon a concrete perceptual dimension, those of normal controls subects were predominantly organized by an abstract conceptual attribute. Networks of AD patients were more complex and chaotic, i.e. consisted of more unnecessary connections and of atypical strengths of association between concepts. These results are unlikedly to be a consequence of random response behavior (e.g. from lack of attention or inability to follow instructions) since AD patients, like control subjects, performed consistently between two identical testing sessions over a 5–7 day period. Also, it is unlikely that the performance of AD patients is due to other factors such as general cognitive impairment,retrieval failure, or a deficit in episodic memory, since the cognitive maps of amnesiac

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patients and Huntington's disease patients were essentially normal when compared with their age-matched counterparts.

Supported by NIA grant AG05131, CUHK Direct Grant, CUHK United Grant, NIA grant AG08203.

75. Taste neurons of *Drosophila melanogaster* in primary cell cultures

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Each labellum of *Drosophila melanogaster* has 32–36 sensory hairs which detect tastants. Extracellular recordings from the taste sensilla of wild type and mutant flies show that the taste neurons respond to sugars, salts and water. Intracellular or patch-clamp recordings from these cells *in situ* are difficult to carry out due to the presence of a hard cuticle and the sheath cell that wraps around the neuronal cell body.

We have developed a method for maintaining the taste neurons from the labellum of *Drosophila* in primary cultures. After a week in culture ~60% of surviving cells are neurons. Neurons were identified on the basis of their morphology, by expression of reporter genes for β-galactosidase or GFP that are driven by neuron specific elements or with the neuron specific antibody 22C10. The neurons remain alive for 18–20 days. They are excitable and carry voltage activated channels. We will describe the morphological characteristics of the cultured labellar cells *in vitro* and results of patch-clamp recordings from neurons.

76. Molecular and physiological evidence for glutamate (*umami*) taste transduction via a G-protein coupled receptor

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Transduction mechanisms for the taste of sugars and certain amino acids and bitter substances are thought to involve G-protein coupled receptors. Our recent molecular analyses have demonstrated that a metabotropic (G-protein coupled) glutamate receptor, mGluR4, is expressed in many cells of taste buds from rat circumvallate and foliate papillae (Chaudhari *et al.*, 1996). Specifically, (i) RT-PCR and RNase protection assays demonstrated the presence of mGluR4 mRNA in taste bud-enriched but not in taste bud-deficient lingual tissue and (ii) *in situ* hybridization showed that mGluR4 mRNA is localized to taste buds and is absent from surrounding epithelium and underlying tissue. Behavioral studies demonstrated that L-AP4, an agonist for mGluR4 and related receptors, mimicks the taste of monosodium glutamate (MSG) in rats. Recent electrophysiological studies (Bigiani *et al.*, 1997) on circumvallate and foliate taste buds from rat show that membrane currents elicited by MSG and L-AP4 are similar. The predominant effect was a sustained outward current, associated with a decrease in membrane conductance. These results imply that glutamate may lead to closure of cation channels and produce hyperpolarizing receptor potentials. In neurons, mGluR4 and related metabotropic glutamate receptors are known to signal through inhibition of the cAMP cascade. We have found that upon stimulation with MSG, circumvallate and foliate taste epithelia contain decreased levels of cAMP relative to controls (Zhou and Chaudhari, this meeting). The synergistic effect of 5′ IMP (inosine 5′ monophosphate) on MSG taste was tested: larger decreases of cAMP were observed following stimulation with the combined tastants. Collectively, these data provide broad support for our interpretation that a metabotropic glutamate receptor may be critically responsible for the transduction of glutamate (*umami*) taste. Supported by grants from Umami Manufacturing Association of Japan, Kraft General Foods and NIH (DC 03013).

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77. A developmental psychology perspective on the detection and influence of social odors in mammals

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The present review analyzes 280 articles from the field of social olfactory communication in animals and humans. Of these, 152 articles are from the years 1971–1975 and 128 from the years 1991–1995. The gender of first authors, the gender and age of olfactory donors and recipients and the topics are analyzed. The proportion of women first authors has doubled from 20 to 40% from the 1970s to the 1990s. Although rodents are still the most studied population (80%), there was an even higher concentration of rodent studies in the 1970s (92%). There is no historical change in the ages of subjects. In both time periods, about three-quarters of the subjects are adults. The genders of the subject populations have changed historically in one respect. In the 1970s, 66% of studies involve some female donors and 63% did so in the 1990s. In the 1970s, 44% of the studies involve female recipients and 61% did so in the 1990s. Topics have changed slightly such that the top three topics studied (in order from most to least) in the 1970s are aggression, sex, and care-taking and in the 1990s are sex, social discrimination, and aggression. There are some gender of author by gender of subject effects. Both men and women are biased to study male subjects. However, the men are even more likely to design studies with only male recipients of odors and there is a tendency for men to have more studies with only female donors. Women are more likely than men to design studies with only female recipients and to design studies with only male donors.

78. Intrinsic membrane potential oscillations in mitral cells of the mammalian olfactory bulb

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Oscillations of local field potential (FP) induced or modulated by odor stimulation have been recorded in the olfactory bulbs of a number of species, and have been proposed to play fundamental roles in the coding and processing of odor information in the central nervous system. However, there have been few studies addressing the mechanisms of these oscillatory activities at the cellular level. We are therefore analyzing the oscillatory properties

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of mitral cells by means of whole-cell recording in rat olfactory bulb slices.

The membrane potential of a mitral cell tended to oscillate at a voltage range that is subthreshold for action potetial initiation. The oscillation frequency varied from several Hz to ~30 Hz. This subthreshold oscillation was not blocked by either combined application of CNQX (20 μM), APV (50 μM) and bicuculline methiodide (30 μM), or zero calcium plus 200 μM cadmium in the medium, and therefore is an intrinsic membrane property. Autocorrelation and power spectrum analysis showed that the oscillation was very sensitive to the membrane potential level, attaining its largest amplitude and fastest frequency near firing threshold. Calcium currents and calcium-activated potassium currents appeared not to be directly involved in the subthreshold oscillation. TTX at 1 μM completely abolished the oscillations. Voltage-clamp analysis showed that the mitral cell had a prominent persistent Na^+ current, which seemed to contribute to both the oscillation and inward rectification that appeared near impulse threshold. When these Na^+ channels were blocked by TTX, inward rectification in the *I–V* relation changed to outward, suggesting that some potassium currents were also active in the subthreshold range. A delicate interplay between the persistent Na^+ current and outward potassium currents therefore appears to underlie the subthreshold oscillatory properties of the mitral cell membrane potential.

Supported by grants from NIDCD, NIMH, NASA and NIDCD (Human Brain Project).

79. Initiation and propagation of action potentials in the mitral cell primary dendrite of the rat olfactory bulb

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The mitral cell receives excitatory synaptic input on the distal tuft of its single primary dendrite. Building on our finding of a dendritic fast prepotential (Mori *et al.*, 1982), we have tested whether tuft-to-soma transfer along the primary dendrite is aided by active dendritic properties, using paired dendrite and soma whole-cell recordings from mitral cells visualized by DIC-infrared microscopy.

The mitral cell primary dendrites were clearly visible in the slices and could be traced from their cell bodies across the external plexiform layer to individual glomeruli. In most experiments, paired recordings were made from the soma and the distal primary dendrite near the glomerular border. At resting membrane potential level, injecting weak-to-moderate current pulses into either the soma or the distal primary dendrite evoked an action potential first in the soma, which then back-propagated into the dendrite. However, when a strong current pulse was injected into the dendrite, or the somatic membrane potential was hyperpolarized by DC current injection, the impulse origin could be shifted to the distal primary dendrite, with forward propagation along the primary dendrite into the soma. Single electric shocks were delivered to the olfactory nerve (ON) layer to elicit synaptic excitation, as occurs during odor stimulation. With weak synaptic excitation, the action potential was first generated at the soma and then back-propagated into the dendrite. However, as the stimulus strength increased, the impulse initiation site shifted to the distal primary dendrite, leading to forward-propagation. With weak ON excitation, dendritic impulse propagation could be changed from backward to forward by synaptic inhibition to the cell body and secondary dendrites evoked by external plexiform layer stimulation.

The results show that (i) action potentials can be initiated in both soma and distal primary dendrite; (ii) both forward propagation and backpropagation occur in the primary dendrite; and (iii) both the site of impulse initiation and direction of propagation can be shifted by synaptic inputs to the mitral cell.

Supported by grants from NIDCD, NIMH, NASA and NIDCD (Human Brain Project).

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80. Expression of a cAMP phosphodiesterase in the vomeronasal organ during mouse development

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Although much progress has been made in our understanding of signal transduction in the main olfactory system, much less is known about how chemical signals are processed in the vomeronasal organ (VNO). In fact, evidence from several laboratories suggests that molecular components of pathways thought to be important for odorant processing in olfactory sensory neurons (OSNs), such as adenylyl cyclase III and the G-protein Gaolf, are absent in the VNO. We have found that a cAMP-specific phosphodiesterase (PDE4a) expressed at high levels in OSNs is also localized in sensory neurons of the VNO in the adult mouse. Furthermore, western blotting using a specific anti-PDE4a antibody has revealed a unique protein species that is present in olfactory and VNO tissue, but in no other tissues examined. Although the specific role of PDE4a is not known, its critical involvement in the removal of cAMP suggests a potential role in transduction. To gain insight into its function in the VNO, expression of PDE4a was examined by western blotting and immunocytochemistry during development. In the adult VNO, anti-PDE4a antibody labeled dendrites and cell bodies of sensory neurons, whereas non-sensory neurons were not stained. During development, labeling of a few VNO neurons could be found as early as embryonic day 14 (E14). At this age, only one or two clearly labeled VNO neurons per section could be identified, but by E17 immunopositive VNO neurons were scattered throughout the sensory epithelium. Notably, these results are in contrast with the expression of olfactory marker protein, which does not appear in the VNO of the rat until postnatal day 4 (*Dev. Biol.*, **74**, 205). The current findings indicate that onset of PDE4a expression does not occur simultaneously, but varies widely among VNO sensory neurons. What factors turn on PDE4a expression in specific VNO neurons during development is not yet clear. However, presence of this enzyme may be a flag for cAMP-regulated processing occurring in at least some sensory neurons early in VNO development.

Supported by NIDCD 03019.

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81. Localization of the high affinity neurotrophin receptors in the rat geniculate ganglion

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Most peripheral and central nerves derive trophic substances from their innervation targets. Recent *in situ* hybridization experiments have demonstrated the localization of two well known neurotrophins in the rat tongue epithelium. Brain Derived neurotrophic Factor (BDNF) has been shown to be specific to the gustatory regions of rat tongue papillae, while neurotrophin-3 (NT-3) resides exclusively in the non-gustatory regions of the same papillae (Nosrat *et al.*, 1996). Moreover, studies in this lab using RT-PCR on RNA pools isolated from tissues containing gustatory papillae have also shown abundant quantities of BDNF, along with nerve growth factor (NGF), NT-3 and to a lesser degree neurotrophin-4/5 (NT-4/5). Because all of these neurotrophins are known to provide trophic support for nerves in other systems, we asked if the neurons of the rat geniculate ganglion, most of whose axons innervate taste buds, contained trkA, trkB and trkC, the neurotrophins' high affinity receptors. RT-PCR and immunohistochemical techniques were performed on whole rat geniculate ganglia. After extracting mRNA from the geniculate ganglion, nested RT-PCR was performed, and a differential expression of the trkA, trkB and trkC transcripts was observed: trkB and trkC were more abundant in the ganglion than trkA. Immunohistochemical studies showed the localization of the trkA, trkB and trkC proteins in the neurons of the geniculate ganglion. In general, trkB- and trkC-positive neurons appear to be more abundant than trkA-positive ones. Furthermore, trkB appears to be mostly localized in the cytoplasm of the neurons, while trkC and trkA are present in both the neurons and the surrounding supporting cells. These results indicate that geniculate ganglion nerves are sustained by one or more of the neurotrophins.

Supported by NIH grant # P60 DC02764.

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82. Timing of α-gustducin expression in differentiating rat vallate taste bud cells

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The G protein, α-gustducin, is expressed in taste cells throughout the rat oral cavity, including the vallate papillae. Recent studies suggest that α-gustducin is involved in the transduction of bitter- and sweet-tasting stimuli. Taste bud cells are thought to arise from proliferating stem cells in the gustatory epithelia. After stem cell division, one of two daughter cells enters the taste bud, differentiates, and exists for a finite period before replacement by a new cell. The average life span of a taste cell is ~10 days in rats. 5-bromo-2'-deoxyuridine (BrdU) is a thymidine analogue which is incorporated into DNA during the S-phase of the cell cycle; DNA containing BrdU can be detected immunocytochemically. Cells expressing BrdU are assumed to have originated from stem cells on the day BrdU was administered. The aim of the present study was to determine when gustducin is first expressed during the lifespan of vallate taste cells. To measure this time, vallate papillae of at least two rats were examined at each of several (2.5–6.5) days after BrdU injection (50 mg/kg i.p.). Vallate papillae were embedded in polyester wax, cut coronally at 4 mm and characterized with antibodies to BrdU and α-gustducin. Immunofluorescent material was examined on a confocal scanning laser microscope (CLSM), using fluorescein- and rhodamine-conjugated secondary antibodies or on a light microscope using an avidin–biotin procedure. The occurrence of α-gustducin- and BrdU-labeled cells was determined for at least 200 taste buds at each survival time. Cells with nuclei immunoreactive for BrdU were seen within the taste buds at 2.5 days, but these cells did not express α-gustducin. However, double-labeled cells were evident at 3.5 days and at longer survival times; all had an elongated, spindle shape characteristic of mature taste cells. These data suggest that the expression of gustducin appears relatively early in the lifespan of vallate taste cells.

Supported in part by NIDCD DC00347 (D.V.S.).

83. A simple test to determine the etiology of hyposmia

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This study was conducted to test the hypothesis that cinnamon and mothballs could form a basis of a simple olfactory test to determine if an olfactory deficit was due to a physical block. It is generally accepted that a loss of olfactory ability accompanies a common cold. Although this loss has been correlated with the amount of nasal congestion, not all odorants are equally affected. In general, polar, low intensity odors are more difficult for subjects with colds to detect than are intense, non-polar odorants. Cinnamon and mothballs demonstrate this effect particularly well. Cinnamon is a polar and low intensity odorant and is very difficult for subjects with colds to identify whereas the identification of mothballs, a more intense and non-polar odorant is much less affected. In Part 1, subjects were presented mixtures of cinnamon and mothballs and ask to identify the predominant smell. Subjects with colds required more cinnamon in a mixture to identify cinnamon as the prominent odorant that did subjects without colds. In Part 2, subjects with and without colds were asked to rate the intensity of various concentrations of each odorant. Subjects with colds gave the same ratings for the intensity of the mothballs solutions as did subjects without colds. However, for cinnamon, subjects with colds rated the solutions as being much less intense than did subjects without colds. These data provide support for the possibility of a cinnamon/mothballs mixture being used as a simple test to determine if an olfactory loss is primarily an access problem.

Supported in part by NIDCD DC00220.

84. GABAergic mechanisms that shape the temporal response to odors in moth olfactory projection neurons

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A critical step in learning how olfactory information is represented

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in the brain is to unravel the complex synaptic interactions underlying odor-evoked responses in the output pathways from first-order olfactory centers [mitral/tufted cells in the olfactory bulb; projection neurons (PNs) in invertebrates]. In the moth *Manduca sexta*, an array of GABA-immunoreactive local interneurons innervates most, if not all, of the antennal lobe glomeruli, and several lines of evidence indicate that these neurons play a key role in regulating spike activity in PNs. GABA and the $GABA_A$-receptor agonist muscimol hyperpolarize and lower input resistance in many PNs, often blocking ongoing spike traffic. The GABA response mimics a short-latency, chloride-mediated IPSP in PNs evoked by electrical or odor stimulation, and the vertebrate $GABA_A$ receptor antagonist bicuculline methiodide (BMI) quickly and reversibly blocks this IPSP. In other insects, however, bicuculline is ineffective in blocking the inhibitory responses to GABA infusion, and there is also evidence that bicuculline blocks nicotinic receptors on the cell bodies of certain motor neurons. In contrast, focal injection of BMI immediately preceding a GABA pulse blocked the hyperpolarization evoked by GABA in moth PNs, but a similar injection of BMI preceding an ACh pulse failed to block the depolarization evoked by ACh. Bicuculline also did not affect the temporal response patterns to odors in the locust antennal lobe, but the temporal pattern of odor-evoked activity in moth PNs is strongly and reversibly altered by BMI. Importantly, the temporal pattern of the response depends on the temporal characteristics of the stimulus: longer-duration odor pulses evoke more complex, sometimes oscillatory responses, whereas briefer pulses can be copied with discrete bursts of spikes. Collectively our results indicate that PNs in the moth possess a $GABA_A$-type receptor that participates at several levels in the temporal representation of odor information in the brain.

Supported by NIH grant AI-23253 to J.G.H.

85. Developmental anatomy of the olfactory system in the opossum *Monodelphis domestica*

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Although olfaction is thought to play a role in guiding the locomotion of the newborn marsupial, the cellular substrates on which this notion is based have only been systematically investigated in a few studies. This project demonstrates the anatomical development of the primary olfactory pathway in the postnatal *Monodelphis* using electron microscopy and immunohistochemistry. The olfactory epithelium appear morphologically immature at birth although some of the olfactory neurons are shown to express olfactory marker protein (OMP). Dendritic knobs are sparse because many of the dendritic processes containing basal bodies are still elongating and have yet to reach the epithelial surface. In addition, mitotic figures are often observed in the superficial regions of the epithelium. During the first postnatal week, there is an increase in the number of OMP positive neurons although the distribution remains uneven. Some OMP-positive fibers are observed to extend deep into the olfactory bulb, beyond the mitral cell layer. Such exuberant growth of olfactory axons which have been previously observed in the perinatal rat and mouse gradually undergo regression.

No distinct morphological lamination is present in the newborn *Monodelphis* olfactory bulb. On the third postnatal day, apposing membrane densities resembling immature synapses are observed. By the end of the first postnatal week, lamination is apparent and this is followed by the first appearance of synapses 2 days after. The findings in this study suggest that the marsupial and eutherian olfactory system share a similar temporal sequence of developmental processes although in the marsupial they typically lag ~10–14 days behind those of the mice.

86. Depolarization-induced upregulation of tyrosine hydroxylase expression in primary cultures of dopamine neurons

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Tyrosine hydroxylase (TH), the rate-limiting enzyme in the biosynthesis of catecholamines, is localized to a population of periglomerular dopamine neurons in the olfactory bulb. TH expression in these neurons appears to be regulated by odor-induced synaptic activation originating from olfactory receptor cells. In the current studies, the mechanisms by which synaptic activity regulates olfactory bulb TH expression were analyzed in primary cell cultures of olfactory bulbs obtained from 2-day-old mouse pups. As previously reported, 48 h (h) of depolarization produced by a single application of high potassium in the medium (50 mM KCl), to mimic neuronal activation, increased the number of immunostained TH neurons 2.5 ± 0.04 fold (n = 19) as compared with control cultures treated with an equimolar concentration of sodium chloride (NaCl).To distinguish general changes in survival of all cell types from increases specific to the number of TH neurons, cellular density was estimated by Nissl staining of nuclei. Depolarization produced no significant changes in nuclear density. Staining of cultures with antisera to either GFAP or neuron specific tubulin Isotype III, also showed no differential treatment-induced effect on the number of either glia or neurons, respectively. A second series of experiments determined if short-term depolarization was sufficient to trigger the molecular pathways involved in TH synthesis or, as the *in vivo* model suggests, continuous depolarization is necessary for expression. Parallel cultures were treated with either KCl or NaCl for 4, 8, 16, 24 and 48 h. All cultures were harvested 48h after initial exposure. No significant difference in the number of TH neurons occurred until 48 h when the typical 2.5-fold increase was observed. These data suggest that, in primary cell cultures of olfactory bulb, a phenotype-specific increase occurs in the number of TH expressing cells requiring prolonged continuous depolarization.

Supported by NIH Grant AG09686.

87. Norepinephrine selectively increases rat mitral cell excitatory responses to weak olfactory nerve input via alpha-1 receptors *in vitro*

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Noradrenergic (NE) inputs to the main olfactory bulb (MOB) arise from nucleus locus coeruleus (LC) and terminate densely in the internal plexiform layer (IPL) and granule cell layer (GCL) and to a lesser degree in the external plexiform layer (EPL). Recent

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studies in our laboratory showed that selective LC activation enhances mitral (Mi) cell responses to weak (i.e. perithreshold), but not strong (i.e. suprathreshold), olfactory epithelium stimulation *in vivo*. Here, we investigated the effects of exogenously applied NE on Mi cell activity in rat MOB slices.

Horizontal MOB slices (400 μm thick) were obtained from 75–110 g rats. Spontaneous and olfactory nerve (ON)-evoked activity were recorded extracellularly from single Mi cells. Single ON shocks elicited a characteristic response in Mi cells consisting of distinct, early and delayed excitatory spiking components separated by a brief inhibitory epoch. Bath-applied NE (1 μM) increased the early spiking component elicited by perithreshold (44% increase, $P < 0.03$), but not by suprathreshold (3% decrease, $P > 0.5$), intensity ON shocks ($n = 10$ cells). NE also decreased the inhibition between the early and late spiking components by 60% ($P < 0.05$). By contrast, NE had no consistent effect on Mi spontaneous discharge rate. These effects were mimicked by the alpha1 receptor agonist phenylephrine (1 μM, $n = 10$ cells, $P < 0.001$) and blocked by the alpha1 adrenergic antagonist WB 4101 (1 μM, $n = 9$). The alpha2 receptor agonist clonidine (100 nM, $n = 8$) and the beta receptor agonist isoproterenol (1 or 10 μM, $n = 6$) had no consistent effects on Mi spontaneous or ON-evoked activity.

These results indicate that NE increases Mi cell excitatory responses to weak but not strong ON inputs *in vitro*. This effect is mediated primarily via alpha1 receptors, a finding consistent with reports that MOB (external plexiform layer) exhibits the highest density of alpha1 receptor in the CNS. Olfactory cues evoke NE release in the MOB and NE release is increased in early olfactory learning and reproductive/maternal behaviors. Taken together with findings *in vivo*, the present results suggest that NE release in the MOB may increase the sensitivity of Mi cells to improve the detection of weak odorants.

Support: NIH DC00347, DC03195, NS29218 and NS29635.

88. Diatom chemotaxis and movement in measured chemical gradients: a component in diurnal migration

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Diatoms are known to move diurnally through benthic sediments, rising to the surface of algal mats in daylight h to photosynthesize, and moving back down in the evening. Although phototaxis has been shown to be a factor in these daily migrations, little is known about the chemical component of this phenomenon. It has been hypothesized that motile diatoms move to deeper, anoxic layers of the benthos at night to retrieve liberated phosphorus, although no direct evidence has been recorded. We hypothesize that the diurnal movement of diatoms through the sediment is mediated by nutrient concentrations. Our experiment records 'choice' in motile diatoms as a function of chemical gradient. Diatoms were placed in minute y-mazes etched in plastic and by two syringe pumps, one with a nutrient load and one with distilled water. Tests were run in both light and dark conditions. Diatom motility is described with respect to variation in both light and chemical gradients, which more completely accounts for the rhythmic movements encountered in nature.

Supported by NSF grant # IBN-9614492.

89. Sensory properties of selected terpenes: thresholds for odor, nasal pungency, nasal localization, and eye irritation

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We tested four normosmics and four anosmics in detection thresholds for six terpenes commonly found indoors: cumene, *P*-cymene, delta-3-carene, linalool, 1-8 cineole and geraniol. Normosmics provided odor thresholds and anosmics provided nasal pungency thresholds. All subjects provided nasal localization (i.e. right/left nostril) and eye irritation thresholds. Each type of threshold was measured eight times per subject-stimulus combination. Stimuli were presented from squeeze bottles in a two-alternative forced-choice procedure via an ascending method of limits. Odor thresholds ranged between 0.1 and 1.0 ppm. Nasal pungency thresholds lay about three orders of magnitude above odor thresholds. Nasal localization and eye irritation thresholds did not differ between normosmics and anosmics, and fell close to nasal pungency thresholds. Olfactory thresholds could be obtained for all stimuli in all repetitions using the criterion of five correct choices in a row. Trigeminal thresholds (i.e. pungency, localization and eye irritation) could be obtained on all repetitions only for some terpenes using that same criterion. Carene and cineol produced nasal pungency and eye irritation on all repetitions. None of the terpenes could be localized on all repetitions, but cineol was localized a higher percentage of instances than were the other stimuli. At the other extreme, geraniol failed to evoke any of the three trigeminal responses in most instances. Overall, the results indicate that the three trigeminal thresholds produce a uniform view of the potency of these terpenes, with nasal pungency and eye irritation being slightly more sensitive than nasal localization. Furthermore, application of a previously derived linear solvation energy relationship to the results reinforced the view that physicochemical properties can predict the chemesthetic impact of volatile organic compounds.

Work supported by research grant number R29 DC 02741 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health, and by the Center for Indoor Air Research.

90. Olfactory identification and response bias in HIV infection

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HIV infection commonly leads to CNS effects such as dementia and organic affective disease. The hallmark of the dementia is cognitive slowing, which is difficult to assess by simple untimed bedside screening tests. Additionally, cognitive slowing and poor memory are features of uncomplicated major depression.

We present data suggesting that olfactory identification testing may provide a simple, convenient method for assessing dementia and depression in HIV-infected patients. Four groups of patients (HIV^+ with normal memory and mood, HIV^+ demented, HIV^+

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depressed patients, and HIV+ with both dementia and depression) and normal controls were tested with a yes/no olfactory identification test. This test yields independent indices of accuracy (Pr) and response bias (Br, the decision rule subjects use when uncertain). Response bias is an aspect of executive function most likely mediated by frontal lobe function. Historical data from depressed and Alzheimer's disease (AD) subjects are also presented.

Results indicate that accuracy is impaired in *all* HIV+ subjects, most markedly in the demented and/or depressed groups, and that response bias is liberal in both demented and depressed subjects. In contrast, patients with uncomplicated major depression have conservative response bias, and minor, if any olfactory accuracy impairment. Euthymic patients with other dementias such as Alzheimer's disease have poor olfactory accuracy and liberal bias.

Thus, the depression of HIV infection appears qualitatively and quantitatively different than primary affective disorder: it has the the olfactory accuracy and bias deficits of dementia.

Supported in part by the Veterans Adminsitration.

91. Do odor maps change after recovery from olfactory nerve transection?

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Recent studies have shown that olfactory receptor subtypes converge onto fixed loci in the olfactory bulb. It has been postulated that this arrangement results in the formation of odor 'maps' that may serve as a basis for odor quality discrimination. Olfactory receptors also have the capacity to regenerate following injury and to reestablish connections with the olfactory bulb. In this study, we asked whether odor maps are restored following recovery from nerve transection. To map projections of a known receptor subtype, we used a genetically altered strain of mice (*P2-IRES-tau-lacZ*) developed in the laboratory of Richard Axel (Mombaerts *et al.*, 1996). In this strain, P2 receptor axons converge onto two fixed loci in the bulb and can be visualized with a blue stain. After left olfactory nerve transection, reconnected axons were examined at recovery times ranging from 2 to 4 months. Only those nerve fibers projecting to the left bulb were transected; nerves projecting to the right bulb served as a control. After recovery from nerve transection some of the regenerated P2 receptor axons retained their capacity to converge. However they established multiple sites of convergence and in new loci. On the control side, axons converged onto the same two fixed loci. These findings demonstrate that odor maps are altered after recovery from nerve transection. This may explain why humans have distorted or altered odor sensations after recovery from head injury. Future experiments are planned to study the mechanisms that guide axons to their proper targets and obstacles that may prevent axons from restoring the original odor map.

This research was supported by NIH grant DC00165.

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92. Robust OERPs produced with fewer trials: effects of age and gender

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The relatively long interstimulus interval (ISI) required to record olfactory event-related potentials (OERPs) necessarily increase testing time which increases subject fatigue. If reliable and robust OERPs could be recorded with fewer trials, considerable experimental and clinical benefit would be obtained. A previous study from this laboratory found that the first stimulus presentation trial yielded larger OERPs than all other blocks of trials. The present study extended these findings by comparing the first trial alone with the average of trials 1–5, 6–10, 11–15 and 16–20. Isoamyl acetate was presented using a 60 s ISI to elicit OERPS, which were recorded monopolarly at the Fz, Cz and Pz electrode sites in 14 younger adults (7 males, 7 females) and 14 older adults (7 males, 7 females). For both N1/P2 and N2/P3 OERP components the largest amplitude was found for the first trial. Younger participants produced larger N1/P2 amplitudes than older participants for all trial blocks, although both subject groups demonstrated similar first trial component amplitudes. Females produced the largest N1/P2 amplitude for trials 1–5 and 11–15 with the young females demonstrating the largest N1/P2 amplitude and the older males producing the smallest amplitude for the same blocks of trials. These results suggest that useful OERPs can be produced with only a few stimulus presentations.

Supported by NIH grant # DC 02064 to (C.M.).

93. Taste–carbonation interactions

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Despite the widespread use of carbonation in beverages, there have been few published studies of its impact on taste. Two studies have, however, suggested that impact may be negligible, manifesting primarily in increases in the perceived intensity of relatively weak salt and sour stimuli. Assuming CO_2 is a pure trigeminal stimulus, this result is not altogether surprising; for example, studies of the impact of the trigeminal stimulus capsaicin on taste have also shown it to be small or non-existent. On the other hand, there are neurophysiological data indicating that CO_2 stimulates gustatory as well as trigeminal fibers. In that case, carbonation might alter the quality profile of a stimulus without producing substantial changes in overall taste intensity—much as occurs when qualitatively different taste stimuli are mixed. To address this possibility, we asked subjects to rate the total taste intensity of moderate concentrations of each of the basic tastes and their binary combinations, with and without added carbonation. In addition, subjects subdivided total taste intensity into the proportions of sweetness, saltiness, sourness, bitterness and 'other taste qualities' they perceived. The addition of carbonation produced only small increases in ratings of total taste intensity. However, rather dramatic alterations in the quality profiles of stimuli were observed, particularly for sweet and salty tastes. The effect of carbonating simple taste solutions was similar to that of adding a moderately intense sour tastant. These data suggest that

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carbonation *does* impact substantially on taste perception. The nature of the interaction is consistent with a direct effect of carbonation/CO_2 on the gustatory system.

Supported by NIH grant P50 DC00214.

94. Postnatal retinoic acid and the expression of messages for retinoic acid receptors in the mouse olfactory system

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The transcriptional activator retinoic acid (RA) is an endogenous morphogen for many regions of the developing nervous system. Systemic exposure of early embryos to high levels of exogenous RA is highly teratogenic. However, the effects of postnatal exposure to RA brain structures that are still rapidly developing postnatally are less well understood. We have previously demonstrated that 1 month after neonatal mice are exposed to RA the olfactory epithelia is reduced in thickness and the olfactory bulbs contain fewer glomeruli. We expect that the expression of specific RA synthetic enzymes, RA binding proteins and RA nuclear receptors determine the vulnerable time period in the olfactory system. To better understand the sequence of events that RA may activate in the developing olfactory system, we have begun to investigate the primary olfactory system after short post-exposure periods. To determine what role the expression of RA receptors may play in the postnatal development of the primary olfactory system we have begun nonisotopic *in situ* hybridization studies of different members of the RA nuclear receptor family, specifically RARs and the RXRs, after relatively short periods following neonatal RA exposure. In contrast to the relative abundance of RA receptor messages in the embryonic nervous system, only subsets of the receptor family (RXRa and RARb) can be detected as late as postnatal day 3 in the main olfactory system. Investigations continue to focus on the extent to which exposure to RA postnatally regulates the expression of the RA receptor family.

This research was supported in part by NIH grants DC00953, HD04147 and EY01938.

95. Alterations in the neurotrophic factors BDNF, GDNF and CNTF in the regenerating olfactory system

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Neurogenesis, axonal outgrowth and synapse formation are usually restricted to specific stages during CNS development but the mature olfactory system maintains these capacities. The cycle of neuronal turnover can be experimentally induced by surgical ablation of the olfactory bulb (OB). We are interested in the growth factor regulation of these processes and the trophic role played by the target tissue, the OB.

We studied the immunohistochemical expression of three neurotrophic factors, glial cell line-derived neurotrophic factor (GDNF), brain derived neurotrophic factor (BDNF) and ciliary neurotrophic factor (CNTF) in the rat olfactory neuroepithelium (ON) and OB and in target-deprived ON at 1, 3 and 12 weeks post unilateral bulbectomy. We found BDNF immunoreactivity (IR) was restricted to the basal cells and did not alter postbulbectomy. GDNF-IR was expressed by mature olfactory receptor neurons (ORNs), their axons and target cells in the OB in controls, but absent from the ON postbulbectomy. Hence, the expression of GDNF by ORNs was found to be target-dependent. CNTF-IR was present in ORNs and their target cells in the OB, in basal cells and some immature ORNs. Postbulbectomy, CNTF-IR was unaltered in the basal cells and very low levels were detectable in maturing ORNs in the ON.

Our results indicate these three factors contribute to the trophic regulation of this neuronal pathway in a coordinated fashion. Previous work has shown that BDNF promotes survival of ORNs *in vitro* (Mahanthappa and Schwarting, 1993) and Trk B expression has been found in both immature and mature ORNs (Deckner *et al.*, 1993; Roskams *et al.*, 1996). Hence, BDNF produced by basal cells may be acting locally on neurons expressing Trk B. Expression of CNTF by both the basal cells and the ORNs suggests it may play an integral role in this neuronal differentiation pathway. Finally, the expression of GDNF exclusively by mature ORNs in the ON, its presence in the target cells in the OB and abolition of expression by bulbectomy, suggests it may be target-derived. This provides a major mechanism by which the bulb could exert trophic influences on ORNs.

Supported by the Garnett Passe and Rodney Williams Memorial Foundation.

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96. Learning odor–visual associations

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Although odors are thought to be strong cues for memory, little is understood about how these associations are formed, and there have been few direct comparisons of the effectiveness of odors versus other modalities as memory cues. The present study examined odors and nonwords as cues for two different types of visual stimuli (abstract art or three-dimensional shapes). In an attempt to decrease verbal labeling, odors were chosen to be relatively unfamiliar and difficult to name appropriately. Nonwords were chosen, rather than real words, to match for stimulus novelty.

In Experiment I, 65 subjects participated in two learning tasks: learning 12 odor–art (OA) and 12 nonword–art (NA) pairs. Each task had four trials in which the 12 pairs were presented, followed by a cued recognition test. For recognition, each odor or nonword was presented, and the subject chose the paired picture from a display of all 12 visual items. Forty-two subjects participated in a one-week delayed recognition test. Results showed that NA pairs were learned significantly better than OA pairs and although it was hypothesized that OA pairs would be better retained over a 1 week delay this was not supported.

Experiment II used the same paradigm, replacing pictures withabstract shapes. Half the subjects participated in a verbal

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interference condition during both learning tasks. Preliminary analyses showed that again nonword pairs were learned better than odor pairs in both conditions. However, verbal interference was found to significantly decrease learning of nonword pairs across trials, while learning of odor pairs was unaffected. Performance at 1 week delay will be presented.

Discussion will address the effectiveness of verbal mediation in odor pairings, as well as the role that stimulus novelty may play in long-term retention of paired associations.

This research was supported by the Medical Research Council of Canada.

97. Human brain function during odor encoding and recognition: a PET activation study

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In previous positron emission tomography (PET) studies we have shown significant cerebral blood flow (rCBF) increases during olfactory stimulation: unilaterally in the right orbitofrontal cortex, and bilaterally in the inferior frontal and temporal lobes (piriform cortex). In the present study we investigated brain function during different stages of olfactory memory processing.

Subjects were scanned during four tasks: odor encoding, long-term odor recognition, short-term odor recognition and a no-odor sensorimotor control task. Subjects were 12 right-handed healthy volunteers (6 men, 6 women). Each subject underwent a training session four days prior to their PET scan to learn the six odors required for the long-term memory scan. PET scans were obtained with a Siemens Exact ECAT HR$^+$ 3D system using $H_2{}^{15}O$ methodology and 60 s scanning intervals. PET images were co-registered with each subject's magnetic resonance imaging scan, averaged, and transformed into standard stereotaxic space. Paired image subtractions were analyzed for rCBF changes.

Preliminary analyses have revealed significant activation of the right orbitofrontal region and bilateral piriform cortices during the long-term odor recognition task compared with the control task. Activation of the right piriform cortex was present during the short-term recognition task. Brain activity during encoding and retrieval tasks also involved prefrontal cortices. PET activation studies of memory in other modalities have led to hypotheses of a hemispheric encoding/retrieval asymmetry in frontal cortex; the generalizability of this theory to olfactory memory will be discussed.

This research was supported by the Medical Research Council of Canada and the McDonnell-Pew Cognitive Neuroscience Centre.

98. Taste sensitivity in cancer patients treated with chemotherapy

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Electrogustometry was used to assess cancer patients' sensitivity before, during and after chemotherapy with a variety of chemically different treatments.Electrogustometry consists of an iontophoretic stimulation of sensory cells with cations contained in saliva and taste pore substance. Thresholds are given in microamperes. A 5 mm^2 stimulation was produced with a metallic spherical electrode applied at the tip of the tongue and at 5 mm behind the right and left eye-teeth. Patients (n = 110) had thresholds between 5 and 250 μA (35 ± 47) at the tip and 45 ± 65 μA on both sides of the tongue. A group of 160 healthy controls showed significantly different thresholds between 5 and 160 μA (P < 0.01). A few discrepancies between right and left sides could be accounted for by teeth surgery. The mode of the distribution was 40 μA in cancer patients under treatment and 21 μA in controls. Thresholds in some individuals could be measured repeatedly before and after treatments. Recovery was observed at 18 days (twice the half time of most taste cells) or sooner following treatments. We hypothesize that some chemotherapies directly destroy taste sensory cells as their turnover is comparable to tumoral cell turnover.

99. Cognitive modulation of adaptation and sensitization to odor

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Exposure-induced changes in the responsiveness to an odorant are commonly attributed to sensory mechanisms. Cognitive determinants of odor perception may be equally important, however. To evaluate the potency of cognitive modulation of sensory processing in olfaction, 135 subjects were given short-term exposure to odors following explicit biasing information about the source and consequences of exposure to the odorant. Subjects, while seated in a chamber, judged the perceived odor and irritation intensity of either (i) methyl salicylate (15 ppm, normative perception of 'healthy'), (ii) isobornyl acetate (45 ppm, normative perception of 'ambiguous') or (iii) butanol (20 ppm, normative perception of 'hazardous') following one of three characterizations of the odorant source and risk from exposure. Ratings of odor and irritation varied significantly with odorant characterization. Subjects for whom the odor was characterized as a natural substance showed the most adaptation to the perceived odor and irritancy during exposure and the fewest health symptoms following exposure. In contrast, subjects for whom the odorant was characterized as an industrial solvent showed initial adaptation followed by sensitization during exposure; they also reported more frequent and more intense health symptoms. For methyl salicylate and butanol, the responses of subjects who were given non-specific information were most similar to those subjects who were given characterizations congruent with the normative perception of the odor (i.e. characterizing methyl salicylate as a natural extract). Changes in response to olfactory stimuli seem to be a joint function of sensory and cognitive processes. The time-course of these changes appears to reflect sensory adaptation combined with cognitively-mediated adaptation or arousal, thus yielding coincident processes of adaptation and sensitization.

Supported by NIH grants F32 DC-00197, RO3 DC02683 and a grant from the Olfactory Research Fund.

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100. Increases in chloride permeability hyperpolarize squid olfactory neurons

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Our previous studies have indicated that odorants such as betaine and dopamine activate changes in chloride conductance which could either depolarize or hyperpolarize squid olfactory receptor neurons (ORNs), depending on the internal chloride concentration. We report here that odorants induce an increase in chloride conductance that hyperpolarizes the ORN *in vivo* due to a very negative natural E_{Cl}. Isolated squid ORNs were voltage clamped using gramicidin perforated patch recording techniques, which allow electrical access without disrupting $[Cl^-]_i$ or second messengers. During gramicidin patch recordings with near equimolar pipette and external chloride concentrations, the E_{rev} of the odorant response was -67 ± 17 mV ($n = 26$) indicating a normal $[Cl^-]_i$ of 35 mM. After rupturing the patch and dialyzing the cell with high Cl^- pipette solution, the E_{rev} shifted to $+5 \pm 13$ mV ($n = 19$). To confirm that membrane conductance was increasing, we applied repeated voltage steps or ramps to nystatin patched ORNs, while spritzing on 5 mM betaine. In all cases, the current response of the ORN was increased during betaine application, indicating an increase in the membrane conductance of the cell. These results demonstrate that, in contrast to most vertebrates, where chloride conductances depolarize ORNs, in the squid chloride mediates ORN hyperpolarizing odorant responses.

This work was supported by NIDCD R29 DC02587-02.

101. Behavioral and single chorda tympani taste fibers responses in common marmoset, *Callithrix jacchus jacchus*

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Gustatory responses of common marmoset were studied using two-bottle preference (TBP) test and single fiber recording from chorda tympani (CT) nerve. Responses of 43 CT fibers were recorded during stimulation of the tongue with three salts, four acids, six bitter compounds and 30 sweeteners. Hierarchical cluster analysis of the fibers' response profiles revealed three major clusters of fibers characterized by predominant sensitivity to sweeteners (S cluster), bitter compounds (Q cluster) or acids (H cluster). NaCl as well as LiCl did not stimulate CT taste fibers.

Activity in particular cluster of CT fibers can be associated with the type of the response in TBP (strong preference, no preference, rejection). Marmosets rejected stimuli with unpleasant taste to humans: quinine, sucrose octaacetate, tannic, and aristolochic acids. These compounds stimulated the Q cluster. Twenty preferred sweeteners stimulated only the S cluster fibers. Stimuli which elicited responses in both the S and the Q clusters were either rejected (stevioside and D-phenylalanine) or non-preferred (ampame, xylitol, saccharin). Marmosets neither preferred nor rejected sweeteners which stimulated none of the CT fibers.

102. Rapid clinical evaluation of anosmia—the Alcohol Sniff Test

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In spite of the availability of clinical tests of olfactory function, the typical cranial nerve examination omits CNI. There is a critical need for a smell test appropriate for a standard neurologic examination, easily administered by all physicians. A simple olfactory test, the Alcohol Sniff Test (AST), which uses an odor stimulus readily available in the medical environment and is rapidly administered was developed at the UCSD Nasal Dysfunction Clinic. It provides a measure of Cranial Nerve I function *in situ*ations where no other functional measure is practical. The present study aimed to determine the ability of the test to (i) discriminate between patients with anosmia or hyposmia and persons with normal function, (ii) to estimate the correlation of the results of this test with another more lengthy, but widely used test of olfactory function and (iii) to estimate the reliability of the test. Participants were 100. Of these, 64 were patients presenting to the UCSD Nasal Dysfunction Clinic for rhinitis, sinusitis and olfactory impairment and 36 were normal controls participating in a longitudinal study of olfactory function. All participants underwent ENT examinations, including nasal endoscopy. A standard 70% isopropyl alcohol prep pad was positioned below the nostrils along a standard tape measure and advanced at a rate of 1 cm per expiration until the subject detected the presence of odor. The procedure was repeated five times. Results were compared with those obtained using a routine, two-alternative, forced-choice, ascending, threshold test. ANOVA demonstrated significantly different AST thresholds for patients and controls, $P < 0.005$. Repeated measurements were stable, showing no significant variation over the five trials, $P > 0.70$. When participants were classified as anosmic, hyposmic or normosmic based on the routine threshold procedure, ANOVA showed these groups to differ significantly on the AST, $P < 0.005$. The AST is recommended as a rapid screening for anosmia in settings where screening would otherwise be omitted.

Supported by NIH grant # AG04085.

103. Target/ganglion cell interactions in the development of rodent trigeminal sensory neurons

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Detailed *in vitro* and *in vivo* studies have revealed that the neurons of the embryonic mouse trigeminal ganglion undergo marked changes in their survival requirements during development. When trigeminal neurons start extending axons towards their targets they survive independently of neurotrophic factors in culture. When the earliest sensory axons approach their peripheral targets most of the neurons are supported by either BDNF or NT3 in culture, but not by NGF. During the earliest stages of target field innervation, the majority of neurons switch dependence from

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BDNF and NT3 to NGF. Consistent with these *in vitro* findings, there is a substantial increase in the number of dying neurons in the early stages of the development of the trigeminal ganglion in embryos that are homologous for a null mutation in the BDNF receptor tyrosine kinase (*trk*B) gene, whereas the increased neuronal death occurring in embryos that are homologous for a null mutation in the NGF receptor tyrosine kinase (*trk*A) gene peaks later in development, coincident with the peak of naturally occurring neuronal death. Unlike the onset of BDNF dependence, which is controlled by an intrinsic timing mechanism that is programmed early in development, the switch to NGF dependence is appears to be triggered by target-derived signals that act during the switchover period. The onset of BDNF and NGF dependence is associated with marked increases in the expression of trkB and trkA, respectively. The loss of BDNF dependence, however, is associated with high levels of expression of non-catalytic trkB variants in neurons which act as negative modulators of BDNF signalling. The expression of neurotrophins in peripheral tissues is correlated with the changing responses of trigeminal neurons to neurotrophins during development. In the late stages of naturally occulting neuronal death, trigeminal neurons additionally acquire *in vitro* responsiveness to CNTF, LIF and OSM.

104. Substance P modulates the activity of taste-responsive neurons in the hamster solitary nucleus

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The rostral gustatory portion of the nucleus of the solitary tract (NST) in the hamster contains substance P (SP) immunoreactive neurons and also receives extrinsic SP immunoreactive afferent inputs. In brainstem slice studies, SP excites ~70% of the cells in the gustatory portion of the NST in both rats and hamsters. Although *in vitro* studies provide valuable information about the biophysical properties of NST neurons, it is not possible to confirm their gustatory responsiveness. In the present study, the effects of SP microinjections on the electrophysiological responses of gustatory NST neurons were examined in hamsters following either anodal electrical or 0.032 M NaCl stimulation of the anterior tongue. A micropipette containing SP (0.25–0.5 mM) was glued to the shaft of a tungsten microelectrode ~120 mm from the recording tip and ~30 nl of SP was delivered by pressure. There was a statistically significant effect of SP on the levels of both spontaneous and anodal- or NaCl-evoked activity in gustatory NST neurons. Following anodal stimulation, the activity of 60% of the neurons (n = 15) was enhanced (14.3 versus 31.9 imp/s) and that of 40% was suppressed (12.7 versus 6.8 imp/s) in the presence of SP. The modulatory effect of SP following repetitive anodal stimulation of the tongue lasted 30–400 s. In the presence of SP, the response of 48% of the neurons (n = 23) to NaCl was increased (6.1 versus 17.4 imp/s) and that of 9% was decreased (19.5 versus 9.9 imp/s). The effect of SP was specific to the stimuli that normally drive the cell; it did not create new sensitivities. These studies demonstrate that SP has a dual modulatory action on the response profiles of taste neurons *in vivo*. SP could directly excite taste-responsive neurons (as shown *in vitro*) or it could excite inhibitory local circuit neurons that, in turn, decrease the responsiveness of gustatory neurons.

Supported in part by NIDCD DC00066 (D.V.S.) and DC00207 and DC00245 (B.J.D.).

105. Calcium changes in necturus olfactory receptor neurons elicited by amino acids

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Amino acids have been shown to be potent stimuli of *Necturus* olfactory receptor neurons with excitatory and inhibitory responses recorded using standard electrophysiological techniques. We wanted to determine the chemosensitivity of dissociated *Necturus* olfactory receptor neurons to amino acids using the less invasive technique of calcium imaging, measuring changes in intracellular [Ca^{2+}] with the fluorescent indicator, fura-2. Only those olfactory neurons with cilia were studied. Individual L-amino acids (at concentrations from 10 μM to 10 mM) were dissolved at neutral pH in bath saline and applied rapidly at concentrations from 10 μM to 10 mM. The cells showed selectivity; some olfactory receptor neurons responded to one or more stimuli, but no cell responded to all of them, and many cells did not respond to any. With several cells in a single field, we have observed some responding to one stimulus and others responding to a different stimulus. IBMX, a potent phosphodiesterase inhibitor that leads to an increase in intracellular cAMP, was also tested. Only one-third of the olfactory neurons showed an increase in $[Ca^{2+}]_i$ in response to IBMX. Interestingly, some of the olfactory neurons that increased $[Ca^{2+}]_i$ in response to IBMX, responded to histidine or proline with a decrease in $[Ca^{2+}]_i$. This suggests that more than one pathway mediates the responses to different amino acids

This work was supported by grants NIHDC00176, DC00256 and NSF IBN 94-21190.

106. Tests of taste preference synergism between glutamate agonists and IMP in rats

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Molecular and behavioral data suggests that a class III metabotropic receptor, mGluR4, may be a receptor involved in taste transduction for glutamate taste, termed 'umami'. Harbaugh *et al.* adapted a model of Rifkin and Bartoshuk to develop a taste preference procedure that can detect synergism between taste stimuli in rats. Synergism between monosodium glutamate (MSG) and nucleotides is a key characteristic of umami taste. Harbaugh *et al.* measured lick rates (LR) of non-deprived rats during 30-s trials in an MS80 Davis lickometer. These investigators found synergism between MSG and inosine 5′ monophosphate (IMP) but not MSG and sucrose. We have extended these experiments to study synergism between glutamate agonists and IMP on taste preference in rats to see if a specific glutamate ligand underlies umami taste transduction.

Experiments were conducted to establish taste preference concentration gradients for NMDA or kainic acid (KA), presented

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to rats at neutral pH. Naive rats were then tested for interactions between NMDA or KA and IMP using an ABA design. During A, the baseline LR for each concentration of a tastant was established for each rat. During B, LR to mixtures of NMDA/IMP, or KA/IMP were measured. Preference ratios were computed by dividing LR for each taste stimulus by the mean LR for water trials. When model ratios were compared with behavioral ratios with ANOVA procedures, no evidence of synergism between IMP and NMDA, or IMP and KA was found, unlike the results for MSG and IMP. We are now extending these procedures to test L-AP4, a specific ligand for the mGluR4 receptor.

Supported, in part, by NIH grant PO1 DC01959.

107. Role of *Pax-6* gene in olfactory bulb precursor cell migration and survival

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Pax-6 is a member of a murine family of developmental control genes important for CNS development. A point mutation in the *Pax-6* gene is responsible for the Small-Eye (*Sey*) phenotype in mice. We recently found that main olfactory bulb volume is significantly reduced in heterozygous (*Sey*/+) animals as compared with wild-type litter mates by postnatal day 42 (P42). In mice, olfactory bulb granule and periglomerular cells arise in the subventricular zone of the lateral ventricles and migrate along the rostral migratory stream to the olfactory bulb throughout life. We hypothesized that *Pax–6* might be important for olfactory bulb precursor cell proliferation, migration and/or survival. To examine progenitor cell migration, *Sey*/+ and wild-type animals were injected on P40 with bromodeoxyuridine (BrdU). Animals were sacrificed 2 days later and numbers of BrdU-immunoreactive cells were counted in the subventricular zone, along the rostral migratory stream and in the olfactory bulbs. Fewer BrdU-immunoreactive neurons were present in the rostral most regions of the migratory stream and in the olfactory bulbs as compared with controls, suggesting that two alleles of *Pax-6* might be required for normal olfactory bulb precursor cell migration. There was also an overall reduction in numbers of BrdU-immunoreactive cells in the brains of *Sey*/+ animals, suggesting that cell pro- liferation and/or survival might also be affected. Therefore, we injected P40 wild-type and *Sey*/+ litter mates with BrdU and sacrificed them 2 h or 30 days later to examine whether these processes also contribute to decreased olfactory bulb volume in *Sey*/+ animals.

Supported by NIH grants NS 09632 (T.L.D.) and R01 DC00880 (M.S.F).

108. Perireception in olfaction: molecular weight filtration by aesthetasc sensillar cuticle limits odorant access to receptor sites in Caribbean spiny lobster *Panulirus argus*

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The responsiveness of chemoreceptor neurons can depend on a combination of perireceptor and receptor events. Olfactory neurons of crustaceans are packaged into distinctive cuticular sensilla called aesthetascs. The cuticle of aesthetascs is thin and permeable even though it does not contain any obvious surface pores or pore tubules. This suggests that this spongy cuticle may act as a molecular sieve that is permeable only to small odorant molecules. We examined whether this is so for the aesthetasc cuticle of the Caribbean spiny lobster *Panulirus argus*. We used a chromatographic column packed with aesthetasc cuticle and connected to a flow-through UV spectrophotometer to measure the elution times of UV-absorbent molecular weight markers between 165 and 2 000 000 daltons. Molecules >9 kDa had similar elution times, indicating that they did not penetrate the cuticle. Molecules <9 kDa had longer elution times that were directly and inversely proportional to their molecular weight. These results suggest that aesthetasc cuticle excludes molecules >9 kDa from entering or leaving the sensillar lymph. We conclude that the molecular sieving capacity of the aesthetasc cuticle of *P. argus* is a perireceptor mechanism that is a critical determinant of the types of molecules capable of stimulating its olfactory receptors.

Supported in part by NIH grant DC00312.

109. Oral irritant effects of nicotine: psychophysical evidence for decreased sensation following repeated application and lack of cross-desensitization to capsaicin

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When delivered to the oral mucosa, a variety of naturally occurring chemicals such as capsaicin from red chili peppers, piperine from black pepper, and nicotine from tobacco, cause a diffuse burning sensation often referred to as irritation. The burning sensation evoked by capsaicin increases when delivered repeatedly at 1 min intervals (sensitization), but then decreases markedly following a 10 min rest period (self-desensitization). It is also interesting that following desensitization by capsaicin, irritant sensations evoked by other chemicals are also reduced (cross-desensitization), suggesting that oral irritation from some agents may be mediated by a population of capsaicin-sensitive trigeminal polymodal nociceptors.

Although nicotine is a major component in tobacco smoke, little is known about its sensory properties. Accordingly, a study of the oral irritant effects of nicotine as compared with capsaicin was initiated.

Whereas capsaicin (0.5 or 3 ppm; repeated at 1 min intervals over 10 min) evoked significantly stronger sensations (sensitization), there was a significant decrement in sensations to repeated application of nicotine (0.1%). After the subjects had received either repeated capsaicin or nicotine on one side of the tongue, a rest period ensued followed by a bilateral application of either capsaicin or nicotine. Subjects were, then, asked to choose which side yielded a stronger sensation (two-alternative forced choice). Following capsaicin pretreatment, all subjects reported that capsaicin evoked a stronger sensation on the previously untreated side (capsaicin self-desensitization). Similar self-desensitization was observed with nicotine. Furthermore, nicotine was reported to evoke a significantly weaker sensation on the side of the tongue pretreated with capsaicin (cross-desensitization). In contrast, equal

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numbers of subjects reported capsaicin to evoke a stronger sensation on either the nicotine-pretreated side or the untreated side, indicating an absence of cross-desensitization. These results are discussed in terms of physiological mechanisms that might underlie the contrasting sensory effects of nicotine versus capsaicin.

Supported by the California Tobacco-Related Disease Research Program, 4IT-0087.

110. Effect of estradiol on the regeneration of olfactory mucosal damage induced by 3-methylindole

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Though women show better performance than men on a wide variety of smell tests and fluctuations in olfactory sensitivity have been documented, it is still unclear whether female gonadal hormone has a direct effect on the olfactory epithelium. This study is to evaluate the effect of estrogen on the regeneration of olfactory mucosa damaged by systemic 3-methylindole (3-MI).

A total of 48 female Sprague–Dawley rats were divided into two groups. At 3 weeks of age, all rats were bilaterally oophorectomized. Half of the rats (experimental group) were administered 1 mg/kg of 17β-estradiol every day until the end of experiment. The other half were administered corn oil alone. Two weeks after operation, 4% solution of 3-MI in corn oil was injected in all rats at a dose of 300 mg/kg. Four rats in each subgroup were killed 3, 7, 14, 21, 28 and 35 days after the injection of 3-MI. Sections cut at 4 mm were stained with H&E, S-100, and PCNA for light microscope.

At 3 days, olfactory epithelial necrosis and extensive ulceration was found with relative preservation of basal cells. Respiratory epithelium was viable. At 14 days, the olfactory area was covered with a few layers of undifferentiated columnar cells. From 21 days, thickness of the olfactory epithelium increased and receptor cells were frequently stained with S-100 in both groups. By 35 days, olafatory mucosa was almost regenerated with focal squamous metaplasia in both groups. The nasal cavity was partially occluded by fibrous and bony tissue in several rats. This result suggests that estradiol doesn't seem to have an influence on the regeneration of damaged olfactory mucosa. Histological study on the higher antomic levels and behavioral study will be followed in the near future.

Supported by Samsung Biomedical Research Institute C-95-051-2.

111. Hypothalamic–pituitary–gonadal axis inhibition in normal men induced by delivery of pregna-4,20-diene-3,6-dione to the vomeronasal organ

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The vomeronasal organ (VNO) of animals has been shown to mediate the effects of pheromones, chemicals which influence sexual and maternal behavior, the estrus cycle, gestation, the onset of puberty, and the recognition of conspecific and heterospecific members.

This abstract reports the changes observed in the release pattern of gonadotropins and testosterone in normal men after administration to the VNO of a steroidal vomeropherin compound, pregna-4,20-diene-3,6-dione (PDD). PDD (250 pg) was delivered to the VNO of 10 healthy men in a randomized, double-blind, crossover clinical trial, with placebo. Administration was repeated every 10 min over 6 h using a vomeronasal applicator. Blood serum was obtained after each vomeropherin or placebo administration, and LH, FSH and testosterone were measured. A peak detection algorithm was used to characterize the pulsatile characteristics of the gonadotropin secretion. Both the total amount of gonadotropins released and the number of 'peaks' detected during the administration of PDD were significantly diminished as compared with the value obtained during placebo administration (LH $P = 0.009$; FSH $P = 0.022$). Mean value (6 h period of sampling) of serum testosterone was also reduced by the effect of the vomeropherin (PDD 3.34 ng/ml; placebo 5.14 ng/ml; $P < 0.05$).

These results demonstrate that very small quantities of vomeropherin acting through the human VNO induce inhibition in the activity of the hypothalamic–pituitary–testicular axis in humans.

112. Odorant-binding proteins of true bugs: generic specificity, sexual dimorphism, and association with subsets of olfactory sensilla

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Odorant-binding proteins (OBPs) in insects occur within olfactory sensilla, and are thought to transport chemical stimuli to receptors on dendrites of sensory neurons. Until recently, knowledge of OBPs in insects was limited to moths and *Drosophila*. We discovered an antennal-specific protein (LAP) with a unique *N*-terminal sequence in the true bug, *Lygus lineolaris*. We localized LAP to antennae, determined its molecular weight (16 kDa), and showed that while it was expressed in nymphal antennae, its levels dramatically increased in adults concurrent with increases in numbers of olfactory sensilla and electrical responses to odors. In our current study, we used immunological techniques to demonstrate in more detail that LAP occurs only in antennae, and to show its expression within *Lygus* species. LAP was expressed more in male antennae than in antennae of females for the *Lygus* species examined. AntiLAP did not recognize antennal proteins of two other genera of bugs. Immunocytological studies showed LAP primarily within the sensillar lymph of type 1 and type 4 sensilla on antennae. These observations strongly suggest LAP to be an OBP, and our discovery and characterization of OBPs in true bugs

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provides a third order for use in the study of evolution of OBPs in insects.

113. Cloning and characterization of human CYP2G1-related genes and transcripts suggest the absence of a functional CYP2G gene in humans

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CYP2G1 is an abundant, olfactory-specific cytochrome P450 isoform highly active in sex steroid hydroxylation in rodents and rabbits. However, in previous studies, CYP2G1-like immunoreactivity was not detected in human olfactory mucosa with an antibody to rabbit CYP2G1, although a short cDNA fragment derived from a CYP2G-related mRNA was detected in human nasal mucosa by RNA-PCR. In the present study, genomic sequences corresponding to 5′- and 3′-untranslated regions of a putative human CYP2G1 gene were obtained from a human genomic library. PCR primers were subsequently designed and used to amplify full length CYP2G1-related cDNAs with RNA preparations from human olfactory mucosa. Three different cDNA clones were obtained, but none contain an open reading frame for a full length P450 protein. Alignment of cDNA sequences with that of rat CYP2G1 gene indicated that all three clones lack exon 2 sequence while one clone lacks an additional 71 bp in exon 9. Moreover, all clones have nonsense substitutions or single nucleotide additions that indicate that they originate from CYP2G pseudogenes or mutant alleles. Thus, all three clones represent aberrant splicing products of CYP2G pseudogene transcripts. Further attempts, including genomic library screening, exon 2-specific RNA-PCR, RNA blot analysis, and allele-specific genomic PCR, did not detect a functional CYP2G1 gene or transcript. It appears that humans may not have a functional CYP2G1 gene. The lack of a functional CYP2G1 gene may be related to some of the species differences in olfactory chemosensory ability between humans and mammalian animals.

This research was supported in part by NIH grant DC02640.

114. Intensity and hedonic valence of everyday odors correlate positively with familiarity: a cross-cultural study

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A question of central interest in both basic and applied olfactory research is to what extent odor perception is determined by physico-chemical properties of the stimulus, by properties of the primary olfactory apparatus, or by subjective, higher-order processes. In a recent comparison of odor perception in Japanese and German subjects, we found culture-specific differences in the familiarity of odors, and that independent of the odor, familiarity correlated positively with hedonic valence and also with perceived intensity. To examine the generality of these results, we extended the study to include an equivalent, age-matched Mexican sample.

The responses of 40 Japanese, 40 German and 40 Mexican women to the odors of 19 natural substances were compared. Odorants were presented in random order in opaque polyethylene squeeze bottles and subjects were asked to rate each sample for intensity, pleasantness and familiarity. Although there were considerable differences in rating behavior between the three groups, clear correlations were found in each of the three populations between familiarity and intensity (Japanese, $r_s = 0.69$; Germans, $r_s = 0.59$; Mexicans, $r_s = 0.53$), and between hedonic valence and intensity (Japanese, $r_s = 0.69$; Germans, $r_s = 0.55$; Mexicans, $r_s = 0.79$).

These results suggest a significant experience-dependent component to odor perception, and in particular, that intensity may not depend solely on stimulus properties. However, whether this is due to experience-dependent changes in central processing or in the olfactory epithelium remains to be investigated.

115. An improved model of the synaptic organization of insect olfactory glomeruli

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The synaptic circuitry between identified neuron individuals or types within cockroach antennal lobe glomeruli has been studied in the electron microscope in order to characterize the putative neuronal pathways underlying first-order processing of odor signals. The anatomical findings are consistent with physiological data from recordings of central neurons. For characterization of the synaptic connections, different combinations of neuron labelings have been applied including (i) HRP-injection of local interneurons (INs) or projection neurons (PNs), (ii) GABA-immunogold labeling and (iii) experimentally induced degeneration of antennal receptor neuron projections (RNs). The following model is proposed:

Axons of afferent antennal RNs form output synapses in a dyadic fashion onto a PN and, in addition, a GABA-positive neuron. The majority of such GABA-positive neurons in the glomeruli belong to multiglomerular INs. These neurons, in turn, are presynaptic to PNs and/or other GABA-positive INs. PNs receive input signals from RNs also via two presynaptic and serially connected GABA-positive INs. Reciprocal synaptic connections exist between (i) GABA-positive INs and PNs as well as (ii) two GABA-positive INs. GABA-positive INs form dyadic output synapses onto axons of RNs and, simultaneously, dendrites of PNs.

These findings support the following hypothetical pathways between glomerular input- and output neurons: (i) a monosynaptic and putatively excitatory route, and (ii) at least two polysynaptic routes, one of them a putatively inhibitory and the other a disinhibitory path. (iii) There exist inhibitory feedback connections from these two paths via GABA-positive INs onto both, RNs as well as PNs. (iv) Since a given GABA-positive IN innervates a large number of antennal lobe glomeruli, inhibitory signals could also be passed to neighbored glomeruli.

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116. Temporal characteristics of mitral/tufted cell spike trains with varying stimulus concentration

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The mitral/tufted cells (MCs) of the olfactory bulb respond to odor stimuli with complex spike trains. The temporal pattern of response varies within single cells with changes in both stimulus molecule and concentration. These patterns cannot always be predicted from one stimulus condition to the next, even varying concentration alone. To understand how individual MCs participate in coding odor quality, it will be important to determine how stimulus concentration contributes to MC response patterns. We analyzed extracellular recordings from 65 cells in the MC layer of the olfactory bulb in tiger salamanders (*Ambystoma tigrinum*) and leopard frogs (*Rana pipiens*), using various concentrations of propyl acetate, butyl acetate and amyl acetate as stimuli. The nasal cavity was intact, ensuring that all connections to the MCs were complete. Cells recorded in this preparation had response properties similar to those recorded with surgically exposed epithelia, but a larger percentage of cells responded: 100% responded to at least two concentrations, as compared with ~30% found by Kauer in 1974. Responses included all types described by Kauer. At low concentration, most cells did not respond; the few that did showed simple suppression. At intermediate concentrations, over half of the responses were suppression, the rest included one or more spike bursts preceded or followed by suppression. At the highest concentration, MCs responded predominantly with complex spike trains including both excitation and suppression. Effects of concentration were observed after, but not before, bursts were sorted into three types, by latency. Neither firing rate nor number of spikes in bursts was significantly affected by concentration for any burst type (as would have been expected for a rate code). Instead, concentration affected latency to spike bursts and inter-spike interval, as well as duration of suppression. With the ability to factor out effects of concentration, we can next examine how responses differ for odors that are perceptually discriminable.

Supported by grants from the ONR and the NIDCD.

117. Influence of scopolamine hydrobromide on odor detection performance of rats

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The influence of scopolamine hydrobromide (0.10, 0.125, 0.15 and 0.20 mg/kg i.p.) on the odor detection performance of 15 adult male Long Evans rats to ethyl acetate was measured using high precision olfactometry and a go/no-go operant signal detection task. The drug conditions and a saline control were administered to each animal in an order counterbalanced by Latin squares, with 2 day intervals interspersed between tests. Scopolamine produced a subtle depression in detection performance at lower odorant concentrations and a subtle enhancement at higher odorant concentrations, as measured by percent correct S+ and S– responses and a non-parametric signal detection analysis measure of sensitivity. Odorant sampling time, as well as a measure of the subject's response criterion, was inversely correlated with sensitivity. This study suggests that scopolamine has a small influence on measures of odor detection performance which varies as a function of the odorant concentration being assessed.

Supported by NIDCD Grant PO 00161, NIH.

118. Multiple sclerosis: influence of cns lesions on odor identification ability

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Multiple sclerosis (MS), the most common neurologic disease in the young adult, is accompanied by focal demyelinating plaques within the brain that can be quantified *in vivo* using high-resolution magnetic resonance imaging (MRI). Thus, MS may be an excellent model for studying the influences of focal lesions on some forms of sensory function. In this study, we determined whether the number of MS-related plaques within the inferior temporal and frontal lobe regions (T&F) correlates with scores on the University of Pennsylvania Smell Identification Test (UPSIT). We administered the UPSIT to 9 men and 17 women (mean age = 42.17 years) with definite MS. Thin section MRI of the brain with gadolinium enhancement was performed on the same day as olfactory testing, and counting of plaques was done without knowledge of UPSIT scores. A strong negative relationship (Spearman $r = -0.94$, $P < 0.0001$) was present between the UPSIT scores and the number of demyelinated plaques within the T&F. No such relationship was present between the scores and plaque numbers in non-olfactory eloquent brain regions ($r = -0.08$, n.s.), implying the relationship was restricted to brain structures directly involved in olfactory processing. Relative to normative data based upon nearly 4000 subjects, 38.5% of the MS patients had demonstrable olfactory loss, with 7.7% exhibiting severe bilateral microsmia, 19.2% moderate bilateral microsmia and 11.5% mild bilateral microsmia. None were anosmic. No sex differences or meaningful left–right asymmetries in UPSIT scores were observed. These findings clarify the ongoing controversy concerning the presence or absence of olfactory dysfunction in MS. Importantly, they support the concept that MS, with its relatively discrete focal lesions of inflammation, demyelination and gliosis, can serve as a useful model for the study of CNS lesions on human sensory perception.

Supported by NIDCD Grant PO 00161, NIH.

119. Olfactory dysfunction in aging and disease

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It is now well documented that olfactory function declines with age. Furthermore, such declines are present in patients with several age-related neurodegenerative disorders, including Alzheimer's disease and idiopathic Parkinson's disease. In this presentation, I review studies of olfactory function in the elderly and in a broad range of neurological disorders, including Alzheimer's disease, amyotrophic lateral sclerosis, the dementia-parkinsonism syndrome of Guam, Down's syndrome, idiopathic Parkinson's

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disease, multiple sclerosis, Huntington's disease, progressive supranuclear palsy and schizophrenia. A discussion of the possible etiological bases of the olfactory loss of age and a number of such disorders will be made. The strengths and weaknesses of the olfactory vector hypothesis, a hypothesis which implicates putative environmental toxins in the etiology of a number of neurological disorders, will also be discussed.

Supported by grants PO1 DC 00161 and RO1 DC 2974 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

120. Glial development in the primary olfactory system

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The glial cells of the primary olfactory pathway comprise astrocytes as well as a glial cell type (i.e. olfactory ensheathing cells) that is unique to the olfactory system. Although the focus of the talk will be on glial cell development, in particular dealing with the olfactory ensheathing cells, there will be some discussion on the adult anatomy in so much as it pertains to specific developmental aspects. The range of phenotypic features expressed by ensheathing cells during development, as well as in adult animals, is only now becoming apparent. Several studies have revealed the phenotype of ensheathing cells at different stages of development, providing an initial glimpse of the changes that occur in these cells as they differentiate into mature non-myelinating cells. This non-myelinating phenotype appears to be the default phenotype, but probably only because the olfactory axons, being small C fibers, do not induce ensheathing cells to assemble a myelin sheath. However, if ensheathing cells are given the opportunity to ensheath larger axons, they will express a myelinating phenotype. When taken in conjunction with their axonal promoting ability, ensheathing cells are beginning to be viewed as ideal candidates for inclusion in therapeutic approaches to treating spinal cord injury as well as demyelinating diseases such as multiple sclerosis.

Supported in part by NIDCD 02370.

121. Sensory responses to 6-*n*-propylthiouracil (PROP) or sucrose solutions and food preferences in young women

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Genetic sensitivity to 6-*n*-propylthiouracil (PROP) has been associated with a greater number of food aversions and reported rejection of bitter-tasting foods. This study was based on 159 women, mean age 27 years and mean BMI 23.2. The women were classified as PROP nontasters (n = 43), regular tasters (n = 70) or supertasters (n = 46) on the basis of their PROP detection thresholds and ratios of perceived intensity of PROP versus NaCl. As expected, PROP tasters tended to dislike coffee, espresso, rye breads, grapefruit juice and selected cruciferous vegetables, notably brussels sprouts. Factor analyses of preference data confirmed that PROP tasters reacted negatively to the sour and bitter components of vegetables and fruit. There was no evidence from food preference checklists that PROP tasters preferred meats or fats. However, stated acceptance of such foods by young women can be subject to numerous biases related to diet and health. Whereas inherited sensitivity to bitter taste may predict food dislikes, hedonic response functions to five sucrose solutions were unrelated to reported preferences for sweet foods. Separating sucrose 'likers' (n = 66) from 'dislikers' (n = 82) using established procedures yielded no major differences in reported preferences for sweet foods or beverages. The distinction between sucrose 'likers' and 'dislikers' was unrelated to PROP sensitivity in this all-female sample.

Supported in part by CA 61680.

122. Multiple arrestins in the mouse olfactory system

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The arrestins are a class of signalling proteins that act directly or indirectly upon several types of G protein-coupled receptors to promote tachyphylaxis and terminate signal transduction. Multiple isoforms of this gene family have already been characterized in the visual system and elsewhere. The diversity of the odorant receptor gene family prompted us to characterize arrestins in the olfactory system. A previous study demonstrated the presence of β-Arrestin2 (βARR2) immunoreactivity in the olfactory epithelium and showed that it promotes desensitization of odorant-induced cAMP responses in cilia isolated from olfactory sensory neurons (Dawson *et al.*, 1993). Consistent with that study, we have been able to detect βARR2 transcripts in mouse olfactory epithelium by RT-PCR followed by cloning and sequencing of the partial cDNAs. However, these same studies *also* resulted in the isolation of a β-Arrestin1 (βARR1) fragment from olfactory epithelium total RNA. *In situ* hybridization experiments showed that βARR1 mRNA is found in the neuronal layer of the olfactory epithelium. βARR1 transcripts are also expressed in the olfactory bulb, especially in the mitral cell layer, but also around the glomeruli, presumably in periglomerular cells. Studies are currently in progress to explore further the potential diversity of this group of signal transduction molecules in the olfactory system.

This work was supported by NIH Grant NS37348.

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123. Cyclic nucleotide-modulated K^+ channels and Ca^{2+} transduce a subset of odorants

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The functional identity of an olfactory receptor neuron is determined in part by the diversity of odorant-modulated second messenger pathways and membrane conductances expressed in the apical dendrite and cilia. We have investigated the effect of mutations in a cyclic nucleotide (CN)-modulated K^+ channel subunit encoded by the *ether a go-go* (*eag*) gene on odorant responsiveness in *Drosophila melanogaster*. *eag* underlies in part the transduction of only a subset of odorants (4/9 tested) using

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electroantennogram recording methods. Loose patch recording techniques applied to a recently developed antennal preparation (Dubin and Harris, 1997) indicate that a population of adult olfactory neurons requires *eag* expression for excitatory responses. While there appeared to be a shift in affinity for a component of the transduction pathway to propionic acid and ethyl butyrate, the responses of single neurons indicate a decrease in a proportion of responding cells to low doses of odorant and a non-specific effect of odorant at non-physiological concentrations. The loose patch technique was used to determine the sensitivity of *eag* and wild-type olfactory neurons to membrane-permeant CN analogs. Since a population of excitatory responses were specifically lost by *eag* mutations and activation of a K^+ conductance would inhibit neuronal excitability under our recording conditions, we tested whether Ca^{2+} influx through Eag channels (Bruggemann *et al.*, 1993) acts as a third messenger to increase excitability. In the absence of extracellular Ca^{2+}, the odorant elicits inhibitory responses presumably due to activation of Eag K^+ channel subunits, but in the presence of Ca^{2+}, these inhibitory responses become excitatory. Furthermore, a class of neurons whose excitability is rapidly increased by CN analogs is absent in *eag* mutants. Taken together, these data suggest that a subset of odorants activate Eag by increasing intracellular levels of CNs, and that subsequent influx of Ca^{2+} through these K^+ channels modulates downstream effectors. This pathway has the potential to alter cell excitability in opposite ways, depending on the ionic composition of the sensillar lymph and Ca^{2+}-modulated effectors expressed in dendrites.

Supported by R03 DC02579 and Whitehall Foundation (A.E.D.), American Heart Association, CA and MDA (G.L.H.).

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124. Accessory olfactory bulb (AOB) examined with respect to organization of input from the vomeronasal organ (VNO) and cellular activation patterns following VNO stimulation

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The topography of the projection from the VNO to the AOB was examined in the rat by injecting fluorescent dyes into discrete portions of the AOB glomerular layer and examining the VNO for retrogradely labeled cells. After injections into the rostral portion of the AOB, sensory cells close to the lumen were retrogradely labeled. Caudal AOB injections resulted in retrogradely labeled VNO cells located close to the VN nerve. Both subpopulations of VNO cells appeared to project dendrites to the lumen. Examination of anterogradely labeled mitral cells (me) in the AOB revealed that rostral injections labeled cells in the rostral part of the mitral layer and caudal injections labeled caudally situated mc.

To determine if this rostral/caudal anatomical gradient could be observed under endocrine conditions which activate the AOB, ovariectomized female rats and intact female mice housed in crowded conditions were exposed to urine from conspecific males. Cellular activation in the AOB was measured by *c-fos* immunocytochemistry. In both species, exposure to male urine resulted in significant increases in the number of *c-fos* immunopositive mc as compared with controls. In female mice, cellular activation occurring in distilled water treated controls was confined to the rostral or rostral and mid portions of the mitral layer. In both rats and mice, exposure to male urine resulted in activation of the entire mitral layer; however the *c-fos* immunopositive cells were more dense and intense in the caudal part.

The finding that both subpopulations of VNO cells project dendrites to the lumen suggests that both are physically capable of detecting stimuli present in the lumen. However, stimuli which cause weak activation of the AOB mc may be detected by VNO cells located close to the lumen whereas stimuli producing strong activation of AOB mc appear to be detected by both populations of VNO cells.

Supported by MH 41784.

125. Taste changes across pregnancy

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We examined taste intensity and preference in 46 pregnant and 41 healthy female controls enrolled in the Yale Pregnancy Study (J. Rodin, PI). Pregnant females were tested non-pregnant (non-pg) and during the first, second and third trimesters; controls, at corresponding time intervals. Subjects rated intensity of and preference for a three-member concentration series of NaCl, sucrose, citric acid (CA), and quinine hydrochloride (QHCl) on a labeled line ('nothing' at the left, 'extremely' at 80%). Variance differences between groups were tested with the F distribution ($P < 0.05$). Controls had significantly greater variance among overall intensity ratings than pregnant females, most pronounced for QHCl. Controls did not have highest variance among overall hedonic ratings, but did have highest variance for sweet and QHCl ratings. Within pregnancy analyses were tested with the Friedman two-way ANOVA. Significant intensity changes occurred for moderate NaCl and QHCL concentrations ($P < 0.005$). For NaCl, intensity fell from non-pg and first trimester to second and third trimesters. For QHCl, intensity rose from non-pg to first trimester, and fell from first to second and third trimesters. In the hedonic ratings, significant changes occurred for top concentrations of NaCl ($P < 0.05$) and.001 M CA ($P < 0.001$), and moderate QHCl ($P < 0.005$). NaCl preference rose from non-pg to third trimester. Preference for CA fell from non-pg to first trimester and then rose from first to second and third trimesters. QHCl became less disliked from non-pg and first trimester to third trimester. Summary: taste intensity and hedonic variance in controls may associate with menstrual hormone fluctuations. Pregnant women were aligned in pregnancy stage which may explain the lower variance. Taste intensity and hedonic changes may support a healthy pregnancy: increased first trimester bitter intensity to avoid ingestion of poison; decreased intensity/increased preference for NaCl later in pregnancy to support ingestion of sufficient electrolytes for expanded fluid volume; increased preference of sour and bitter qualities to allow ingestion of a varied diet.

Supported in part by 9603745 NRICGP/USDA (V.B.D.), NIH Grant DC00283 (L.M.B.) and NIH Grant NS00168 (J.R.).

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126. The relationship between intelligence and olfaction in old age

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Recent research has shown a relationship between measures of cognitive and sensory (i.e. visual and auditory acuity) functioning in old age. This has led a number of investigators to hypothesize that there is one common factor (i.e. overall reduction in central nervous system functioning) mediating age-related decline in sensory and cognitive ability. However, the assessment of the relationship between sensory and cognitive decline may be confounded because measures of intelligence typically rely on the visual and auditory modalities. Olfaction is independent of this measurement confound. Previous studies suggest an average decline in intelligence begins as early as the mid-50s. This study examined the proposed convergent decline of cognitive functioning and olfactory sensitivity using an odor threshold test and various intelligence and cognitive measures. Olfactory thresholds for butanol were obtained for 97 non-demented elderly subjects using a two-alternative, forced-choice, ascending method of limits. All subjects were tested in seven cognitive domains: speed of information processing, visuospatial skills, reasoning, knowledge, memory, verbal fluency, and global cognitive functioning. Sub-test scores from the Weschler Adult Intelligence Scale—Revised provided the measures for calculating overall intelligence. A series of simple and multiple regression analyses revealed a significant correlation between odor threshold scores and overall intelligence and between all seven cognitive factors and odor threshold scores. The findings provide support for the hypothesis that there is one common factor reflecting neuro-degenerative processes due to aging.

Supported by NIH grant # AG04085-11 to C.M.

127. Changes in chemosensory dysfunction and nutritional state in the first two years of dialysis

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The Chemosensory Complaint Inventory (CCI) has been described previously. This inventory has now been used prospectively to evaluate the changes in chemosensory disorders that occur in the first 2 years of dialysis treatment. Olfactory function was measured at six month intervals with the University of Pennsylvania Smell Identification Test (UPSIT). Blood biochemistry indexed nutritional state.

Thirty-eight patients were enrolled within three months of outpatient dialysis. Ages ranged from 29 to 80, with a mean of 51.2 ± 2.4 (SEM) years. Twenty-five patients were on hemodialysis and 13 on peritoneal dialysis. At enrollment, the mean score on the CCI was 10, out of a possible 26. The mean score on (UPSIT) was 31 (±1) out of a possible 40. Three patients (8.6%) were anosmic and 18 (51.4%) were hyposmic. Because performance on this test differs between the sexes and declines with age, test scores were converted to percentile scores, based on age and sex-matched norms. The mean percentile score was 31 (±4) and 22 of the 38 subjects (57%) had UPSIT scores less than the 25th percentile for their age and gender. Mean serum albumin and transferrin scores at enrollment were not abnormal, but 14/32 serum albumin (38%) and 7/12 (58%) of serum transferrin values were below the usual standard set for malnutrition.

CCI data collected at 3, 6, 9 and 12 months indicates that these scores may stay fairly constant. Percentile scores on the UPSIT were 24 (±4) at 6 months and even lower at 12 months (16 ± 6) following the onset of dialysis. Correlations of these measures with biochemical indices of malnutrition are in progress and may suggest multiple mechanisms of sensory dysfunction.

128. Ultrastructural analysis of developing circumvallate taste buds from rats

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Previous studies of the gustatory system using light microscopy have indicated that a significant amount of development takes place during the first 2 weeks of postnatal life in rodents. Most developmental studies have focused on taste buds of the fungiform papillae. We are now interested in elucidating the ultrastructural changes found in developing taste buds of the circumvallate papillae of rats. Animals aged 0–14 days were examined using light and transmission electron microscopy. At day 0, developing taste buds are present near the basal lamina in the gustatory epithelium. These developing taste buds contain undifferentiated cells that cannot easily be distinguished as type I or type II cells. The undifferentiated cells are moderately electron-dense and contain polyribosomes and intermediate filaments. Using standard ultrastructural criteria, we have also identified electron-lucent type I and electron-dense type II cells as early as day 0. We have observed large accumulations of glycogen in electron-dense cells at birth. The first taste pores do not appear until day 2, and these taste buds are much smaller, contain fewer cells and appear less organized than adult taste buds. At day 2, only a few of the taste cells have assumed the spindle-shape that is characteristic of the taste cells found in adult taste buds. The most common synapses found in developing taste buds are afferent synapses. These synapses are associated with both type I and type II cells, but most often form connections with type II cells. There are also neuro-neuronal synapses present at day 1 as well as efferent synapses. Developing taste buds contain many neurons that apparently form a nerve plexus at the base of the taste bud. This nerve plexus is most apparent at days 1–4. A sequential course of events is difficult to establish because taste buds do not develop at a synchronized rate. At any given day during the first two weeks of postnatal life, taste buds at different developmental stages can be observed. Our studies provide evidence that taste buds of the circumvallate papilla may develop much earlier than previously thought and mature faster than taste buds found in the fungiform papillae.

This work was supported by NIH grants DC00285 and DC00244.

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129. Taste preferences in the emerging Indonesian food market

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Indonesia is a rapidly growing country with potential for importing much high-value processed and semi-processed food in the near future. A study of adult food consumers in the most affluent consumer category ('A+') was conducted in March 1996 and consisted of three structured discussion sessions (focus groups) of 10–12 participants and a questionnaire given to 114 adults who had not been in the focus groups. Qualitative information was obtained about the following: shopping habits; food preparation in the home; social eating; snacking; typical meals; attitudes to religious strictures concerning food; and attitudes to branding, labelling, canned, fresh and frozen foods, traditional foods, healthy food, price of food, packaging, restaurants and imported foods. The questionnaire provided numerical data on the following: Spending on food, shopping habits, favourite foods (breakfast, lunch and dinner); favourite sweet, sour and salty foods; favourite drinks; and traditional and non-traditional meals. The priorities people put on attributes of a range of foods when making purchasing decisions was measured. Taste was one of the main drivers. Twenty-two Western-style flavours were rated for appropriateness for use in three types of food: confectionery, ice cream and biscuits (cookies). The flavours were considered appropriate for some foods but less so for others: e.g. Ginger was assessed as very appropriate for confectionery but not for ice cream or biscuits. Chocolate scored highly appropriate for all three foods. Watermelon and papaya was considered inappropriate for all three. Some interesting effects emerged when the data was partitioned by age, religion and gender. This data should help food companies prepare their market entry or expansion strategies in this important Asian food market, and emphasizes the importance of understanding cross-cultural sensory perceptions and preferences when doing so.

130. Antibody to fasciclin II recognizes a subset of olfactory receptor axons and glomeruli during development in *Manduca sexta*

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The astonishing ability of animals to detect and discriminate olfactory signals in the environment depends entirely upon the specificity of connections between olfactory receptor axons and their targets in the brain. Recent studies in mice have indicated that olfactory receptor molecules in the membranes of receptor neurons probably play a role in guiding axons to their appropriate glomerular targets, but that these molecules are unlikely to be the sole guidance mechanism (Mombaerts *et al.*, 1996). In developing moths, we have found that an antibody (Carr and Taghert, 1988) that binds to fasciclin II, a cell adhesion molecule in the IgG superfamily, selectively recognizes a subset of olfactory receptor axons throughout the period of glomerulus development, which lasts ~1 week; staining in the antennal (olfactory) lobe is absent before and after glomeruli form. The labeled axons project to 12–15 glomeruli, which are interspersed among lightly labeled and unlabeled glomeruli. To determine whether the same glomeruli stain from animal to animal and acro ss developmental stages, we currently are performing 3-D reconstructions of images from optical sections taken through labeled brains examined as wholemounts in the laser scanning confocal microscope. Preliminary examination reveals that certain uniquely identified glomeruli have reproducible staining patterns during the entire period of their development. Thus, fasciclin II may play a role in guiding a subset of olfactory axons to their targets in the moth's brain. Experimental perturbations of fasciclin II during ingrowth of receptor axons will provide a direct test of this hypothesis.

Supported by NIH grant # NS20040 to LPT.

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131. MHC and olfactory communication in humans

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The major histocompatibility complex (MHC) is a gene cluster that is involved in antigen recognition and tolerance induction in the anticipatory immune system of vertebrates. The most prominent feature of the MHC, its outstanding genetic diversity seems to be selectively maintained in contemporary natural populations, although the strength and nature of this selection are currently unclear. Due to the critical role played by proteins encoded by the MHC in immune recognition, it is generally assumed that some form of parasite- and pathogen-driven selection takes place, namely negative frequency dependent selection and overdominance. However, the high degree of heterozygosity found in natural populations of most species seems to be promoted not only by overdominance but also by non-disease-based selection such as mating preferences. Results which confirm that MHC-associated olfactory signals in mice contribute to imprinted mating preferences which serves to promote heterozygosity stem from studies of inbred strains as well as semi-natural populations. First hints of similar effects of the MHC in humans will be presented. They come from four types of studies: (i) experimental studies on the expression of HLA-associated urine odors using trained rats in a computer-controlled olfactometer; (ii) gas-chromatographic analyses of HLA-associated profiles of volatile substances in pooled human urine samples; (iii) studies in natural settings on the occurrence of HLA-associated body odors and social effects of HLA-similarity; and (iv) experimental studies on the expression of HLA-associated body odors using humans as subjects.

This work was supported by the Volkswagen Foundation.

132. Using olfaction to study memory

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In 1972, Walle Nauta wrote that the olfactory system provides a

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'flow diagram' for studies of sensory–limbic interactions. Nauta's insight is proving very useful for our understanding of memory mechanisms through a model of olfactory–hippocampal pathways. This presentation will focus on three stages of odor memory processing in rodents: the olfactory cortex, the parahippocampal region, and the hippocampus. A combination of neuropsychological and neurophysiological studies indicates that each stage makes a unique contribution to odor memory. The olfactory cortex, composed of piriform and orbitofrontal areas, is critical to many forms of odor learning and memory, and neurons in these areas encode specific odors and their significance, as well as associations among odors. The parahippocampal cortical region is critical to extending storage for single odors beyond immediate memory, and can support simple odor recognition even in the absence of normal hippocampal function. Correspondingly, neurons in the parahippocampal region encode and store specific odor representation. The hippocampus itself is not critical to learning and memory for single odors, but is critical for forming representations of relations among odor memories, and for the capacity to express these representations in many novel situations. Results from newly developed odor paired-associate and serial order learning paradigms show that rats with hippocampal damage can acquire odor memories, but cannot express them when challenged to make inferences based on previous learning. Correspondingly hippocampal neuronal activity reflects abstract relations between odor items or between odors and other information. These studies that exploit the exceptional qualities of olfactory learning and memory in rodents help to clarify the nature of distinct forms of memory representation in animals and humans.

133. Clinical evaluation of olfactory disorders

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Odorant molecules interact with receptor neurons in the olfactory epithelium located in the upper part of the nasal cavity leading to a sensory message which is conveyed, via the olfactory bulb, to cerebral structures. Olfactory disorders may result from disturbances at different levels of this sensory process. Lesions or anatomic abnormalities of the nasal cavity may limit odorant molecules access to the sensory epithelium. Abnormalities of the olfactory epithelium, transection of olfactory nerve fibers or lesions of central olfactory pathways may also lead to a decrease of olfactory perception. In the present study we have associated olfactory threshold determinations and rhinomanometric measurements with computed tomographic data and endoscopic observations. More than 600 subjects with or without olfactory complaints were examined. We present some examples to illustrate the usefulness of this converging approach for the diagnosis of olfactory disorders. The olfactory acuity was assessed by measuring detection thresholds for five odorants using a computer-controlled olfactometer (Eloit and Trotier, 1994). Rhinomanometric measurements of the nasal permeability to airflow allows the detection of functional abnormalities of the nasal cavity. Computed tomography provides detailed structural information about the nasal cavities, particularly in the superior parts, the cribriform plates and the anterior cranial fossa.

All these data are currently analyzed to examine to what extent correlations can be established between olfactory sensitivity, nasal permeability and anatomy of the nasal cavities. We are particularly interested in determining how the structural organization of the nasal cavities controls the dynamics of the nasal airflow towards the olfactory cleft where the sensory epithelium is located.

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134. Odor identification and recognition in the oldest-old

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An examination of odor recognition among the oldest old was performed on 21 community-dwelling centenarians aged 100–115. Participants were given an odor threshold task with phenethyl alcohol and menthol, an odor memory task, odor recognition task, and an odor identification task. Other measures for evaluating the participants included the Geriatric Depression Scale, Wechsler's Vocabulary module, the EPAT and Wolinsky's Nutritional Measures. Results demonstrate better than expected performance on the thresholds, memory and identification tasks, approximating the reported performance of a 60- to 70-year-old.

135. Glutamate and synaptic plasticity at mammalian primary olfactory synapses

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Chemoanatomical studies suggest that glutamate is the major excitatory transmitter at mammalian olfactory nerve (ON)–mitral (Mi) cell synapses. In agreement with this, our electrophysiogical studies in rat olfactory bulb slices demonstrate that ON-evoked excitation of Mi cells is mediated by glutamate. ON shocks elicit distinct early and late spiking components separated by a brief epoch of inhibition. The early and late spiking are blocked, respectively, by AMPA/KA and NMDA receptor antagonists. Field potentials recorded simultaneously in the glomerular layer (GL) also consist of early AMPA/KA-dependent and late NMDA-dependent components that paralleled the early and late spiking. These and other results suggest that ON-evoked field potentials in the GL reflect monosynaptic excitation of Mi apical dendrites, and trigger the early and late spiking responses to ON shocks. Consistent with these results, computer modeling showed that the presence of both AMPA/KA and NMDA receptors on Mi apical dendrites were required to generate both the early and late Mi spiking responses to ON shocks.

Since most forms of synaptic plasticity involve NMDA receptors, the presence of glutamate and NMDA receptors at primary olfactory synapses raise the possibility that the strength of neurotransmission at these synapses is modifiable. Our results support this notion. Brief, tetanic ON stimulation *in vitro* induced robust LTP of the late, NMDA spiking; the early AMPA/KA spiking was unaffected. Both the induction and expression of LTP are NMDA receptor-dependent. Modeling experiments disclosed several potential mechanisms underlying the selective LTP of NMDA receptor-dependent spiking: (i) increased AMPA/KA

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conductance; (ii) increased NMDA conductance; (iii) increased presynaptic glutamate release; and (iv) decreased GABA-A receptor-mediated granule to Mi cell inhibition. These mechanisms are currently under investigation.

Taken together, our findings demonstrate that communication from the ON to Mi cells exhibits a novel form of plasticity whereby high frequency synaptic activity induces selective LTP of NMDA receptor-dependent spiking of Mi cells. Activity-dependent plasticity may provide a mechanism for rapid, persistent facilitation of Mi cell responses to odors, and play a role in formation and/or storage of odor-specific memories.

Support: NIH DC00347, DC03195, NS29218, NS29635 and NS36940.

136. Mechanisms underlying Ltp at olfactory nerve-mitral cell synapses: electrophysiology and computational modeling

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Activity-dependent modification of excitatory transmission at the first synapse in the rat olfactory bulb was studied with neurophysiological techniques *in vitro* and computational modeling. Single olfactory nerve (ON) shocks elicited a characteristic response in mitral (Mi) cells consisting of distinct, early and late spiking components separated by a brief inhibitory epoch. Simultaneously recorded field potentials evoked in the glomerular layer (GL) consisted of an early (N1; peak 9–13 ms) and late components (N2; peak 50–150 ms) that paralleled the early and late spiking responses. Both the early and late spiking and GL field potentials were blocked respectively by AMPA/KA and NMDA receptor antagonists. These and other results suggest that N1 and N2 reflect monosynaptic excitation of mitral apical dendrites, and trigger the early and late spiking responses to ON shocks. In agreement with these results, computer modeling showed that the presence of AMPA/KA and NMDA receptors on mitral apical dendrites replicated the early and late spiking responses.

Brief, tetanic ON stimulation induced LTP of the late, NMDA spiking while the early AMPA/KA spiking was unaffected. Unexpectedly, tetanic stimulation induced LTP of the early component (N1) of the ON-evoked GL field potential. In some experiments, LTP of N1 was also accompanied by LTP of N2. Modeling simulations disclosed a possible mechanism underlying selective LTP of NMDA mediated spiking: increased AMPA/KA receptor-conductance increased NMDA-, but not AMPA/KA-mediated spiking. This is due to interneuronal GABAergic inhibition which normally truncates AMPA/KA-mediated spiking and prevents the increased conductance from generating additional spiking. However, after increased AMPA/KA-mediated depolarization, the truncating inhibition fails to return the membrane potential back to resting potential. As a result, the NMDA receptor-dependent depolarization arises from a higher potential, and triggers increasing spiking because of the reduction of the voltage-dependent block of NMDA receptor channels by Mg^{2+}. Taken together, these results suggest that brief, tetanic stimulation induces robust, LTP of mitral responses to sensory inputs. Activity-dependent plasticity may provide a mechanism for rapid, persistent facilitation of mitral cell responses to odors, and play a role in formation and/or storage of odor-specific memories.

Supported by NIH DC00347, DC03195, NS29218 and NS29635.

137. Amino acid immunocytochemical characterization of the rat vomeronasal organ

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The rat vomeronasal organ (VNO) is a blind-ended, tubular structure, 6–7 mm long, containing a thick medial sensory epithelium (M) and a thinner lateral non-sensory epithelium (L). We used amino acid pattern recognition (Marc *et al.*, 1995) to study differences in the sensory and non-sensory walls of the VNO. Rabbit polyclonal ABs against arginine (arg), D-aspartate (D-asp), citrulline (cit), GABA, glutamate (glu), glutamine (gln), glutathione (gsh), glycine (gly) and taurine (tau) were used. VNOs of male rats were dissected, fixed, embedded in plastic and serially sectioned. The amino acids were visualized with primary antibody and nanogold secondary antibody with silver intensification. Several striking differences in staining were noted in the organ. (i) In general, epithelial tissue stained more intensely with all the ABs than did extra-epithelial tissue. (ii) Whereas the L wall and superficial luminal layer of the M wall stained relatively uniformly with many of the ABs, the deeper basal compartment of the M wall showed great variation among several of the ABs. Tau was one of the most striking in that the basal half of the M wall showed a distinct mosaic of cell profiles that ranged over a broad spectrum of staining intensity. Although less vivid, glu, gln and gsh staining revealed discernible, lighter profiles in the basal layer which contrasted sharply with the uniformly darker staining lining the lumen. Striae from these light profiles were traceable to the luminal surface. (iii) Arg staining was notable in that it was a unique marker for the microvillar surface of the M wall. These results suggest that the sensory wall of the VNO is composed of cells with diverse amino acid immunoreactive profiles, especially notable are differences in the luminal and basal compartments. Args unique reactivity in the microvillar region is provocative since it is a NO precursor and its location is suggestive of a role in transduction.

We thank R. Marc for the antibodies and C. Burns for her assistance. Partially supported by NIH DC 01418.

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138. Identification and characterization of human fructose or glucose taste variants with hypogeusia for one monosaccharide but not for the other

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Human psychophysical functions for sweetness are similar for sucrose and fructose, but different for glucose, and suggest different mechanisms for fructose and glucose. *Drosophila*

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behavioral and electrophysiological data are similar to the human data and indicate separate receptor cell mechanisms for the monosaccharides. Moreover, fructose 'nontasters' (NTs) and glucose NTs have been identified in two *Drosophila* species. Identification of human NTs would confirm separate mechanisms and could lead to identification of proteins in human sweet taste by molecular genetic techniques. To identify human NTs, we first obtained responses for sucrose, fructose and glucose from 20 subjects. They tasted seven concentrations of each sugar (2–128 mM), paired with water, and indicated the sweeter of each pair. Functions for Recognition Indices (RI) (proportion of subjects recognizing the sugar as sweeter) were similar for sucrose and fructose and different for glucose; this result agrees with the previous studies and supports different mechanisms for the monosaccharides. At 128 mM, RIs for all three sugars were 1.0; this result is consistent with the monogeusia reported by Breslin *et al.* for concentrations higher than tested here. Eleven rising-phase concentrations (10–35 mM fructose, 10–90 mM glucose) then were tested on 32 subjects. A statistically significant interaction indicated different regression slopes and supported different monosaccharide mechanisms. From these data, Positive Identification values (PID) (lowest concentration at which the sugar always was judged sweeter than the water) were determined for each subject. The fructose log(PID) and glucose log(PID) data were not well correlated; thus separate mechanisms were supported further. Next, NT traits were defined by log(PID)s ±2 SD above the mean for one sugar while the PID for the other remained within 1 SD of the population mean log(PID). Ninety two subjects were screened to identify 12 glucose NTs and four fructose NTs. Two glucose NTs and three average subjects were tested in six additional sessions. The NTs showed an experience-induced change: there was a statistically significant reduction of glucose PIDs, but not of fructose PIDs. No change occurred in PIDs of the average subjects for either sugar.

Supported by NIH DC/OD02663.

139. Odor representations in the bee brain

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We investigated representations of odors in the CNS of honeybees by calcium imaging *in vivo*. In insects, the antennal lobe (AL) is the first neuropil that processes olfactory information. It is the structural and functional analogue of the olfactory bulb in mamals. The AL consists of 156 glomeruli, that receive input from ~60 000 chemoreceptor cells. After staining the brain with the calcium sensitive dye Calcium-green-AM we were able to record spatial activity patterns upon odor stimulation for several hours.

Each tested odor evoked a specific activity pattern in the AL with single activity foci corresponding to distinct glomeruli. Varying odor concentration led to almost the same patterns with different signal amplitudes. Mixtures of two or more components resulted in patterns containing elements of all single components. Additionally we observed nonlinear effects, in particular inhibitory interactions. These seem to increase with the number of components in the mixture.

By comparing the odor evoked activity maps of both ALs in single individuals we found bilateral symmetric patterns. This shows that genetic instructions are involved in forming the 'odotope map' of the AL. When comparing odor maps between individuals we found a high degree of similarity only for the pheromone component citral. For ordinary odors the degree of similarity was smaller indicating possible effects of individual experience on the odor representations.

We also investigated odor evoked activity in higher centers of the brain namely the calyces and alpha lobes of the mushroom bodies. At this level we could not find as clearly distinct patterns for different odors as in the AL. As the mushroom bodies are involved in associative learning imaging experiments might reveal changes in activity patterns induced by olfactory learning.

140. Tyrosine phosphorylation down-regulates a potassium current in rat olfactory bulb neurons and a cloned Kv1.3 channel

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We are studying the regulation of ion channels by protein tyrosine kinases (TKs) using olfactory bulb neurons (OBNs) and a cloned voltage-dependent potassium channel, Kv1.3 as models. Rat OBNs, which express Kv1.3 channels, had whole-cell outward currents that were suppressed by picomolar quantities of margatoxin and showed a slow inactivation that increased over a 10 min period. These same pharmacological and kinetic properties were found also in human embryonic kidney (HEK 293) cells transfected with rat Kv1.3 cDNA. The insulin receptor TK was detected in the OB by Western analysis. Exogenous application of insulin was found to suppress whole-cell outward current in all OBNs tested. Perfusion of the non-receptor Src kinase likewise suppressed outward current. Current was not suppressed with heat-inactivated Src or when ATP was excluded from the pipette solution. The membrane permeant tyrosine phosphatase inhibitor, pervanadate, was found to suppress current in a subset of neurons, implying the presence of an endogenous tyrosine kinase in these neurons. Using site-directed mutagenesis of six tyrosine residues contained within good recognition motifs for tyrosine phosphorylation, we constructed conservative Y to F mutations in the Kv1.3 cDNA and expressed the Kv1.3 channels in HEK 293 cells. We found that Tyr449 is a target for both the pervanadate- and Src-induced suppression of Kv1.3 current; Tyr111, 112 and 113 are also important for modulation by pervanadate; and Tyr137 is a target for modulation by Src. In summary, tyrosine phosphorylation of Kv1.3 and related channels may be involved in the modulation of OBN excitability.

Supported by research and training grants from the NIH.

141. Does Olfactory Marker Protein participate in olfactory neurogenesis?

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Olfactory Marker Protein (OMP) is a phylogenetically conserved, 19 kDa, acidic, soluble protein found abundantly in mature

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olfactory sensory neurons. Its function has been enigmatic although recent evidence from studies on OMP null mice suggests that neurons lacking OMP exhibit altered physiological activity, including prolonged onset and recovery kinetics following stimulation (Buiakova *et al.*, 1996). We have reported increased expression of OMP in individual surviving sensory neurons that have been deprived of their target, the olfactory bulb (*Soc. Neurosci. Abstr.*, **22**, 653). Because olfactory epithelia deprived of their target also exhibit an increased rate of cell division we investigated the effect of recombinant OMP on cell division in organotypic cultures of fetal rat (embryonic day 19) epithelium grown for 3 days *in vitro*. After 3 days, cultures were given a 1 h pulse of a mitotic marker, bromodeoxyuridine (BrdU), fixed and prepared for immunohistochemistry to determine the number of proliferating cells. We found a dose-dependent increase in the number of BrdU-positive cells/100 mm length of epithelium. The number of labeled cells increased incrementally, reached a plateau at 20 nM OMP/ml culture medium, 50% higher than in cultures with no OMP added, and remained at that level at 50 and 100 nM doses. Controls included trypsinized OMP and addition of equivalent volumes of tris buffer lacking OMP. These results, taken together with previous studies on several growth factors (Farbman and Buchholz, 1996) indicate that regulation of neurogenesis in olfactory tissue is a multifactorial process and that OMP may play a role.

Supported by NIH grants # R01 DC02126 and P01 DC00347.

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142. fMRI study of taste cortical areas in humans

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We used 3 Tesla fMRI with echo planar imaging to map taste projections in the human. In order to retrieve brain activations, we introduced a psychophysical measurement of the taste sensation in the magnet with an amagnetic finger span device producing an on line time intensity profile. In order to interpret a number of activations in different regions of different subjects (n = 13) for a number of stimuli (n = 6), we used multidimensional analysis.

Activation maps allowed a synthesis between clinical observations and electrophysiological studies in the monkey. Activations were found surrounding and buried in the Sylvian fissure; the upper part of insula, the frontal operculum, the foot of pre and post central gyri were usually activated. Moreover, we could describe, for the first time, a lateralized associative projection located in the lower part of the dominant hemisphere of the subject (n = 10). We also observed activations in anterior cingulate gyrus, centromedial thalamus, hippocampal structures and other areas related to emotional or cognitive processes.

Meanwhile, 35 subjects were submitted to a 10 week familiarization to a series of unknown stimuli chosen to sample neophobic and non-neophobic stimuli, semantically or not semantically described stimuli. Measurements of isointense concentrations, magnitude estimates and hedonic values were assessed repetitively twice a week, 3 fMRI were performed, before, during and after familiarization, for 6 of these subjects.

Psychophysical data showed a relationship between extinction of neophobia and the intensity of the perception whereas the hedonic factor seemed to partly depend on the existence of a semantic label.

fMRI results are tentatively interpreted with respect to the biological and cognitive significance of the stimuli unraveled by the psychophysical experiment.

143. Glutamate neurotransmission in the primary gustatory nucleus of the goldfish

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The vagal gustatory portion of the nucleus of the solitary tract in goldfish takes the form of a large, laminated lobe of the midmedulla called the Rvagal lobeS. The vagal gustatory fibers, innervating taste buds on the specialized palatal organ, form a distinct, large nerve root penetrating the ventrolateral portion of the lobe and terminating in distinct superficial layers. The anatomical arrangement of this root and the overlying sensory layers is conducive to an *in vitro* slice preparation for examining the physiology and pharmacology of the vagal gustatory afferent system. In this preparation, bipolar electrodes are used to stimulate incoming primary gustatory afferent fibers; evoked population responses are recorded from the various sensory layers. These evoked responses show complex waveforms with negative peaks at 3 and 5 ms post-stimulus. Repeated electrical stimulation of the nerve root shows facilitation of responses within 50 ms of the first stimulus, but suppression thereafter.

Application of low concentrations of non-NMDA glutamate antagonists, DNQX or NBQX, abolish or greatly diminish the short latency responses evoked by nerve stimulation. This indicates that the immediate post-synaptic responses in the primary gustatory nucleus rely on excitatory amino acid transmitter receptors. In addition, in Mg^{2+}-free solutions, a slower, negative-going evoked response is evident. The time course of this slower and smaller waveform is ~50 ms. These longer potentials are abolished by administration of APV, a known NMDA receptor antagonist but are unaffected by the non-NMDA antagonists. The coincidence in time course between the NMDA response and the facilitation with fast, repeated stimuli indicates that this facilitation may be mediated via an NMDA mechanism.

Radioligand binding studies confirm the presence of both NMDA and non-NMDA receptors in the sensory layers of the vagal lobe. In summary, sensory processing in this primary gustatory nucleus entails both rapid responding, non-NMDA excitatory amino acid receptors and more sustained NMDA components.

144. Symposium: Mechanisms of sensory transduction—olfaction

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Keeping pace with the rapid advances made in olfactory

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transduction, this symposium will bring together representatives from leading laboratories investigating physiological aspects of olfactory transduction. The entire range of physiological data will be covered, from ion channel biophysics to single cell recordings, whole epithelium recordings and calcium imaging. The focus will be on a mechanistic understanding of the first steps in olfactory transduction, including second messenger systems, odor sensitivity, and adaptation. The symposium will present not only the current level of our physiological knowledge, but will attempt integrate this information with recent advances in biochemistry and molecular biology to develop a comprehensive, state-of-the-art appreciation of olfactory transduction.

145. Activated microglia increase in the rat olfactory bulb after early unilateral naris occlusion

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Physiological cell death ensures that appropriate functional and trophic balances are maintained among interacting cell populations in the developing brain. Removal of the remaining cellular debris is believed to involve the phagocytic actions of microglia. Enhanced cell death has been observed in the olfactory bulbs of rats in which airflow through one side of the nasal passage has been blocked on the day after birth (P1). The cell loss ultimately contributes to an ~25% reduction in the volume of the bulb by P30. Previous studies using isolectin B_4 histochemistry reported that the bulb is densely populated by microglia, supporting the hypothesis that there is considerable ongoing cell death. However, no changes in the density of microglia were observed after unilateral naris occlusion, possibly due to high resting levels. To examine the dynamics of microglial responses to alterations in patterns of cell survival, we have used an antibody to the CR3 complement receptor, which becomes upregulated on the surface of *activated* microglia. Neonatal rat pups had a single naris occluded on P1 and brains were removed 10, 20 or 30 days later. Immunocytochemical labeling was performed using a monoclonal antibody (Serotec) to CR3. Initial results reveal increased levels of activated microglia in the granule cell and deep glomerular layers of experimental olfactory bulbs, precisely the regions known to be areas of increased cell death after naris occlusion. Further work is being done to clarify these results and to examine normal developmental patterns of microglial activation.

Supported by Grant DC-00338 from the NIDCD.

146. Regulation of Na,K-ATPase in the mammalian olfactory mucosa by aldosterone

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Na,K-ATPase maintains the ionic gradients necessary for resting and odorant-induced ion flux across the olfactory mucosa. In addition, this enzyme is likely necessary for secretion and modification of olfactory mucus from Bowman's glands. In many other tissues Na,K-ATPase activity is modulated by corticosteroid hormones. Following establishment of baseline data, the effect of alteration of serum aldosterone levels on olfactory Na,K-ATPase activity in the guinea pig was investigated. A high sodium/low potassium diet offered ad libitum for 5 days was utilized to significantly decrease serum aldosterone in male Hartley guinea pigs compared with controls. An injection of aldosterone (10 μg/100 g body wt) 24 h prior to sacrifice resulted in significant elevation of serum aldosterone over that obtained with the high sodium/low potassium diet. Binding of radiolabelled ouabain, a specific inhibitor of Na,K-ATPase, was significantly elevated in the olfactory mucosa of aldosterone-injected versus sham-injected animals. Serum electrolytes were elevated in animals on the high sodium/low potassium diet and unaltered by the administration of exogenous aldosterone. The enhancement of binding in the aldosterone-injected animals, therefore, did not appear to reflect an alteration of serum electrolytes per se. The results of these experiments are consistent with the hypothesis that aldosterone increases the activity of Na,K-ATPase in the mammalian olfactory mucosa.

147. The effects of topical anesthesia on oral burning in burning mouth syndrome

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Burning mouth syndrome (BMS) is an oral pain disorder of uncertain origin. We postulate that physiological factors attributable to either central or peripheral pain mechanisms may play a role in the oral burning of BMS. In order to investigate which mechanisms may be involved, we administer a topical anesthetic test to patients complaining of oral burning. Prior to anesthetic application each patient provided a baseline intensity rating, on a scale from 0 (none) to 9 (maximum), for oral burning, taste dysgeusia and two chemical stimuli, 1.0 M NaCl and 1.0 M sucrose. The patient then rinsed with 15 ml of 1% dyclonine. HCl for 2 min. Following a distilled water rinse, each patient reassessed the intensities of the chemicals, oral burning and dysgeusia once every 2 min for 10 min. A total of 33 patients (9 male and 24 female, average age 60 years) and 13 age-comparable controls are included in this analysis.

The anesthetic reduced the perceptual intensity of both chemicals in these patients on four out of five post-anesthesia trials ($P < 0.01$). In addition, the BMS cohort included 12 patients whose burning increased ($P < 0.001$), 14 patients whose burning did not change, and seven patients whose burning decreased ($P < 0.001$) after anesthesia. baseline dygeusias ($n = 13$) decreased in intensity ($P < 0.001$) after anesthesia, suggesting BMS dysgeusia is related to the activation of peripheral taste mechanisms. The results also suggest that BMS oral burning may be a disorder of peripheral pain pathways in some patients.

Supported by NIH Grant DC00168.

148. Developmental analysis of olfactory neurogenesis in the antenna of the moth *Manduca sexta*: role of ecdysteroids

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The antenna of the moth *Manduca sexta* bears over 100 000 olfactory sensilla segregated into different morphological and

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functional classes. Each sensillum arises from a single sensory mother cell that divides to produce two neurons and three support cells (thecogen, trichogen and tormogen). Neurogenesis occurs early during the adult development, between days 1 and 3 after the pupal ecdysis and is coordinated by a rise in ecdysteroids levels.

Using *in vivo* BrdU incorporation, we show that the onset of neurogenesis occurs in a spatial/temporal wave that correlates with the establishment of sensillar spatial domains. The wave of mitotic activity starts on day 1, at the edges of each annulus which corresponds to the adult position of the long pheromone-sensitive hairs. Mitotic activity continues until day 3 and moves towards the center of the annulus where the short general odorant-sensitive hairs will arise. A similar spatial/temporal wave of DNA replication is observed after apolysis when the antennal epithelium detaches from the pupal cuticle. This second wave starts at day 5, ends at day 7 and represents an increase in ploidy level among the trichogen support cells. In addition, before apolysis, a similar spatial/temporal pattern is observed for the expression of ecdysone receptors (EcRs), suggesting that EcRs might be involved in the regulation of the neurogenic responses to the ecdysteroids. In order to further study the role of ecdysteroids in the regulation of the neurogenesis, we have developed culture experiments and are currently looking at the antennal BrdU pattern in presence or absence of ecdysone agonist.

Supported by NIH NIDCD DC-00588.

149. Evolution of the olfactory receptor gene family

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The chemospecificity of olfactory sensory neurons is supposed to be determined by their specific receptor types which are capable of interacting with structurally distinct odor molecules. In mammals, the repertoire of olfactory receptor types is extremly large consisting of as many as a thousand different subtypes, whereas in fish the number of receptor types appears to be considerably smaller. This difference may reflect a phylogenetic trend of augmenting the receptor repertoire, however, it is also conceivable that the small repertoire of receptor types in fish may reflect the odor complexity in aquatic versus terrestrial environments. To gain some insight in the phylogenetic relationship and functional implication of olfactory receptor diversity, receptor-genes were studied in amphibia (*Xenopus laevis*) which are not only ranked at an intermediate position between fish and mammals on a phylogenetic scale but are capable of smelling airborne as well as water-soluble odorants. It was found that Xenopus possess a gene repertoire encoding two distinct classes of olfactory receptors: one class related to receptors of fish and one class similar to receptors of mammals. Although originating from common ancestors, both receptor classes apparently evolved differently. Exploring the olfactory receptor genes from *Latimeria chalumnae* revealed that the 'living fossil' already disposes the two receptor classes; however, the diversity of class II receptors is much smaller than in amphibia. Analysis of receptor genes in species such as lungfish, lampreys and marine mammals will contribute to better understand the phylogenetic mechanisms underlying diversification of olfactory receptor genes during vertebrate evolution.

Supported by the Deutsche Forschungsgemeinschaft.

150. Nerve dependency of developing sensory receptor cells

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The degree to which sensory cells without an axon are dependent on an afferent innervation for their formation and maintenance has been extensively debated in the last 100 years. In this paper we will review recent evidence (i) in the electrosensory system development and regeneration of axolotl and bony fish and (ii) inner ear development and taste bud development of control and mutant mice with a targeted disruption of neurotrophins and neurotrophin receptors. The most recent data in all systems, except the taste buds, suggest strongly that all sensory cells can be induced and undergo a morphological maturation in the absence of any innervation. In the ear, there is maturation of different types of hair cells in all sensory epithelia irrespective to the absence or presence of afferents or efferents. These studies also showed that the ganglia in the VII, IX and Xth cranial nerve which innervate the taste buds, are critically dependent on the neurotrophin BDNF and its cognate receptor TrkB for survival. In mutant mice lacking TrkB there is a rapid and complete loss of these ganglion neurons before they even reach the developing taste buds at embryonic day 12.5. Consistent with recent developmental data on induction of taste buds, many taste buds were present in embryonic TrkB null mutant mice. However, only few taste buds in a topologically restricted distribution remain recognizable in 16 day old mice, the longest time these mutants survive. Electron microscopic investigations show a limited degree of cytological differentiation of cells in the these taste buds, no taste pore, and the presence of extragemmal fibers to the apex.

Supported in part by NIDCD P50 DC00215-09.

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151. Cross adaptation and molecular modeling study of receptor mechanisms common to four taste stimuli in humans

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Psychophysical cross adaptation experiments were realized with two carbohydrates, sucrose (SUC) and fructose (FRU), and two sweeteners, acesulfame-K (MOD) and dulcin (DUL). Seven subjects were asked to match concentrations eliciting the intensity of a sucrose reference (30 g/l). Cross adaptation levels were calculated as the percentage isointense concentration change for a given stimulus before and under adaptation. On average, cross adaptation between SUC and FRU was low and symmetrical (~10%, $P < 0.10$). By contrast, cross adaptation between SUC and MOD was clearly asymmetrical: SUC adapted MOD significantly (24%, $P < 0.01$), but MOD failed to adapt SUC (2%, $P < 0.40$). Significant and symmetrical cross enhancement was observed between DUL and MOD (~–20%, $P < 0.02$), and also between

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SUC and DUL (~–15%, $P < 0.05$). In parallel, molecular modeling of the four tastants was performed in order to look for the 12 common binding motifs that were previously isolated on 14 other tastants (Froloff *et al.*, 1996). These motifs consist in hydrogen-bonding atoms and hydrophobic areas. SUC and FRU appeared to display numerous binding motifs (10 each) whereas DUL and MOD only displayed 4 and 5 distinct binding motifs resp. FRU and SUC share a majority of binding motifs and show mutual cross adaptation. Four motifs of MOD are found among the 10 motifs of SUC, which may explain why SUC cross adapts MOD but not *vice versa*. By contrast, DUL and MOD do not share any motif and do not cross adapt. The various molecular mechanisms that may be responsible for cross adaptation and/or cross enhancement are discussed in light of our results.

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152. Development and clinical application of a jet-stream olfactometer

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We have developed a new olfactometer named a jet-stream olfactometer. This olfactometer is using three compounds of T&T olfactometer as odorants, including phenyl ethyl alcohol, methyl cyclopentenolone, and isovaleric acid. The technique stimulating olfactory receptors is changed a blast method from a sniff method. It consists of an air pump, a cylinder with 1.5 ml of chemicals and a nozzle, can separate each parts. A stimulation time is 1.0 s. The purpose of this study is to identify the clinical usefulness of this olfactometer from the results of comparison with other olfactory function tests such as the smell identification test, T&T olfactometer, and olfactory detection threshold test. There were remarkable correlation between the results of this olfactometer and others. A T&T olfactometer has some problems such as an environmental pollution in the lavatory and the adherence of odorants on operator's clothes. As the odorants are closed up in a duralumin tube with absorbent tightly, they were improved. Our results indicate that a jet-stream olfactometer is very useful for a mean of olfactory function tests.

Supported in part by the Japan Rhinology Society.

153. Structure–activity relationships of nucleotides in the taste receptor cells of the fleshfly

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Nucleotides applied to the labellar chemosensillum of the fleshfly *Boettcherisca peregrina*, could induce impulse-discharge of the taste receptor cells. While 5'ADP evoked a large response of the sugar receptor cell (sugar response), 5'GDP evoked a large response of the salt receptor cell (salt response). On the other hand, the salt response to 5'ADP and the sugar response to 5'GDP were relatively small. The sugar response to 5'ADP was independent over a range of pH 5–9 and the salt responses to 5'GDP and 5'IDP were inhibited at pH 6–7. Hill plot analysis of dose response data showed that Hill coefficient value of 5'ADP was ~1 and that of 5'GDP was ~2. These indicated that the nucleotide receptor site of the sugar receptor cell was particularly different from that of the salt receptor cell. The nucleotide receptor site of the sugar receptor cell was specific to adenosine nucleotides (5'ADP, 5'AMP, 5'ATP). Ranking of potency was: 5'ADP > 5'AMP = 5'ATP. Not only 5'GDP, but also 5'NMPs and cNMPs (N=A,G,I) evoked the salt response. While the potency ranking of 5'NMPs was: 5'GMP > 5'IMP > 5'AMP, the dose response curves of cGMP, cIMP and cAMP were almost the same.

154. Morphological peculiarities of taste buds development in early ontogenesis in beluga (*Huso huso* L.)

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The goal of the work is to study the development of the receptor part of taste system in early ontogenesis of beluga (*Huso huso* L.). Electron-microscopic investigations allowed to discover the differences in taste buds morphological structure of extraoral and intraoral systems. Three types of taste buds was distinguished and each of them had its distribution on the taste zones. According to our dates there is definite distinction in morphometric values of intra- and extraoral beluga taste buds where the sizes of intraoral taste buds exceed extraoral ones that is apparently connected with their functional meaning. The diameter of taste buds gradualy increases by transition moment of larvae beluga on the full external feeding both inside and outside of the mouth except taste buds arranged on the barbells. It means that the size of the taste buds, connected with mouth region, increases, dependent on the ecology and biology of the species, and reflects characteristic peculiarities of beluga taste buds development.

Therefore extra- and intraoral groups of taste receptors of larvae beluga distinguish not only by their morphological peculiarities but they have different growth dynamics in ontogenesis.

155. Pattern of innervation of fungiform papillae by geniculate ganglion cells in the hamster

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Gustatory nerve fibers in the tongue are known from neurophysiological recording to branch to innervate a number of fungiform taste papillae. At the same time, individual papillae are innervated by numerous nerve fibers from the geniculate ganglion. The present study uses fluorescent labeling of single fungiform papillae to determine the number and topography of geniculate ganglion cells that innervate individual taste buds. Fluorescent labeling of sensory fibers also permitted their collaterals to be traced to determine the topographic relations of buds that share innervation. Tetramethylrhodamine dextran was iontophoresed through a micropipette into the pore region of single fungiform papilla buds in 12 animals; one papilla per animal was heavily labeled. After 4–7 days survival, sections of tongue and ganglia

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were examined (with epifluorescence and a green, G2A-Nikon filter cube) for labeled axons and ganglion cells, respectively. Seven animals were analyzed. In every animal, the injection site was confined to a single fungiform papilla. Labeled axons typically extended to the taste buds of a number of nearby buds. Labeled geniculate ganglion cells were entirely ipsilateral and varied in number (5–35 cells) depending upon the location of the injected papilla on the anterior tongue. The more anterior injections tended to label a larger number of cells than more posterior injections. In the geniculate ganglion, cells were widely scattered in the rostrocaudal and mediolateral axes. More anteriorly-injected buds had their ganglion cell sources located mainly in the ventral two-thirds of the ganglion. In general, however, there was no evidence of a topographic organization within the ganglion.

This research was supported by NIH grant DC01091.

156. Measures of sensitivity for estimation tasks

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Garriga-Trillo developed a sensitivity measure, M, based on the proportion of correct ordered responses which could be applied to quantitative responses in magnitude tasks. This measure has been validated using Stevens' exponent, the correlation between the natural logarithm of the stimulus magnitude ($\ln S$) and the natural logarithm of the corresponding response ($\ln R$), d' and the proportion of correct responses and applied across different modalities. Although M seems to be quite robust as a sensitivity measure and as a measure that probably reveals what the subjects do in a magnitude task it does not differentiate between the correct order of the responses when the two stimuli are quite close or when they are farther apart. To solve these issues a new measure of sensitivity, M_R, was defined considering a weighted proportion of correct ordered responses that takes into account the distance between stimuli in the chosen comparison pair. This work studies the distribution of M and M_R considering olfactory data ($n = 270$) from 63 normal subjects. The distributions were negatively skewed and non-normal. A criterium was set to determine very low, low, average, high and very high olfactory sensitivity in normal subjects eventough the high scores obtained could indicate that when people quantify the physical continuum most of them do it in the right way, when considering just an ordinal measure.

This research was supported in part by the Spanish Government's DGICYT grant PB94-0389 and Cooperaci\n Iberoamericana grant 020484.

157. Normative data, verification of olfactory bulb and tract volumes

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We sought to verify magnetic resonance (MR) volumetric techniques and define normative values for the olfactory bulbs and tracts (OBT) across various deciles of age. Twenty-six subjects who underwent 3 mm thick surface coil magnetic resonance imaging had unilateral OBT volumes measured by two individuals in two separate sessions on an ISG Technologies workstation. Two phantoms were used to determine accuracy using water displacement techniques. Within and between individuals, correlation coefficients and Spearman rank order correlations ranged from 0.858 to 0.977 for OBT volumes. Using two phantoms the maximum variation in measured volume versus water displacement ranged from 0.9 to 4.8%. The overall error for large and small phantoms was 5.7%. Phantom interobserver variability ranged from 0.1 to 3.7%.

Mean OBT volumes peaked in the 4th decade of life (mean 157 mm^3, SD 23.3) and then declined into the a nadir in the 7th and 8th decades (mean 118.4 mm^3, SD 28.7). These findings were present in men and women with mean volumes larger in men. These volumetric measurements varied across age in a manner similar to that observed for UPSIT scores. Statistically significant differences were noted between OBT volumes in the fourth decade and seventh and eighth decades.

Supported by NIDCD research grant PO1 DC 00161, NIH.

158. Investigation of expression of olfactory metabolic enzymes and heat shock protein 70 in the young, adolescent and aged male Long–Evans rat

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We have shown that the olfactory mucosal toxicity caused by the neurotoxicant 3,3'-iminodipropionitrile (IDPN) was dramatically increased in aged (10–21 months of age) compared with young adult (8–10 weeks of age) male rats. Age-related alterations in the expression of xenobiotic metabolizing enzymes may provide a basis for this observation. In the present study we examined the expression of various metabolic enzymes known to be present in the rat olfactory mucosa and believed to be involved in the metabolism of IDPN in rats ranging from 4 weeks to 9 months of age. In addition, because of the known protective effect of the heat shock proteins against the toxicity of various drugs, the expression of heat shock protein 70 (HSP 70) was also evaluated. Phase I enzymes of interest include CYP 2E1 and the liver form of the flavin-containing monooxygenases, FMO-1, as these enzymes have been implicated in the bioactivation of IDPN. Northern blot analysis suggests that the expression of FMO-1 may increase with advancing age. The expression of microsomal epoxide hydrolase, a Phase II enzyme, increased after puberty, but appeared to be unaffected subsequently. In contrast to the results of previous studies in human olfactory mucosa, HSP 70 expression, previously reported to decrease in aged human olfactory mucosa, appears to remain constant across this age range in rats. These results suggest that the enhanced toxicity of IDPN in older rats may be attributable to increased levels of FMO-1, an enzyme likely responsible for the formation of the *N*-hydroxy metabolite of IDPN, which has been demonstrated to be a more potent neurotoxicant and olfactory toxicant than IDPN itself.

Supported in part by AG 13837.

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159. The effects of complex mixtures on odorant-receptor binding and olfactory transduction

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The response magnitude to a mixture is dependent not only on which transduction cascades are activated by each component of the mixture, but also on the interactions between components. In the spiny lobster *Panulirus argus*, biochemical and electrophysiological studies suggest that inhibitory binding interactions can shape the responses of ORNs to binary mixtures. In the present study, we examined the binding interactions of complex mixtures containing up to seven compounds. Biochemical techniques were used to examine the binding of radiolabeled adenosine-5′-monophosphate, L-glutamate, or taurine in the presence of mxtures of up to six other odorants. Our results show that the magnitude of binding inhibition by complex mixtures is often large and is not usually predictable from inhibition values for the individual components. The magnitude of binding inhibition is influenced more by the mixture's composition than complexity. Electrophysiological responses of ORNs to these complex mixtures show generally similar effects. Modeling using these data will be used to examine the role of binding inhibition on responsiveness of olfactory neurons to complex mixtures.

Supported by NIH grant DC000312.

160. Chorda tympani transection increases KCl detection threshold in rats as measured by a two lever operant conditioning paradigm

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It is well established that, in the rat, the chorda tympani nerve (CT) is critical in the taste detection of low concentrations of NaCl. In the experiment outlined here, we used a two-lever operant conditioning procedure to measure taste sensitivity to KCl in rats before and after CT transection. Water-deprived rats were trained to press the appropriate lever after sampling either KCl or distilled water, with water reinforcement following each correct response. KCl concentrations ranged from 0.003 to 0.4 M in multiples of two (eight concentrations in all) prior to surgery with five KCl concentrations per session. After surgery, concentrations ranged from 0.05 to 0.8 M (five total) for nerve-transected animals, while the concentrations used for the control animals remained unchanged. At least one of the higher concentrations of KCl (0.2–0.8 M) was included in the stimulus array for each session in order to both measure and maintain stimulus control. Threshold was defined as the concentration that produced half asymptotic performance on a psychophysical logistic function fit to the data. The proportion of correct responses adjusted for the false alarm rate served as the dependent measure. Results indicated that for histologically confirmed CT-transected rats, KCl detection threshold increased by 0.5 $\log_{10}$ units following surgery ($P < 0.001$). The control group showed no significant change in KCl taste sensitivity after sham surgery. Thus, transection of the CT impairs the rat's ability to detect KCl, but to a much lesser extent than reported for NaCl (1–2 $\log_{10}$ unit increase in threshold).

Supported by NIH grants: R01-DC–01628 & K04-DC-00104.

161. Transforming growth factor-α regulation of cell cycle protein expression in the olfactory epithelium of transgenic mice

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In proliferative tissues, growth factors regulate the progression of quiescent cells in the G0/G1 phase of the cell cycle to the checkpoint prior to the duplication of genetic material in the S phase. To examine growth factor regulation of olfactory epithelial cell renewal, we analyzed transgenic mice in which the keratin 14 promoter drives expression of the TGF-α gene (K14 TGF-α T) and mice that lack the TGF-α gene [TGF-α (–/–)]. Using ^{125}I-radioimmunoassay, we demonstrated a 73% increase in expression of TGF-α protein in the nasal epithelium (NE) of 6-week-old K14 TGF-α T compared with non-transgenic (NT) littermate controls, and a 93% greater expression of TGF-α over that of TGF-α (–/–). Also measured was a 38% greater expression of TGF-α protein in the NE of 3-week-old T over that in 6-week-old T, suggesting developmental regulation of gene expression. We used Western blot analysis to compare the levels of expression of epidermal growth factor receptor (EGFR), the receptor for TGF-α, and 2 early cell cycle regulatory proteins, retinoblastoma (pRb) and cyclin D1. EGFR in the NE of T > NT, whereas in the control tissue skin, T ≈ NT. pRb in the NE of T > NT whereas in skin, T > NT. In the skin of 3- and 6-week-old mice, cyclin D1 in T > NT. In contrast, in 6-week-old mice, cyclin D1 in the NE of T ≈ NT. The results of our study demonstrate the usefulness of the TGF-α transgenic mouse models for studies of cell proliferation and the role of EGFR and regulatory proteins in early cell cycle progression in the olfactory epithelium.

Supported by research grant number 1 R01 DC 00159-16 (T.V.G.) from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

162. The cloning and characterization of nitric oxide synthase in the olfactory system of the moth *Manduca sexta*

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Nitric oxide stimulation of soluble guanylyl cyclase has been suggested to play a role in mediating olfactory processing and development in both insect and mammalian species (Breer and Shepherd, 1993). This suggestion has been strengthened by the demonstration of high levels of NADPH-diaphorase activity in insect antennal lobes (Elphick *et al.*, 1995; Müller and Hildebrandt, 1995) and antennae (Stengl and Zintl, 1996). Since NADPH-diaphorase staining is, at best, an indirect way of determining the activity of nitric oxide synthase (NOS), we have sought to understand better the role of nitric oxide in the olfactory system of *Manduca sexta* by cloning and characterizing the NOS expressed in that system. We have obtained a 6.0 kb cDNA clone which contains a 4.0 kb open reading frame which codes for a protein with very high similarity to mammalian and

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Drosophila NOS. Northern blots demonstrate that only one 6.0 kb species of NOS mRNA is expressed in the olfactory system of *Manduca sexta*. Here we will show the complete sequence of this clone, its relationship to previously identified NOS, and its localization within the olfactory system using in-situ hybridization.

We gratefully acknowledge the support of the NIH training grant NS07363 to A.N., NIH NS29740 to D.B.M, and NIH NS28495 to J.G.H.

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163. Vividness of olfactory mental imagery: correlations with sensory response and consumer behavior

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In this study, we measured olfactory imagery ability using a newly developed questionnaire and related individual differences on this measure to response to odor stimuli and to measures of consumer behavior. Marks's Vividness of Visual Imagery Questionnaire (VVIQ) has been previously adapted to an olfactory context. The VVIQ and the Vividness of Olfactory Imagery Questionnaire (VOIQ) were administered to 154 subjects between 20 and 40 years of age. The frequency distribution of VVIQ and VOIQ scores was similar to previous data. Correlations between the two scores (r = 0.53, $P < 0.0005$) were also similar to earlier findings. Order of test presentation did not affect scores on either questionnaire. Subjects from the top (n = 25) and bottom (n = 27) thirds of the VOIQ distribution were called back for a second experiment. They rated twelve odor stimuli for strength and hedonics using 9-point labelled scales, and completed a usage habit questionnaire about scented toiletries and household products. There were few significant differences between high and low olfactory imagers on the sensory measures. However, olfactory imagery status was associated with marked differences in consumer behavior. High imagery women were more likely to be frequent users of perfume, eau de toilette, and air fresheners (AF); they used more perfume brands; and they were less likely to be exclusively users of nonscented antiperspirant/deodorant (AP/DEO). High imagery men were less likely to be frequent users of aftershave/cologne; however they were more likely to be frequent AF users, used more cologne brands, and were less likely to be exclusive users of nonscented AP/DEO.

We thank Dragoco, Inc. for providing the fragrances.

164. Dietary fat preferences are inversely correlated with peripheral gustatory fatty acid sensitivity

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We previously demonstrated that taste receptor cells (TRCs) respond to *cis*-polyunsaturated fatty acids (PUFAs) through an inhibition of delayed rectifying K channels (K_{DR}; Gilbertson *et al.*, 1997) which may represent the transduction mechanism for dietary fat. To determine if there is a link between the sensitivity of fungiform TRCs to PUFAs and dietary fat preferences, we compared the PUFA-sensitivity of TRCs using patch clamp techniques from Osborne–Mendel (O-M) and S5B/Pl rats which display dietary preferences for fat over carbohydrate and carbohydrate over fat, respectively (Okada *et al.*, 1992). In isolated TRCs, the PUFAs, linoleic (C18:2), linolenic (C18:3) and arachidonic acid (C20:4), inhibit K_{DR} in a concentration-dependent manner in both strains, while the unsaturated lauric acid (C12:0) was ineffective. K_{DR} from TRCs of S5B/Pl rats were significantly more sensitive to inhibition by all three PUFAs (10 μM) than were TRCs from O-M rats. We are currently investigating whether this differential responsiveness is due to (i) the relative affinity of the interaction between cis-PUFAs and the delayed rectifying K channels or (ii) the relative density of delayed rectifying K channels in the two rat strains. Whatever the mechanism, these data suggest an inverse correlation between peripheral gustatory sensitivity to PUFAs and the dietary preference for fat. This finding may provide insight into the mechanism for sensing dietary fat which allows the S5B rats to reduce fat intake on a high fat diet and avoid the obesity which results when O-M rats eat a high fat diet.

Supported in part by NIH DC02507 (T.A.G.) and DDK32089 (G.A.B.).

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165. Fate of rat olfactory receptor molecules expressed in heterologous cells

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The study of functional properties of the presumed olfactory receptors has been difficult due to lack of functional expression in heterologous cells. We have previously found that the rat olfactory receptors are expressed in *Xenopus* fibroblasts and melanophores, but are not present on the plasma membrane. In this study, immunoflourescence microscopy showed that heterologously expressed olfactory receptors were contained within the staining pattern of a marker for endoplasmic reticulum but not with markers for the medial Golgi apparatus or the nucleus. Creating a simpler protein by truncation of an olfactory receptor after the second or third predicted cytoplasmic loop did allow plasma membrane expression in fibroblasts. We conclude that olfactory receptors are likely to be misfolded in heterologous cells and therefore become trapped in the endoplasmic reticulum. Functional expression of olfactory receptors may require the protein folding activity of a chaperon or enzyme that is absent from most cells.

The research was supported by NIDCD award DC02736 to T.S.M.

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166. Taste preference in non-human primates to compounds sweet in man

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Primates have stimulated more interest than any other group as humans are ranked in this same mammalian order. Gustatory responses of human and non-human primates have already been compared for compounds such as monosaccharides, oligosaccharides, polyols, amino acids, dipeptides, proteins, dihydrochalcones, sulfamates, saccharin, acesulfame, diterpenes or urea derivatives, all known to be sweet in man. But no rational comparison in primates has been attempted. Using a structure–activity relationship study in primates, it is now possible to classify the primate sweetness receptors into four types according to the behavioral responses observed from various selected compounds sweet in humans. The four types are represented by (i) the Callitrichidae and (ii) the Cebidae, both from the infraorder Platyrrhini (New World monkeys), (iii) the Lemuridae and Lorisidae, from the suborder Prosimii (prosimians), and (iv) the Cercopithecidae (Old World monkeys), Hylobatidae (lesser apes), Pongidae (great apes) and Hominidae (humans) from the infraorder Catarrhini (Old World simians). By a comparative study of the putative receptor recognition sites postulated for each type of receptor, it is inferred that the Callitrichidae (marmosets and tamarins) have retained the most primitive sweetness receptor among primates. As we believe that the evolution of the sweetness receptor is a key factor involved in the raising of non-human primates from a 'primitive grade' towards a more 'advanced' or 'simian grade', the possible phylogenetic implications of these findings will be discussed.

167. Sustaining olfaction at low salinities: generating an ionic/osmotic microenvironment by diffusion

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The olfactory sensilla (aesthetascs) of the blue crab, *Callinectes sapidus*, each contain the branched outer dendritic segments (ODS) of olfactory receptor neurons. The 'exposed' portions of these dendritic processes are separated from the external environment by the thin, permeable cuticle of the aesthetasc. We propose that in low salinity conditions the ODS are bathed by a sensillar lymph in which the diffusive loss of ions to the external milieu is matched by ion efflux from the hemolymph, thus dynamically providing ionic/osmotic conditions suitable for sustaining the structural and functional integrity of the exposed ODS. Two lines of evidence suggest that the net efflux of Na^+ through the aesthetascs in freshwater is largely driven by diffusion down the concentration gradient established between the hemolymph and the external medium—a gradient maintained by the active transport of NaCl into the hemolymph via specialized epithelia of the gills. First, the observed flux ratio for Na^+ (i.e. influx/efflux = 0.038) falls within the range of that predicted for passive diffusion based on the Ussing equation (i.e. calculated flux ratio = 0.101). Secondly, assuming a passive diffusion model, the measured level of Na^+ efflux in freshwater-acclimated crabs approximates that predicted from the Na^+ efflux measured in seawater-acclimated animals and corrected for the difference in trans-antennule potential. While vital to sustaining olfactory function, the diffusive loss of Na^+ from both antennules of a freshwater-acclimated crab (based on a rate 167.77 fmol/s/ aesthetasc) during a 24 h period represents just 0.1% of the total Na^+ available in the hemolymph.

Supported by NSF Grants BNS 9121568 and DCB 8916412.

168. Symposium: Experience-induced changes in chemosensory responsiveness–underlying mechanisms and functional implications

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There is increasing evidence that experience modifies sensitivity to specific chemosensory stimuli. Prolonged exposure to, or deprivation from, specific stimuli can markedly alter subsequent responsiveness to the same stimuli, and in some cases, even different stimuli. This plasticity has been observed in the gustatory, olfactory and somatosensory (trigeminal) systems, and differs from more transient processes like sensory adaptation because it has a relatively long latency to onset and offset (several hours to weeks). This symposium will examine this new and exciting area of study, by considering the following questions. (i) What is the extent to which responsiveness to environmental stimuli can change? (ii) Are there fundamental similarities in the mechanisms underlying this plasticity across the different chemosensory systems? (iii) Are there critical periods during which animals are particularly vulnerable to experiential effects? (iv) What is the functional significance of this plasticity to animals (including humans) in their natural environment?

169. Experience-induced changes in responsiveness of the mature gustatory system

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Most animal species (including humans) exhibit substantial individual variation in sensitivity to specific sapid stimuli. However, the mechanistic basis and functional significance of this individual variation is poorly understood. Despite evidence that genetic factors contribute to this variation, the relative importance of genetic versus epigenetic factors remains unclear. Over the past 3 years, I have explored the extent to which one epigenetic factor, dietary experience, can alter gustatory sensitivity to sapid compounds. The majority of this work has been with a herbivorous insect: tobacco hornworm caterpillars, *Manduca sexta*. I have examined several days of dietary exposure to one bitter compound can modify the responsiveness of the caterpillar's (i) bitter-sensitive taste receptor cells (TRCs) and/or (ii) behavioral rejection response to the same compound. This caterpillar rapidly rejects diets treated with compounds humans describe as bitter. Results to date indicate that dietary exposure can markedly attenuate the taste-rejection response to some, but not all, bitter

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compounds. The attenuated taste-rejection response appears to be mediated in some cases by reduced responsiveness of bitter-sensitive TRCs, whereas in other cases, it appears to be mediated by changes within the central nervous system. These findings demonstrate that recent dietary history can contribute significantly to individual variation in sensitivity to sapid stimuli. The functional significance of this diet-induced plasticity for herbivores lies in the fact that many nutritious plants contain harmless bitter-tasting compounds. A herbivore such as *M. sexta* would benefit by having a mechanism for overcoming its normal taste-rejection response to these harmless plant tissues. Otherwise, it may unduly restrict its dietary options.

Supported in part by NIDCD 02416.

170. Anterograde transport from olfactory epithelium to olfactory bulb after partial lesions of the epithelium

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We have examined the effects of olfactory mucosal lesions on projections of sensory neurons to the olfactory bulb. The left olfactory sac of neonatal (3–6 days old) or adult rats was exposed by removal of the nasal bone and lesions of the dorsal epithelium were produced using a cautery. One to eight weeks later both olfactory sacs were injected with 6 µl of 1% horseradish peroxidase coupled to wheat germ agglutinin and, 24 h later, the animal was sacrificed and every third 50 micron thick section was reacted using TMB. In the right (control side) olfactory bulb virtually all glomeruli were filled with dense reaction product. In adult operated rats, many olfactory glomeruli in the left (experimental side) bulb, particularly those on the dorsal surface, had only light reaction product. In contrast, all glomeruli of experimental bulbs of long survival neonatal operated rats were filled with dense reaction product. However, the left olfactory bulb of these rats was smaller than the right bulb. In sample sections, its width (measured from the superficial edge of the medial and lateral EPL) was 20–25% less than that of the right bulb.

171. How many second messengers mediate olfactory transduction in vertebrates?

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It is widely believed that two intracellular second messengers mediate excitatory olfactory transduction in vertebrate species, with some odorants being detected by a cyclic AMP-dependent mechanism, and other odorants being detected by an IP_3-dependent mechanism. In this lecture, I will summarize and critique the evidence that cyclic AMP and IP_3 both contribute to excitatory olfactory transduction.

Supported by NIH grant DC-00505.

172. Characterization of a cell line derived from rat olfactory epithelium

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The olfactory epithelium (OE) is remarkable in its capacity to produce neurons continually throughout life. Substantial evidence indicates that proliferative cells within the globose basal cell (gbc) population give rise to neurons in the undamaged epithelium and in epithelium where the neuronal population has degenerated and is being reconstituted. However, GBCs are biochemically heterogeneous and cells with broader differentiative capacities are activated when damage to non-neuronal as well as neuronal populations is incurred. A cell line derived from early OE progenitors would facilitate our understanding of OE cell production and the regulatory mechanisms acting to control the process. We have used multiple cell type-specific markers, some of which are novel, to examine a cell line designated NIC, which is derived from postnatal rat OE. Our findings demonstrate that the morphologically undifferentiated cells express antigens characteristic of GBCs, neurons, sustentacular cells and horizontal basal cells. However, the NIC cells do not express all of the markers for these differentiated cell types. Instead, the NIC cells share the phenotype of more immature stages in the differentiation of the various cell types found in the intact epithelium. Thus, the pattern of marker expressionby the NIC cells is atypical of GBCs in the normal OE. However, cells of mixed phenotype of this sort are observed *in vivo* when both neurons and non-neuronal cells have been ablated via direct, MeBr induced damage to the epithelium. The cell line can be pushed towards neuronal differentiation by the removal of serum, and proliferation ceases as long as serum remains absent. Other culture conditions favor an alternative differentiation pathway. For example, the addition of FGF2 to base medium promotes cell growth. Moreover, FGF2 prevents the development of a neuronal phenotype that occurs in the absence of serum, and increases the percentage of cells that express GBC-1 and Mash-1. Thus, the NIC cell line will prove useful in dissecting the molecular pathway regulating progenitor cells in OE. In turn, an increase in our understanding of progenitor cell biology may contribute to the development of novel therapeutic modalities for certain dysosmic conditions.

Supported by NIH grants K04 DC00080 and R01 DC02167.

173. Cross-talk between signal transduction pathways in rat and human olfactory receptor neurons

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It is generally accepted that there are two odor signal transduction pathways in olfactory receptor neurons (ORNs), mediated by cAMP or IP_3. Using freshly isolated ORNs from the adult human and rat olfactory epithelium and calcium imaging techniques to measure odor-elicited changes in intracellular calcium, we investigated the involvement of the elements of both second messenger pathways in signal transduction. Cells were stimulated with odorants or biochemical activators that have been shown to initiate cAMP or IP_3 production. By using metabolic blockers of the elements of transduction (cAMP: L-cis-diltiazem; IP_3: neomycin) and signal termination (H–89 and *N*-myristoylated EGF receptor, specific inhibitors of protein kinase A and C, respectively), we determined that odor responses of individual human ORNs involve only one of the two pathways. In rats, however, some cells showed a functional separation between these

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two pathways while other cells did not, indicating that there is a possibility of cross-talk between the two transduction pathways in rat ORNs. This suggests that components from one transduction pathway may play a modulatory role in signal transduction of the other pathway, depending on the species under investigation. Thus current models of signal processing may have to be modified to accommodate the possible effects of cross-talk between the two signal transduction pathways.

Supported by NIH DC00566 and DC00214.

174. Carbonic anhydrase activity in the tongue epithelium and its relation to taste abnormality in zinc-deficient rats

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It is well known that zinc deficiency causes abnormalities in general taste sensation. We have reported that rats fed a zinc-deficient diet have a significantly higher preference for carbonated water and NaCl than those fed a zinc-sufficient diet, and that the responses of both the lingual trigeminal and chorda tympani nerves to carbonated water are lower in zinc-deficient rats than in zinc-sufficient rats. We have also reported that reception of carbonated water through these nerves needs the participation of carbonic anhydrase (CA), a zinc-containing enzyme. Furthermore, it has already been shown that CA is localized in the rat submandibular gland and in the taste bud epithelium, and it is considered to play a role in taste sensation. We therefore investigated the effect of zinc deficiency on the CA activity in the tongue epithelium and submandibular gland of the rat. Twenty male SD rats, 4 weeks old, were divided into four groups (Zn-Def, Low-Zn, Zn-Suf, Pair-fed). After taking part in preference tests for42 days, the rats were sacrificed and the tongue and submandibular gland excised. These were homogenized with Tris–HCl buffer (pH 7.2) and centrifuged (1000 *g* for 15min). The CA activity in the supernatant was measured by Maren's micromethod. The CA activity of the tongue epithelium and the submandibular gland was significantly lower in Zn-Def rats than in Zn-Suf and Pair-fed rats. These results indicate that CA activity, considered to be an indispensable factor for the maintenance of normal taste sensation, is affected by the dietary zinc content.

Supported in part by Urakami Foundation, Japan.

175. Long-term potentiation of inhibition in gustatory nucleus of the solitary tract

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Synaptic potentials evoked in neurons of the rostral (gustatory) nucleus of the solitary tract (rNST) are complex mixtures of excitatory and inhibitory potentials (Grabauskas and Bradley, 1996). The excitatory component is mediated by glutamate and the inhibitory component by GABA. By using the glutamate receptor blockers 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) and D-2-amino-5-phosphovalerate (APV, 50 μM) it is possible to elicit 'pure' inhibitory postsynaptic potentials (IPSPs). We have studied the properties of these IPSPs in horizontal brainstem slicesof the rat rNST. Normally IPSPs are elicited by single shockstimuli but input to the gustatory nucleus usually consists oftrains of high frequency action potentials. We have therefore used tetanic stimulation at frequencies that mimic the *in vivo* firing rate of afferent taste fibers to characterize the properties of the IPSPs.

Single shock IPSPs were evoked every 30s for 10 min followed by a single tetanic stimulus (50 Hz for 2s) at the same stimulus strength. Single shock IPSPs were then elicited at the same stimulus strength every 30s. In all 35 neurons tested using this paradigm the tetanic stimulation resulted in a marked potentiation of the evoked IPSP amplitude (mean amplitude is 275% ofcontrol immediately after the tetanic stimulation) and also resulted in an increase in the amplitude and frequency of the spontaneous IPSP activity. In the majority of neurons the potentiation lasted ~5 min before returning to control levels, but in 17 neurons the potentiation lasted >10 min, and in four neurons the potentiation was sustained for >1 h. After the potentiation had declined to control levels it could be induced again after a second tetanic stimulation. Thus, tetanic stimulation can induce long lasting changes in rNST inhibitory synapses. In the hippocampus changes in the effectiveness of synapses is related to learning mechanisms and it is thus possible that the long term potentiation of inhibition in rNST may be the cellular mechanism of learned taste behaviors.

Supported by NIDCD, NIH Grant DC 00288.

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176. Effectiveness of continuous bilateral sampling for robot chemotaxis in one turbulent odor plume

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The American lobster (*Homarus americanus*), like many marine and terrestrial animals, lives in an environment in which odor arrives at its chemoreceptors as a temporal series of discontinuous odor patches resulting from the action of turbulence. We have beenexploring chemo-orientation strategies alternative to concentration gradient ascent with a lobster-sized autonomous robot that uses odor concentration from a single source as the sole directional cue. The robot senses its environment with two chemical sensors which sample the external concentration of tracer chemicals with a temporal resolution which exceeds that of lobster chemosensory cells. Using algorithms which make instantaneous comparisons of the concentration signals arriving at the sensor pair to guide the robot we tested the effects of sensor separation on the accuracy of robot orientation in a turbulent odor plume. Starting close to the source (<60 cm), increasing sensor separation made for significantly greater accuracy by the measures of hits defined as successful navigation to the source (A2: $P < 0.001$), time to closest approach (F: $P < 0.001$), distance traveled (F: $P < 0.001$) and path tortuosity (F: $P < 0.05$). Further from the source (>100 cm) sensor separation had significant effects only on path tortuosity (F: $P < 0.0005$). Close to the source, hits at a level >50% occurred with sensor separations >5 cm. This result is surprising as the lobster's primary distance chemoreceptor organs

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are separated by ~3 cm. The lobster's 3 cm sensor separation, though sub-optimal close to the source, may facilitate processing of cues at greater distance including sequential analysis of odor encountered.

This work was supported by NSF award and BES-9315791 to J.A.

177. Factors affecting 'stimulus induced recovery': capsaicin concentration, frequency of exposure, and site of stimulation

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Capsaicin desensitization of the tongue can be reversed by repeated applications of capsaicin or its congener piperine. This phenomenon, termed 'stimulus induced recovery' (SIR), has been observed even when desensitization is strong enough to last 24 h or longer. Results will be reported from a series of experiments that were designed to investigate further the characteristics of SIR. In the first two experiments we examined whether SIR depends upon the relative concentrations of the treatment (desensitizing) and post-treatment stimuli. The tongue tip was desensitized with one concentration of capsaicin before being tested repeatedly with the same, a higher, or a lower concentration of capsaicin. SIR approached completion in both experiments only when the concentration of the post-treatment stimulus equaled or exceeded that of the desensitizing stimulus. In a third experiment we investigated whether the frequency of stimulus exposure (inter-stimulus interval, ISI) dictates the rate and amount of recovery. The results showed that SIR was nearly complete for all but the longest ISI tested (60 s). In a final experiment the site of stimulation was moved to the face to learn if SIR could be observed outside the oral cavity. Although its temporal characteristics differed from those on the tongue, SIR also developed on the cheek. Viewed together these results suggest that SIR is a general characteristic of capsaicin desensitization, that its magnitude depends upon the relative concentrations of the desensitizing and post-treatment stimuli, and that post-treatment stimuli must be presented with brief ISIs to produce it. The implications these findings have for theories of the mechanism of capsaicin desensitization and for the role it plays in oral chemesthesis will be discussed.

Supported in part by NIDCD 002499.

178. The odorant and pheromone binding proteins of *Epiphyas postvittana* (Lepidoptera: Tortricidae), a horticultural pest in New Zealand

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Odorant binding proteins are involved in the highly sensitive and specific semiochemical detection system used by insects to detect food, oviposition sites and potential mates. Airborne pheromone and odorant molecules diffuse from the air through wax-filled pores in the antennal olfactory cells into the aqueous lumen of these sensilla where they are sequestered by pheromone binding proteins (PBPs) and general odorant binding proteins (GOBPs) which facilitate the transport of these lipophilic molecules to receptors on the underlying dendritic membranes. These binding proteins of the lightbrown apple moth, *Epiphyas postvittana*, were isolated from homogenized antennae by size exclusion and ion exchange HPLC. Five odorant binding proteins were detected by native PAGE (14–17 kDa) and their N-terminal sequences obtained. The two distinct male-specific proteins showed similarity to other lepidopteran PBPs. The one female specific protein showed similarity to one class of lepidopteran GOBP and the remaining proteins, common to both sexes, have similarity to the other GOBP type. Oligonucleotide primers, designed to the N-terminal sequences and conserved C-terminal regions of these proteins, were used to amplify the *E. postvittana* pheromone binding protein genes from cDNA. Although the predicted amino acid sequences of these antennal proteins are more divergent from pheromone binding proteins so far isolated from other lepidopterans, they do share a similar predicted secondary structure. We are cloning these genes into expression vectors to aid in characterizing the structural and binding properties of these proteins.

Funded by the NZ Foundation for Research Science and Technology.

179. The effects of odour priming on visual evoked potentials

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In 1993 Lorig *et al.* reported a study investigating the effects of odour labelling on Visual Event Related Potentials through the analysis of the P300 component, However, other ERP work has suggested that the N400 component which has been widely reported in tasks involving semantic congruity may be worth considering as an appropriate measure for investigation. An earlier study suggested that the N400 component may not be solely related to linguistic congruity. The study examined the effects of presenting an olfactory priming stimulus which was either congruent or incongruent with a visually presented food stimulus. The main finding that the N400 component was greater in the non congruent condition as found in the studies involving semantically related stimuli. The present investigation was conducted in order to extend these findings using different types of odours, e.g. lemon, rose, leather and grass.

Subjects were presented with one odour for 4 s via an olfactometer which delivered odours from a common source. This was followed by the presentation of a back projected slide for 800ms which depicted a colour picture which was either congruent or incongruent with the priming odour. The ratio of congruent to incongruent stimuli was maximized by selecting the ratio of 80:20; congruent:incongruent. ERP single trial data was recorded using a 32 electrode channel Neuroscan 32 Synamps (model No. 50835/N) using standard electrode placements. Following recording, ERP data trials were sub-divided into congruent and incongruent scores and a repeated measures analysis of variance was used to investigate differences between electrodes, conditions, hemisphere and sex. The results are discussed with reference to the Lorig *et al.*'s findings.

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180. Reward comparison in single cells of the nucleus of the solitary tract

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Rats typically lick more for a high than a low concentration of sucrose when the two concentrations are experienced during separate daily sessions. When given the opportunity to compare the two closely in time, however, the magnitude of this concentration effect is exaggerated by simultaneous contrast. That is, the number of licks made for the low concentration is reduced (negative contrast) and the number made for the high is enhanced (positive contrast) relative to the between-day condition. A combination of decerebrate and focal lesion data show that the brainstem is sufficient for this type of comparison process and, more specifically, that the nucleus of the solitary tract (NST) may be the critical structure. This conclusion has been substantiated by simulating the simultaneous contrast procedure in an anesthetized preparation and recording from single cells in the NST. The results showed that, like the behavioral data, the magnitude of the concentration effect (i.e. the difference in the number of spikes elicited for the low and the high concentration of sucrose) also was exaggerated when the two concentrations were presented closely in time. These contrast effects were general, occurring with sucrose, NaCl, and citric acid concentration pairs, but were restricted to units that were 'best' for those stimuli. Finally, evidence suggests that contrast in this simulated procedure reflects a central, rather than a peripheral, process. When the low and high concentrations of sucrose were applied to separate receptor fields (e.g. the anterior tongue and the nasoincisor ducts), NST neurons that received convergent afferent input continued to exhibit the exaggerated concentration effect. These data show that reward comparison effects are reflected, and perhaps mediated, by the activity of single units in the NST.

Supported by DC 02016, DC 00240, and MH 00653.

181. Parabrachial nucleus lesions block conditioned odor aversion

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Rats with bilateral ibotenic acid lesions centered in the gustatory zone of the pontine parabrachial nuclei (PBN) failed to acquire a conditioned taste aversion. They also failed to acquire a conditioned odor aversion (COA) when ingesting distilled water in the presence of almond extract that was paired with intraperitoneal injections of LiCl. The deficit in COA persisted over four trials using either a 15 or a 30 min inter-stimulus interval. The failure to acquire an aversion was not due to anosmia per se, because the rats with PBN lesions initiated licking more slowly and ingested less of an strong capsaicin solution when it was cued by an odor. In the first experiment, the odor source was attached to the water spout. In the final experiment, COA was re-evaluated with the odor (2% vanilla extract) dissolved in the water. Even under these circumstances, the rats with PBN lesions failed to acquire an aversion whether measured by the latency to begin licking or by the volume of fluid consumed. As with taste aversion learning, neurons in the PBN appear to play a critical role in associating an olfactory stimulus with a LiCl unconditioned stimulus.

Supported by NIH grants DC 00240, DC 02016, and MH 00653.

182. Chemical orientation in crayfish under stream conditions: effect of substrate on signal dispersion and orientation behavior

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Odor plumes provide important environmental information for many crustaceans. Lobsters and blue crabs use the distribution of chemicals within odor plumes to forage for prey items. The distribution of chemicals within odor plumes will depend upon the turbulent structure within flowing water. Turbulence within flowing water will be greatly influenced by roughness elements (such as sand versus cobble stones) on the bottom of the stream. We studied the orientation behavior of the crayfish, Orconectus rusticus, to fish odors within artificial streams with different substrates. Our results show that the crayfish responded positively to fish odors in both the sand and cobble bottomed streams. The substrate type, while influencing the turbulent structure of flow, did not influence the ability of the crayfish to find the odor source. Analysis of the orientation tracks showed that paths were different for the two different substrates. Thus, it is possible for crayfish to use chemical signals to locate potential prey items under a variety of habitats. With further analysis, we hope to provide some insight into the mechanisms by which crayfish orientation to chemical sources within streams.

Supported by NSF grant # (IBN-9614492).

183. Structure–odor activity relationships of earthy smelling alkylpyrazines

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The odor threshold values of 50 alkylparazines, most of them were synthesized, were determined by gas chromatography–olfactometry. Trimethylpyrazine (I) had the lowest threshold (50 ng/l air) in the line mono, di-, tri- and tetramethylpyrazine. Substitution of the methyl group in position 2 of I by an ethyl group yielded 2-ethyl-3,5-dimethylpyrazine (II) showing a 5000-fold lower threshold than I. The thresholds of 2-ethenyl-3,5-dimethylpyrazine (III), 2,3-diethyl-5-methylpyrazine (IV) and 2-ethenyl-3-ethyl-5-methylpyrazine (V) were as low as that of II. The threshold of 3-ethenyl-2-ethyl-5-methylpyrazine was 8100 times higher than that of V indicating that an ethenyl group was only tolerated in position 2, but not in 3. A further increase was found, when the ethenyl or the ethyl group was located in position 5. Substitution of the ethyl group of II by a (Z)-1-propenyl group increased the threshold only by a factor of 6, whereas the (E)-isomer and the 2-propenyl group enhanced the threshold by factors of 180 and 30 000, respectively. Also a propyl, butyl, pentyl, isobutyl or hexyl group in position 2 of II was too bulky;

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the odor threshold was at least 2400-times higher then that of II. The geometrical shape of a theoretical receptor was obtained by superposing the minimized structures of pyrazines with low thresholds (CHEMX force field minimization). Sterical forbidden regions in the resulting model were found by superposing pyrazines with high thresholds.

184. The arginine receptor: physiology, biochemistry and molecular biology

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The amino acid, L-arginine, is a potent taste stimulus for the channel catfish, *Ictalurus punctatus*. Receptor binding studies demonstrated a high affinity binding of L-Arg to putative taste receptor sites. This binding could be inhibited by preincubation of the tissue in the lectins PHA and *Ricinus communis agglutinin I* (RCA). Neurophysiological studies demonstrated that the L-Arg receptor is a stimulus-gated ion channel type receptor whose conductance was stimulated by L-Arg and inhibited by D-Arg. To purify the receptor we subjected CHAPS solubilized partial membrane preparation from barbel epithelium to RCA lectin affinity chromatography. The bound proteins were eluted with D-galactose. When these proteins were reconstituted into lipid bilayers, L-Arg activated single channel currents with conductances between 45 and 85 pS. SDS–PAGE of the eluted protein showed a distinct band at ~83 kDa. Polyclonal antibodies raised against this 83 kDa band in guinea pigs reacted with numerous small (~1 mm) sites within the taste pore of every taste bud when applied to fixed non-permeabilized barbels. This observation suggests that the antibodies recognize an externally-facing epitope of the putative Arg receptor. The antibodies also inhibited L-Arg-stimulated currents in reconstitution studies. Sephacryl S-300 HR chromatography of the eluant from the affinity column showed a high molecular weight peak (>700 kDa) which was recognized by the antibodies. Reconstitution of the protein from this peak into a lipid bilayer resulted in L-Arg stimulated channels which could be inhibited by D-Arg. This high molecular weight component may be aggregates of the arginine taste receptor.

Supported in part by NIDCD, DC-00356, DC-00014 and DC-01838, and the Department of Veterans Affairs.

185. Threshold distributions of phenylthiocarbamide (PTC) in the Chinese population

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The ability to taste phenylthiocarbamide (PTC) is a well-documented Mendelian trait. Mapping and cloning the gene(s) responsible for the PTC tasting ability would help to delineate the molecular basis for the variations in PTC tasting ability in humans and to shed new insight into taste chemosensory functions. In view of the spectacular successes in genome science, the positional cloning strategy seems to be a feasible approach to the isolation of the gene(s) underlying the PTC tasting ability. As a first step toward mapping the gene(s), we collected PTC taste threshold data on 106 university students in Shanghai, China. Using various parametric and non-parametric statistical methods, we have found that the data set is best described by a bi-modal distribution. The frequency of PTC nontasters is estimated to be 10%. This is consistent with the view that the PTC non-tasting ability follows a recessive mode of inheritance. Several authors had previously reported PTC data on Chinese living outside China. Our data are, to our knowledge, the first ever collected from Chinese population within China.

Supported in part by NIH grants R29 GM52205 (S.W.G.) and R01 GM 56515 (S.W.G.).

186. A new technique of electrogustometry using variable durations of single pulses

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The aim of the study is to develope a new subjective electrogustometric method for testing human sense of taste by using stimuli of variable durations. An easy to use system was achieved by stimulus generation and evaluation using a personal computer.

The thresholds of electric taste are determined in 14 steps in dependence of current pulse duration over a time range of 0.1–2000 ms. In a diagram with a double logarithmic system of co-ordinates characteristic curves are obtained having following segments: (i) the sensitive range of saturation with high threshold at short stimulation times; (ii) the sensoric range of transition with decreasing threshold at medium stimulation times (e.g. 10–500 ms); and (iii) the sensoric range of saturation with low threshold at long stimulation times (characteristic curve electrogustometry—CCE). The points of transition between the ranges are determined by an algorithm. The points of transition yield values for the sensoric and sensitive thresholds and the corresponding stimulus durations.

85 volunteers were participated in the experiments. We found small intraindividual and large interindividual variations of the following parameters, e.g. in the age group of 51–60 years (low threshold: (13.6 ± 15.5) gustatoric decibels (dBg); beginning of threshold transition at stimulus duration: (2.82 ± 0.49) logarithmic units of milliseconds) for n = 14. Side differences in the same subject were detected able to reproduce over several years. Age dependence of the electrogustatoric dynamic range (=difference between sensitive and sensoric threshold) was demonstrated.

In interpreting these results we conclude that this method is suitable to describe the gustatory system more precise as using standard electrogustometry. The new technique prove to be very useful in the clinical environment.

187. Expression of Sonic Hedgehog (Shh) and Patched (Ptc) in developing taste papillae of the mouse

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Taste buds develop from local epithelial cells with papillary

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development beginning between embryonic days 12 and 16. This development occurs in a specific spatial pattern in which rows of fungiform papillae form on the anterior portion of the tongue, and a single circumvallate papillae forms on the midline at the oral–pharyngeal border.

Two genes involved in developmental signaling and epithelial patterning, the signaling molecule Sonic Hedgehog (Shh) and its receptor Patched (Ptc), have been reported to be expressed in the tongue during this time. The present study investigates the timing and pattern of expression of these genes in relation to papillary morphogenesis. Embryos were staged according to crown–rump length and *in situ* RNA hybridization was performed on both whole mount and sectioned tissue using Shh and Ptc probes. Both genes were found to be broadly expressed in the early tongue primordia (E11) and were progressively localized to the developing fungiform and circumvallate papillae by E14. Sectioned material showed that Shh expression in fungiform papillae at E15 was confined to the central epithelial cells of the developing papilla. Although Shh and Ptc expression appeared coincident at early stages of fungiform papillary formation, by E16, Ptc expression appeared as an annulus surrounding a central non-expressing area. In contrast, expression of Shh appeared only in the central region corresponding to the presumed location of the taste bud. This pattern of expression is reminiscent of the center–annular pattern of expression of different growth factors in the developing papilla. The pattern and timing of expression of Shh and Ptc in developing papillae indicates that they may be involved in papillary development, possibly helping to establish papillary boundaries or spacing.

Supported by NIH Grant PO1 DC00244 to T.E.F.

188. Characterization of Ca^{2+}-activated Cl^- channels in excised inside-out patches of mammalian olfactory receptor neurons

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Mammalian olfactory receptor neurons contain specific receptor proteins which respond to odorous stimuli. The binding of odorants to these proteins stimulates G_{olf}, producing cAMP and activating cyclic-nucleotide gated channels in the cell membrane. Entry of Ca^{2+} and Na^+ into the cell causes depolarization and action potential generation. In the cilia of frog ORNs, it has been suggested that Ca^{2+}-activated Cl^- channnels exist as a result of Ca^{2+} entering the cilia and activating these Ca^{2+}-activated Cl^- channels. Using inside-out patches, we have shown that these channels are also present in rat ORNs, with a putative role in their olfactory transduction, and have characterized some of their properties. Current recordings across these patches have indicated the presence of Ca^{2+}-activated Cl^- channels in these mammalian ORNs, and in particular on their dendritic knobs. The *I/V* curves at different concentrations of Ca^{2+} were linear indicating that the channels were open at all voltages applied (–80 to +70 mV). Conductances ranged from 30–75 nS. The dose response curve showed an EC_{50} of 25.5 mM Ca^{2+} and a Hill coefficient of 0.98. Lowering $[NaCl]_i$ resulted in a reduction in current amplitudes and negative shifts in the reversal potentials, which implied that these Cl^- channels have a P_{Na}/P_{Cl} of ~0.035, indicating that Cl^- was considerably more permeable than Na^+. The halide permeability sequence was $P_{Cl} > P_F > P_I > P_{Br}$ and the channels were also permeable to large organic anions like SCN^-, acetate$^-$ and gluconate$^-$, with their permeability sequence being $P_{Cl} > P_{SCN} > P_{acetate} > P_{gluconate}$, indicating a pore diameter of at least 5.8è.

Supported by the Australian Research Council.

189. Selection and description of a MSG-containing soup in a normal living context prior to informed consent

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Subjects unaware of the experiment were individually invited to a residential apartment where they were told that a future dinner was planned, that the soup was yet to be selected, and were asked to taste the soups in three bowls and to pick the bowl containing soup that differed from the other two. The soup was prepared Campbell's® Condensed Soup Healthy Request Chicken Noodle, containing in each 237 ml sample, according to the label, disodium inosinate and disodium guanylate, both at concentrations >1%, 0.474 g Na [comparable to 0.088 M NaCl (0.52%)], but no monosodium glutamate (MSG). The soup was prepared according to directions and served at 38° C. One bowl contained soup with 0.023 M (0.38%) monosodium glutamate (MSG) added; it was discriminated from soup without MSG by 14 of 15 subjects. All 15 completely consumed the soup with MSG added. Although no descriptions were explicitly requested, all subjects volunteered that the soup with MSG provided 'more taste' or was 'tastier'. After the selection and volunteered descriptions were completed, subjects were informed of the nature of the experiment, and provided written consent for the use of their data.

Adding the MSG increased the Na content from 0.088 M NaCl (0.52%) to 0.108 M (0.63%). Although this difference could be discriminable in aqueous solutions in a laboratory context, it is unlikely to be discriminable in a soup served in the context of this study. The discriminability, palatability, and positive descriptions of the soup with MSG added are attributed to gustatory effects of the glutamate plus the Na or 5′-ribonucleotides of the unmodified soup.

190. Differential distribution of amygdalar input across solitary nucleus subdivisions in rat

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The orosensory nucleus of the solitary tract (NST) receives input from the amygdala, a key node in the forebrain feeding-related network. Despite numerous studies documenting the existence of this pathway, however, too little is known about the input organization to the gustatory brainstem to allow definitive conclusions about its functional role. Therefore, towards the long-term goal of characterizing such descending regulatory pathways, the purpose of the present study was to describe the distribution of input arising from the amygdala. The anterograde

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tracer, biotinylated dextran, was injected into the central amygdala based on stereotaxic coordinates in seven adult male rats. Following a 2 week survival time, the animals were sacrificed. Transverse sections of the brains were processed to visualize transported tracer and NST anatomical topography. Labeled fibers were differentially distributed among subdivisions throughout the rostrocaudal extent of NST. Within the rostral NST, the medial (M) subdivision had the highest density of terminal-like endings and swellings (30% of total density), followed by the ventral half of rostral central (vRC, 29%), ventral (V, 25%), dorsal half of rostral central (dRC, 12%) and rostral lateral (RL, 4%). In conclusion, it appears that amygdalar input preferentially overlaps with NST subdivisions (M, V, vRC) containing neurons with local efferent projections to the caudal NST and reticular nuclei that are implicated in medullary reflex circuits, rather than with subdivisions (dRC, RL) receiving primary orosensory afferent input and containing neurons having ascending efferent projections to the parabrachial nucleus. Thus, descending feeding-related pathways may be positioned to act as regulatory substrates controlling the output gain of brainstem circuits which may serve to modulate sensorimotor and autonomic reflexes in response to ingestive behaviors.

This study was supported by OSU College of Dentistry and NIH grants CD02016 to C.B.H. and CD00416 to S.P.T.

191. Incidence of dye coupling in the mitral cell and granule cell layers of adult salamander olfactory bulb

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Dye imaging during whole cell recording has provided evidence that some but not all cells in the mitral cell layer (MCL) and granule cell layer (GCL) are functionally coupled in intact isolated preparations of the adult salamander olfactory bulb (Hamilton and Maloney, 1996). In the present study the incidence of coupling in each layer was quantified using methods similar to those used with the developing rat olfactory bulb (Paternostro *et al.*, 1995). Subsurface clusters of cell bodies in serial 300-mm-thick slices of lightly-fixed *Ambystoma maculatum* and *A. tigrinum* preparations were viewed with a 40× objective under bright-field illumination, and single cells were injected with Lucifer Yellow CH (–2.5 nA, 100 ms pulses at 1 Hz). High contrast 640X480 pixel images of fluorescence at 540 ± 25 nm were obtained at regular intervals using a CCD camera, Silicon Graphics software, and an Indy computer.

Analysis of the image sequences showed that cells in both MCL and GCL were dye coupled to adjacent cells, and that there was a higher incidence and extent of coupling in the GCL. After 20 min and 40 min of dye injection, 2–3 brightly flourescent adjacent cells were observed in 97 and 100% of GCL injections. The dimly flourescent adjacent cells that were observed near the onset of dye injection appeared to be coupled cells that were beginning to fill. Those that wer observed after 20 and 40 min of injection appeared to be cells that were less tightly coupled than the adjacent brightly fluorescent cells.

Supported by the Whitehall Foundation and departmental funds.

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192. Odor learning and memory in Huntington's disease

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Previous research has demonstrated olfactory impairments in patients with Huntington's disease (HD). These deficits include impaired detection, discrimination, identification, and recognition memory. Odor recall has yet to be assessed. On tests of verbal learning and memory, HD patients consistently demonstrate a retrieval deficit. Often they perform poorly on measures of recall, while performing relatively normally on measures of recognition. To better understand odor memory in HD patients, we compared the performance of seven mildly affected HD patients to seven age- and education-matched healthy controls (NC) on an odor learning and memory test (COLT) and a verbal learning and memory test (CVLT).

Results demonstrated that both HD and NC participants learned less on the COLT than the CVLT with no interaction of group ($P = 0.001$). On a measure of recall memory savings following an interference list, the groups did not differ on either the COLT or the CVLT. On a signal-detection measure of recognition discrimination, both groups performed more poorly on the COLT than the CVLT ($P < 0.0001$). In addition there was a significant group by test interaction ($P = 0.004$). Follow-up analyses revealed that the HD participants did not significantly differ from the NC participants on the CVLT, but performed significantly worse than the NC participants on the COLT ($P = 0.003$).

In order to investigate whether olfactory testing provides a sensitive discriminator in cases of early HD, logistic regressions were conducted to compare the classification ability of absolute threshold, COLT recognition discriminability, and CVLT recognition discriminability. Results indicated that absolute threshold had 100% sensitivity and specificity ($P < 0.0001$), the COLT had 85.7% sensitivity and specificity ($P = 0.0006$), and the CVLT had 42.9% sensitivity and 57.1% specificity ($P = 0.55$). Our results suggest that olfactory testing may provide a more sensitive measure of the early disease process in HD than conventional neuropsychological tests.

Supported by NIH grant # A608203 to C.M. We gratefully acknowledge the assistance of Dr Neal Swerdlow of the UCSD GHPP.

193. The crypt cell: a microvillous ciliated olfactory receptor cell in teleost fishes

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The receptor cells of the olfactory epithelium in vertebrates are bipolar neurons with an apical dendrite reaching into the lumen of

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the olfactory cavity and an axon passing through the basal lamina to form the fila olfactoria. All teleosts hitherto examined showed two morphologically distinct receptor cell types: ciliated receptor cells and microvillous receptor cells. Receptor cilia as well as receptor microvilli may differ in length, width and number. During the course of our ultrastructural studies on several diverse groups of teleosts—the catfishes, *Ictalurus punctatus* and *Plotosus lineatus*; the zebrafish, *Danio rerio*; and the cyprinodonts *Xiphophorus helleri* and *Aplocheilus lineatus*—we have identified a unique morphological type of receptor cell, the 'crypt cell', bearing both microvilli and occult cilia. Studies on catfish entailed retrograde labeling of receptor cells by postmortem placement of DiI crystals into the olfactory bulb followed by a 10–14 day diffusion period. Studies on other species included transmission and scanning electron microscopy of normal material. In all species the crypt cells were similar in appearance. The cell bodies of these receptor cells are small (~5 μm), egg-shaped receptor cells situated in the upper quarter of the epithelium. Numerous large mitochondria are located above the nucleus. The most striking characteristic of these cells are occult cilia, ~2–4 μm long, contained within the apical half of the cell and extending a short distance into an apical crypt indented into the surface of the cell. The cilia never reach outward from the crypt to extend above the surface level of the surrounding epithelium. These cilia are shorter than olfactory receptor cilia but show the typical arrangement of microtubules. The apical end of the crypt cell has no olfactory knob; microvilli extend outward from the crypt. Axons of the crypt cells enter the lamina propria to join the fila olfactoria. The crypt cells usually are surrounded by unusual electron-lucent supporting cells with microvillous apices. Further studies to determine whether this receptor cell type is an overall feature in the olfactory epithelia of aquatic vertebrates or whether it is typical only for certain groups of teleosts are in progress.

194. New aspects of the chemoreception in flies

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For studies of insect taste the sensory cells of labellar taste hairs on the proboscis of calliphorid flies (e.g. *Phormia*) are preferred objects. Three sensory cells are electrophysiologically characterized: a sugar cell, a water cell and a cation or salt cell. Studying hairs on legs and proboscis of other families of flies we recently obtained results that extend our knowledge about the complexity of food detection in flies.

The hoverfly *Eristalis tenax* (Fam. Syrphidae) feeds on nectar and pollen of flowers. While nectar is recognized via the sugar cell, the so called salt cell is activated by low concentrations of a water soluble substance in extracts of pollen. From several tested pollen constituents only proline, an essential amino acid for pollen germination, stimulates the salt cell. The discrimination between salt and pollen in behavioral tests can be explained by the finding that the water cell remains active in the presence of pollen extract, but is inhibited by salts. Obviously the water cell activity is involved in the feeding decision. We already described a similar situation in *Phormia*, where the salt cell activity only elicits feeding behavior when the water cell is active too.

In contrast to the sugar cells of species of flies studied so far the sugar cell of the house fly *Musca domestica* (Fam. Muscidae) is highly sensitive to lactose, a disaccharide [Gal-β(1→4)Glc] naturally occurring only in the milk of mammals. Thus sugar spectra adapt to special environmental food situations.

The fifth tarsomere of the forelegs of *Musca* bears besides two D-hairs 46 hairs of the B-type. The B-hairs are functionally not uniform in contrast to their structural identity. Only two hairs, the 'water hairs', contain a water cell beside a spontaneously firing cell, but no sugar cell. Other examples of diversity are hairs that contain apart from the classical sugar cells, additional cells which react either to *p*-nitrophenyl-β-galactoside, but not to the chemically related lactose, or to *p*-nitrophenyl-α-glucoside. Therefore the rule established for the labellar taste hairs that each hair type contains the same physiological equipment of sensory cells, does not hold for the taste hairs of the legs.

195. Sex pheromones and amino acids evoke distinctly different spatial patterns of electrical activity in the goldfish olfactory bulb

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Until now, electrophysiological studies of the vertebrate olfactory bulb have tested only 'generalist' cues. These studies suggest that odorants are discriminated by a broadly distributed spatial map. In this study, we tested for the first time in a vertebrate the hypothesis that 'specialist' cues (pheromones) are discriminated by a more restricted component of the olfactory bulb. Our model is the male goldfish, *Carassius auratus*, for which five sex pheromones with both behavioral and physiological activity have now been identified. Electrical activity (EEG) was recorded over an 18 point grid from the surface of the olfactory bulb while fish were exposed to one of ten stimuli including: five sex pheromones, two amino acids, two bile acids and one control. Evoked activity was evaluated by time series analysis. Power ratios were calculated by dividing the power of the dominant frequency in the power spectrum during stimulation, by the power of the dominant frequency before stimulation. Next, the average magnitudes of odorant responses were compared using ANOVA. The spatial patterning of these responses was also described using cluster analysis which grouped odorants based on the similarity of their spatial patterns of activity. Although all odorants elicited EEG responses with similar dominant frequencies, odorant-specific differences were evident in the size and distribution of these responses. Sex pheromones and bile acids elicited relatively small responses that were spatially restricted in distinctive manners, although some overlap was evident. In contrast, amino acids consistently produced large responses at all positions. These results are consistent with the hypothesis that vertebrate pheromones are discriminated by a distinctive sub-component of the vertebrate olfactory system comprising a relatively small number of olfactory neurons.

Supported by the University of Minnesota Graduate School and the Minnesota Agricultural Experiment Station.

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196. Olfactory responses to guanidine compounds in teleost fishes

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Fish have generally developed an olfactory system highly sensitive to amino acids. However, the sensitivity and specificity of olfactory receptors vary in different species. In salmonids, two major subclasses of receptors are present, neutral and basic amino acid receptors. The former is best stimulated by L-cysteine and the latter by L-arginine (ARG). To date, no specific role is known for these chemical cues. Behavioral responses to amino acids remain largely controversial. This raises a question concerning their genuineness as natural chemical stimulants, i.e. whether more specific, potent chemical compounds other than the common amino acids exist. In the present study, we focused on the olfactory response characteristics of guanidine compounds in six teleost species using EOG recording. The compounds tested are metabolic products present in tissues of animals and plants. Of nine compounds tested, agmatine (AGM), decarboxylated ARG, was the most potent, with thresholds of 0.01–1 nM. In goldfish (*Carassius auratus*) and walleye (*Stizostedion vitreum*), the response magnitude at 10 mM exceeded that of the standard (STD), 10 mM L-serine. The response was relatively low in four salmonids, and in rainbow trout (*Oncorhynchus mykiss*) it remained below 20% of the STD over the range of concentrations tested. L-α-Amino-β-guanidinopropionic acid and L-α-amino-γ-guanidinobutyric acid, potent gustatory stimulants, were also very stimulatory to most species. Their olfactory thresholds were similar to that of ARG (1–100 nM), which is far lower than those for the gustatory counterpart. Changes in carbon chain (homoARG, octopine), esterification (ARG methylester) and amidation (ARGamide) of the a-carboxyl altered the effectiveness. Cross-adaptation indicates close relations between the guanidine compounds. Species-dependent, and possibly sexually dimorphic, responses to AGM suggest its potential roles as kairomones or pheromones in fishes.

Supported by NSERC grant OGP0007576.

197. Developmental changes in distribution and function of taste buds on the soft palate in the rat

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Taste bud distribution at different postnatal age on the the soft palate and within three types of tongue papillae (fungiform, foliate and circumvallate) were examined histologically in the rat (Sprague Dawley). After paraffin embedding, serial sections with 10μm thickness were made and stained by HE. The existence of a taste pore was used to identify mature taste buds. At birth, the mean number of taste buds on the soft palate (SP) was 126.7 ± 23.7 (n = 3), which contained 67.7 ± 18.8 (52.9 ± 6.8%) taste pore. The mean number of taste buds within fungiform papillae (FP) at birth was 110.3 ± 13.7 (n = 3), whereas only 13.7 ± 4.2 (12.2 ± 2.6%) contained a taste pore, that is significantly ($P < 0.001$, two tailed t-test) smaller than that for SP. The number of mature taste buds rapidly increased, whereby after the fist week, 90% of the SP and 80% of the CT taste buds contained taste pores. In contrast, no taste buds with pores were observed at birth withing foliate and circumvallate paillae. The increase in the number of mature taste buds occurred later in these two latter types of papillae, whereby at two to three weeks, 70–80% of the taste buds contained pores. Integrated responses in adult rats to taste stimuli recorded from the greater superficial petrosal nerve (GSP) innervating palatal taste buds and from the chorda tympani nerve (CT) innervating taste buds within fungiform papillae have been compared previously. Based on the total response magnitude, various sugars produced significantly larger responses in the GSP than in the CT. Also in the GSP, various D- and L-amino acids were more stimulatroy than in the CT. These results suggest that at an early stage of development, taste buds within the soft palate play an important role in the detection of nutrients in the neonatal rat.

198. Quinine and PROP avoidance share a common polygenic basis in mice

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Quinine and PROP sensitivities are correlated among inbred strains of mice (*Mus musculus*). Neither correlates with PTC sensitivity. Several studies have suggested polygenic control of quinine sensitivity. Monogenic control of PTC sensitivity has been reported. Only one of the quinine sensitivity genes has been identified, *Soa* (or *Qui*) on distal chromosome 6. It affects PROP sensitivity, but not PTC sensitivity.

In two-bottle tests, C57BL/6J mice avoided 0.1 mM quinine sulfate and 1mM PROP. C3H/HeJ mice did not avoid either bitter. Means for 12 BXH/Ty recombinant inbred strains, derived from these inbred strains, ranged from strong avoidance through indifference for each compound. Intermediate BXH/Ty means indicated recombination and polygenic control of each phenotype. The BXH/Ty means were highly correlated across compounds (r = 0.98), suggesting common polygenic variation.

Quantiative trait loci affecting each phenotype were then found by comparing the BXH/Ty means to their genotypes at 212 chromosome position markers distributed throughout the genome. Genotypes for seven markers, on five chromosomes, were significantly correlated with each phenotype. Six of these markers were correlated with both phenotypes, again suggesting common polygenic variation. Marker *Prp*, very near *Soa*, had the highest correlations, consistent with a major bitter sensitivity gene at this locus.

Polygenic control of PROP sensitivity, uncorrelated with PTC sensitivity, suggests a distinct difference in gustatory organization related to bitterness between mice and humans.

Supported in part by NIDCD grant DC00150.

199. Exploratory sniffing as an index of olfactory functioning

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As it has become increasingly apparent that the sense of smell is compromised early in individuals who develop neurodegenerative diseases such as Alzheimer's Disease and Parkinson's Disease, the

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question has naturally arisen as to whether olfactory testing could be used as a diagnostic tool for such disorders. However, many of the olfactory tests presently being used with neurodegenerative diseases are cognitively demanding, and therefore, it is difficult to determine whether an impairment in task performance should be attributed to a loss of olfactory functioning, a decrease in cognitive ability, or a combination of the two. The present study investigated the potential of using exploratory sniffing as a cognitively-unbiased index of olfactory functioning. In this study, 50 neurologically normal individuals were asked to smell three concentrations of a very unpleasant odor (methylthiobutyrate) while their sniffing patterns were being monitored. A statistically significant inverse relationship between methylthiobutyrate concentration and sniff magnitude was evidenced, with individuals cutting their sniffs short whenever this unpleasant odor was presented. A strict linear relationship between concentration and sniff magnitude was also apparent in the majority of cases, with individuals decreasing the magnitude of their sniffs the more concentrated and unpleasant the stimulus. Ten anosmic individuals were presented with the same unpleasant stimuli; however, none of these participants showed a linear relationship between stimulus concentration and sniff magnitude, as they sniffed comparably to all stimuli. These preliminary data suggest that exploratory sniffing may be useful in discriminating between individuals with defective and intact olfactory functioning, and thus may be an appropriate behaviorally oriented, cognitively unbiased tool that could aid in the diagnosis of neurological disorders in which cognitive deficits are present.

200. Pre-settlement cues for cyprid larvae of the barnacle *Balanus amphitrite*?

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Most barnacles begin life as free swimming larvae and settle as cyprids, the final larval stage. Settlement is an active process whereby a surface is 'tested' through temporary attachment and subsequent surface exploration. When an adequate surface is found, the cyprid will attach permanently and metamorphose to the adult form. In nature, settlement is gregarious and cues that induce settlement are typically associated with conspecifics. One example of this type of cue is a protein present on the cuticle of barnacles, referred to as barnacle settlement factor (BSF). Larvae will settle on surfaces treated with BSF, but their behaviour preceding this settlement is less well known, nor has it been established whether the animals are able to detect the presence of BSF in the water column. We describe a simple olfactory system in the cyprid of *Balanus amphitrite* which could allow for the detection of waterborne cues. We have also characterized the pre-settlement swimming behaviour of *B. amphitrite* in zero-flow conditions and observed the cyprids response to dissolved BSF in concentrations ranging from 0.0002 to 5.0 μg/ml. In control solutions (seawater), cyprids swim freely and occasionally make contact with the surface. Three different modes of swimming can be recognized: circular swimming, straight line swimming and vertical swimming in which the animals move towards the surface and fall passively down. Exposure to test solutions of BSF/seawater leads to a significant effect on both swimming and contact behaviour. The frequency of vertical swimming was shown to be concentration dependent with a maximum at 0.02 μg/ml BSF. Similarly, the frequency of contact behaviour reached a maximum, in this case at 0.2 μg/ml BSF, with the response of cyprids diminishing with age. These results indicate that cyprid larvae of *B. amphitrite* respond to dissolved BSF in a concentration dependent manner raising the possibility that, in nature, this cue acts to promote initial contact near conspecifics.

201. Analysis of a polymodal sensory circuit in *Caenorhabditis elegans*

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The molecules and strategies used by sensory neurons to detect and encode sensory stimuli are poorly understood, yet they are likely to be similar in vertebrates and invertebrates. In *Caenrhabditis elegans*, cellular ablation experiments demonstrated that the ASH sensory neurons are required for response to diverse noxious stimuli—including touch to the nose, high osmolarity and volatile repellants. Yet, analysis of the GLR-1 glutamate receptor suggested that synaptic output from ASH in response to nose touch is different than output in response to osmotic or chemical stimuli (Hart *et al.*, 1995).

To further analyze this circuit, we have identified mutations which specifically perturb response to just one stimulus, leaving intact response to other stimuli detected by ASH. We have cloned the *osm-10* gene which is specifically required for high osmolarity avoidance. The OSM-10 protein has no homology with previously identified proteins, but contains an N-terminal domain with multiple putative phosphorylation sites. Using GFP reporter constructs and polyclonal sera, cytosolic OSM-10 is only detected in the ASH, ASI PHA and PHB sensory neurons. Cellular ablation, behavior and genetic analyses are underway to test the potential role of OSM-10 in signal transduction in these cells. Additional mutations which perturb response to the stimuli detected by ASH are also being identified. These analyses should reveal common molecules and mechanisms in sensory signal encoding.

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202. Associative olfactory learning of sex pheromone and flower odor in the moth *Spodoptera littoralis*

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In moths, olfactory signals play an important role in different behavioral contexts. Both sexes use plant odors to locate nectar rich flowers, and additionally males use female emitted sex pheromone to find a suitable mate. Recently we showed that moth are cabable of learning to associate a flower oder with a food reward, using olfactory conditioning of the proboscis extension reflex. In this study we asked whether moth can be 'reprogrammed' by associative learning to associate an odor that normaly elicites

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an innate behavior with a new response, such as associating a smell of sex pheromone with a food reward.

Male and female S. littoralis were olfactory conditioned using either Z,E9,11-tetradecenyl acetate, the main pheromone component of this species, or geraniol, a flower odor, for conditioned stimulus. The pheromone was used in a behavioral relevant concentration. After 10 repeated conditioning trials 36% of the females (n = 30) and 44% of the males (n = 25) have learned to respond to the pheromone stimulus with proboscis extension, whereas 55% of females and males (n = 44, respectively) learned to respond to geraniol.

Moth indeed learn to combine a smell of sex pheromone with a food reward, although with a less extend than they learn a flower odor. Olfactometer experiments were started now to investigate whether this new learned behavior can influence the naturally shown behavior to sex pheromone.

Supported by a TMR grant of the European Commission to E.H.

203. The therapeutic effects of fluticasone propionate on recovery of function in the olfactory system

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While it is usually assumed that the reduction or loss in olfactory function that frequently accompanies nasal allergy and/or nasal polyposis is due primarily to nasal passage obstruction, other factors may be involved. This is especially true with the loss of olfactory function that can accompany upper respiratory infections. In such cases administration of topical steroids may have other effects in addition to simply reducing inflammation. In 1991 Kimura *et al.* found that topical steroid administration enhanced recovery of olfactory function after intranasal $ZnSO_4$. We examined the effects of fluticasone propionate (FP), a new topical steroid, on recovery of olfactory function after administration of the olfactotoxin methimazole (ME). Three groups of adult ICR mice were trained on an olfactory discrimination task using operant techniques. Once trained, two groups of mice were injected ip with ME (300 mg/kg); the third was injected with the vehicle (DMSO). The mice were then tested daily for the next 22 days on the odor discrimination task. FP or FP-vehicle (50 ml of a 1 mg/ml suspension) was administered intranasally for the first 10 days post ME exposure.

Mice receiving ME/FP-vehicle showed a loss of olfactory function, with gradual recovery over the next 5–15 days. The mice receiving ME/FP initially showed rapid recovery, but then displayed a precipitous decline in olfactory function. FP was terminated on day 10; performance remained depressed for an additional 5 days and then showed rapid improvement. The DMSO/vehicle group was unaffected. Although overall thickness of the olfactory epithelium was not different among the treatment groups, the number of sensory olfactory bipolar cells was reduced at days 10 and 15 in the ME/FP group. The adverse effect observed with concurrent FP and ME exposure most probably results from interference with the clearance of FP by ME metabolism. The buildup of steroid in turn depresses the hypothalamic–pituitary–adrenal axis.

Supported by GlaxoWellcome Pharmaceutical.

204. Two types of cyclic nucleotide gated channels in human olfactory receptor neurons

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The stimulation of vertebrate olfactory receptors by odorants results in the formation of intracellular cAMP which directly gates cation membrane ion channels leading to membrane depolarization and spike initiation. Since the channel is known and the primary structure of the channel has been identified, biophysical and pharmacological properties of native and cloned channels have been studied in detail in different vertebrates. Our interest was to characterize a cyclic nucleotide gated channel on olfactory neurons in humans and so to demonstrate a similar molecular mechanism of transduction. The human olfactory epithelium is hard accessible for biopsy and olfactory neurons are difficult to isolate and to identify. Our aim was therefore first to demonstrate the routinely access of olfactory neurons from patients who were already undergoing tissue removal in course of HNO surgery, and second to identify the sensory neurons by using OMP the first olfactory marker protein isolated and cloned and third to do patch clamp recordings on these neurons to show the existance of the CNG channel.

Patch-clamp recordings on isolated and identified olfactory receptor neurons reveal the presence of a non-desensitizing cyclic nucleotide-gated channel and of fast-desensitizing non-specific cation channels activated by nucleotides on human supporting cells. Cyclic nucleotide-gated channels on olfactory receptor neurons showed selective channel activation by cAMP ($K_{1/2}$ = 5 μM) and cGMP ($K_{1/2}$ = 2 μM), a unitary conductance of 20 pS, a reversal potential of single-channel currents close to 0 mV, a linear current-voltage relationship over the range of –80 to 80 mV and a strong extracellular but a weaker intracellular blocking effect of Ca^{2+}. The channel activity outlasted the cyclic nucleotide pulses for hundreds of milliseconds when higher agonist concentrations (>50 μM cAMP) were applied. The duration of the response was longer than in cyclic nucleotide-gated channels from other species studied so far. The plateau duration and the decay remained constant for pulses with a length of 50–150 ms, whereas pulses shorter than 50 ms successively reduced the time required by shortening the plateau phase. A larger difference for the $K_{1/2}$ values of cAMP ($K_{1/2}$ = 22 μM) and cGMP ($K_{1/2}$ = 2.5 μM) were found for a small group (n = 3) of cyclic nucleotide-gated channels, pointing to the selective expression of the alpha-subunit in a small subgroup of olfactory receptor neurons.

205. Olfactory evoked responses and identification tests in neurological disease

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To assess the value of smell testing we used olfactory evoked potentials (OEP) and an identification test in multiple sclerosis, Parkinson's disease, motor neuron disease and Alzheimer's disease. Methods: the OEP to H_2S (20 ppm) was obtained using an olfactometer designed to stimulate olfactory nerve endings only. Odour recognition was assessed by the University of Pennsylvania Smell Identification Test (UPSIT). In all instances the disease was

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'definite' based on standard diagnostic criteria. Controls were derived from 154 healthy members of hospital staff or British Telecom. Results: (i) multiple sclerosis: 11/72 patients (15%) were abnormal on UPSIT. For OEP there was significant increase of latency and decrease in amplitude in 6/26 patients (23%). (ii) Parkinson's disease: 126/155 (81%) patients had an abnormal UPSIT score. 12/37 (32%) had prolonged latency with normal amplitude measurement on OEP but 27 had absent or unclear readings. 4/10 with normal UPSIT displayed abnormality on OEP. (iii) Motor neuron disease: 9/58 (16%) were abnormal on UPSIT. There was significant delay in 1/10 (10%) patients on OEP. (iv) Alzheimer's disease: UPSIT scores were abnormal in all eight patients examined. OEP was normal in four of these who could be tested. Conclusion Smell dysfunction was found in all four conditions but most severely in Parkinson's disease (>80%). The UPSIT in general showed abnormality more frequently than OEP. The olfactory defect probably involves peripheral structures in all diseases tested except Alzheimer's. A patient with normal olfaction is unlikely to have idiopathic Parkinson's disease.

206. Ascending connections to the thalamic taste relay in rats

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The thalamic taste relay, the parvicellular part of the posteromedial ventral thalamic nucleus (VPMpc) receives taste inputs from the pontine parabrachial nucleus (PB) in rats. The PB is divided into several subnuclei having different connections. The medial and ventrolateral subnuclei include the taste relay which has been shown anatomically and physiologically to project bilaterally to the VPMpc. However, some recent studies by Saper's group reported that the VPpc (VPMpc and VPLpc) receives PB inputs mainly from the contralateral external medial subnucleus, where weak taste responses or their absence was observed. Hence, we anatomically re-examined ascending connections to the VPMpc from the PB subnuclei and other brainstem nuclei. When WGA-HRP was injected into taste responsive portions in the VPMpc, we found in the PB retrogradely labeled cell bodies in the medial, ventrolateral and external lateral subnuclei and in the brachium conjunctivum; in the former two subnuclei the number of labeled cell bodies was slightly larger in the ipsilateral side, but in the external medial subnucleus they were seen predominantly in the contralateral side. Besides PB labels, we saw retrogradely labeled cell bodies contralaterally in the caudal subnucleus of the spinal trigeminal nucleus; they were confined to superficial layers of the dorsal portion of the subnucleus which have nociceptive input sfrom mandibular portions. Tracer injections into taste responsive region in the PB medial and ventrolateral subnuclei bilaterally labeled terminal fields in the VPMpc. Injections into the external medial subnucleus also labeled terminal fields in the VPMpc with contralateral dominance. After injections into the caudal subnucleus of the spinal trigeminal nucleus, we saw diffuse labels of terminal fields in the contralateral VPMpc. The present findings indicate that the VPMpc receives not only bilateral taste inputs from the PB taste relay but also contralateral PB input of unknown modalities and contralateral trigeminal inputs related nociception.

207. Responses of mouse taste cells to monosodium glutamate and analogues recorded by optical imaging

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Monosodium glutamate (MSG) elicits a unique taste called umami. Whole-cell patch-clamp recordings from taste cells isolated from mouse vallate and foliate taste buds have indicated that MSG elicits several different types of responses, suggesting that multiple pathways may be involved in umami taste. We have also used fluorescent dyes and digital imaging techniques to measure MSG-evoked changes in membrane potential and intracellular calcium in taste cells from C3H mice. In this study, similar imaging techniques were used to record the responses of vallate and foliate taste cells from B6 mice to MSG (10 mM), the metabotropic receptor agonist, L-AP4 (2-amino-4-phosphono-butyrate) (1 mM) and the ionotropic receptor agonist, NMDA (1 mM). Cells in isolated taste buds were doubly stained with the voltage-sensitive dye, di-8-ANNEPS, and the Ca^{2+}-sensitive dye, fura-2. Of the 296 cells examined, ~65% responded to glutamate of the other glutamate receptor agonists. Increases in $[Ca]_i$ were observed in 23% of the cells (46/202) stimulated with MSG, 38% of the cells (74/194) stimulated with L-AP4 and 14% of the cells (7/50) stimulated with NMDA. Thirty-eight% of the cells changed the membrane potential by the MSG, 28% by the L-AP4 and 7% by the NMDA. Since mGluR4 receptors in the CNS have been shown to activated Gi, blocked K channels and depolarize the cells, we examined the effect of the K channel blocker, tetraethyl ammonium (TEA), on taste cells responses. Addition of TEA (10 mM) to the bath solution resulted in membrane depolarization in about half of the taste cells. In addition, TEA markedly reduced the number of taste cells showing increases in $[Ca]_i$ in response to MSG (5/68 cells) and L-AP4 (4/68 cells). None of 68 cells tested showed a change in membrane potential when stimulated with L-AP4. In contrast, the number of taste cells displaying responses to NMDA was not reduced by TEA; 9/68 cells showed an increase in $[Ca]_i$ and 12/68 cells showed a change in membrane potential. In the absence of TEA, approximately half of the cells displayed changes in membrane potential when stimulated with L-AP4. In the presence of TEA, membrane potential changes were elicited by L-AP4. These results are consistent with previous patch-clamp data suggesting that MSG activates several different mechanisms in taste cells, including processes mediated by ionotropic and metabotropic types of receptors.

Supported by the Monbusho International Scientific Research Program and NIH grants DC001838 and DC00566.

208. Coexpression of two odorant-binding protein homologs in *Drosophila*: implications for olfactory coding

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Odorant-binding proteins (OBPs) are small, soluble proteins

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present in the aqueous medium surrounding olfactory receptor neurons. Their function in olfaction is unknown: they have been proposed to facilitate the transit of hydrophobic molecules to olfactory receptors, to deactivate the odorant stimulus, and/or to play a role in chemosensory coding. We have examined the genomic organization and expression patterns of two olfactory-specific genes (*OS-E* and *OS-F*) of *Drosophila melanogaster*, the products of which are members of a protein family in *Drosophila* sharing sequence similarity with moth OBPs. We found that the *OS-E* and *OS-F* transcription units are located >1 kb apart. They are oriented in the same direction and display a similar intron–exon organization. Expression of both OS-E and OS-F proteins is spatially restricted to the ventro-lateral region of the *Drosophila* antenna. Within this region, both OS-E and OS-F proteins are expressed within two different types of sensory hairs: in most, if not all, sensilla trichodea and in ~40% of the interspersed small sensilla basiconica. We consistently observe that OS-E and OS-F are coexpressed, indicating that an individual sensillum can contain more than one odorant-binding protein. This finding has potential implications for the roles of odorant-binding proteins in olfactory coding.

Supported by NIDCD R01 DC02174-10 (J.C.) and DC00139 (D.H.).

209. Glossopharyngeal and behavioral taste responses in common marmoset, *Callithrix jacchus jacchus*

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Responses of some 40 glossopharyngeal nerve (NG) fibers were recorded during taste stimulation with three salts, four acids, six bitter compounds and 30 sweeteners. A number of chemically modified sweeteners was included to explore the relationship between chemical structure and the multipoint attachment theory of sweetness. Hierarchical cluster analysis of the fibers response profiles revealed two major clusters with about equal number of fibers characterized by predominant sensitivity to sweeteners (S cluster) and bitter compounds (Q cluster). Acids evoked little activity; NaCl, LiCl and KCl almost no activity. As in the chorda tympani, activity in the S-cluster was associated with significant preference in two-bottle preference tests, while activity in the Q-cluster was associated with rejection. Thus quinine, sucrose octaacetate, tannic and aristolochic acids with unpleasant taste to humans stimulated the Q cluster. The 20 preferred sweeteners stimulated only the S cluster. Stimuli which elicited responses in both the S and the Q clusters were either rejected (stevioside and D-phenylalanine) or non-preferred (ampame, xylitol, saccharin). Neither preference nor rejection was recorded to sweeteners which did not stimulate any NG fibers. The relationship between the results of the conditioned taste aversion tests and the response in the Q- and S clusters will be discussed.

210. Actions of serotonin agonists on the inhibition of calcium-activated potassium currents in taste receptor cells

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We have previously reported that serotonin (5-HT) applied by bath perfusion to dissociated posterior rat taste receptor cells produces an inhibition of outward potassium currents that appears specific to calcium-activated potassium currents. Using whole cell patch clamp recordings, we continue this line of investigation with the application of serotonergic agonists and antagonists to help delineate receptor type. Cells were tested using a command pulse from –50 to +40 mV that results in an outward potassium current that is highly enriched in calcium-activated currents. Drugs were bath applied, and reversible so that drug application was usually applied twice within a recording session (~60 min). Maximal drug effect was noted within five min of application. The general serotonin receptor agonist, trifluoromethylphenyl piperazine (TFMPP), tested at 100 and 500 μM produced large (up to 80%) and fully reversible inhibitions of the test current. The drug methoxyphenyl piperazine HCl (2-MPP, 100 μM), a 5-HT_1 receptor agonist, was less effective and less reversible. Naphthyl piperazine, a moderate 5-HT_1 agonist and a 5-HT_2 antagonist, tested at 100 μM, inhibited the current up to 80%, when bath applied. Inhibitions were reversible. Buspirone, a 5-HT_{1A} agonist, tested at 100 μM, produced moderate and reversible inhibitions of ~33%. Finally 8-OH-DPAT, a selective 5-HT_{1A} agonist, produced large and reversible inhibitions (~60%, 100 μM). Interestingly, many agonists also produced noticeable inhibitions of sodium currents when tested from holding potentials of –80 mV. Voltage-dependence of the inhibition of outward currents was noted. Studies with antagonists are ongoing. Collectively these data would suggest that the most likely candidate for receptor will be of the 5-HT_{1A} subtype.

Supported by NIDCD 00401.

211. Are odors the best cues to memory? A cross-modal comparison of associative memory cues

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The claim has long-been made that odors are the 'best' cues to memory. To examine this issue I have conducted a number of cross-modal experiments where odors were compared with verbal, visual, tactile and musical stimuli as associated memory cues for emotional pictures. The stimulus dimensions of familiarity and namablity were also examined across olfactory, musical and visual modalities. Each experiment comprised two sessions (acquisition and test). At the acquisition session, a series of olfactory stimuli and a series of stimuli from at least one other modality were incidentally associated to a set of emotionally arousing pictures. At the test session, subjects were re-presented with the stimuli (no pictures were seen) and asked to describe what picture was originally associated to it. Memory at test was scored for accuracy and emotionality. At both sessions, written and physiological emotional responses to the stimuli and pictures were assessed. Across studies, results showed that odors were equivalent to other stimuli in their ability to elicit accurate recall, but that odors evoked memories that were more emotional than any other

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stimulus evoked memory. Notably, at the acquisition sessions, emotional reactivity to the scenes and stimuli did not vary as a function of stimulus modality. These data demonstrate that (i) odor-evoked memories are distinguished from other memory experiences by their emotional potency; (ii) the impression that odors are 'better' memory cues is based on their emotional saliency, not on their ability to elicit more accurate memories; and (iii) the distinctive emotionality of odor-evoked memories is a phenomenon of retrieval not encoding.

212. Circuitry of taste neurons of the hamster solitary nucleus deduced from *in vivo* extracellular recordings of neuron pairs

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The rostral tip of the brainstem solitary nucleus is a focal point for the relay of taste information from the tongue. The numerous small and densely packed neurons process taste signals from the periphery and send to higher brain centers information about the quality and intensity of taste stimuli. We have used closely spaced paired microelectrodes (<150 mm apart) to record extracellular activity simultaneously from pairs of neurons. The data have been used to determine (i) whether nearby neurons have the same or different stimulus profiles; (ii) if spike trains of recorded neuron pairs are correlated; and (iii) whether the cells are synaptically coupled, the strength of the coupling, and which cell serves as input to the other. Of 19 neuron pairs recorded from the solitary nucleus, most gave significant responses to at least one of the taste stimuli (0.03 M NaCl, 0.1 M sucrose, 0.1 M KCl) applied to the anterior tongue. The two neurons often had different stimulus profiles, with one neuron much more active than the other. Six neuron pairs (32%) showed positive cross-correlations involving 2–55% of the spikes. No evidence was found for inhibitory coupling of neuron pairs. Identification of spike combinations, especially of doublets, is important for interpretation of correlograms. Small timing differences (≤2 ms) of cross-correlated spikes allowed identification of synaptically connected cells. Examples of both spike failure and spike summation were found for coupled neuron pairs. Studies of correlated neural activity with closely spaced electrodes will help establish the functional circuitry of brainstem taste nuclei, especially in combination with ionophoretically applied neurotransmitters.

Supported by NIH grant DC-00853.

213. Biophysical properties of rat olfactory bulb neurons: afterhyperpolizations involving multiple K^+ conductances

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Little is known about the biophysical properties of mammalian main olfactory bulb (MOB) principal output neurons, the mitral cells. In particular, intrinsic hyperpolarizing mechanisms limiting action potential generation have not been studied. We have investigated afterhyperpolarizations (AHPs) at the mitral soma, using *in vitro slice* preparations of mature rat MOB, with whole-cell patch-clamp methods.

Under current clamp conditions, mitral cells spontaneously generate action potential activity punctuated by AHPs. The spikes involve Na^+ and Ca^{2+} currents, and voltage dependent K^+ currents, but do not appear to involve Ca^{2+}-activated K^+ channels. Trains of action potentials, evoked by passing depolarizing current, are associated with three forms of AHP, each insensitive to blockade of excitatory neurotransmission by kynurenic acid, or of $GABA_A$ergic neurotransmission with bicuculine. A fast AHP (fAHP) follows initial events during spike trains. The fAHP inactivates rapidly and is abolished by 4-AP but not Co^{2+}, consistent with the activity of an A current. Spike trains are followed by long-lasting AHPs made up of two distinct components: a slow AHP (sAHP), lasting up to several seconds in proportion to spike train duration, and an event of intermediate duration (mAHP), available for activation after sustained depolarization independently of spike generation. The mAHP is non-inactivating, and not sensitive to Co^{2+}, TEA, 4-AP, or the BK-channel-specific agent iberiotoxin, and is classified as a voltage-activated delayed rectifier. The sAHP component is insensitive to 4-AP, but is blocked by 5 mM Co^{2+} or 100 nM apamin, identifying an SK type Ca^{2+}-activated K^+ channel. We conclude that a compound slow AHP, composed of potentials generated by a voltage-activated delayed rectifier and an apamin-sensitive SK Ca^{2+}-activated K^+ current, follows repetitive activity in mitral cells. This AHP acts to determine repetitive firing characteristics, and therefore the responses of mitral cells to olfactory input, independently of interactions with interneurons of the MOB.

Supported by NIH DC29218, DC00347, DC03195 and NS29128.

214. Correlation between the loci of mouse taste bud cells in single taste buds and their electrophysiological properties

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We investigated the correlation between the loci of mouse taste bud cells in single taste buds and their electrophysiological properties. In order to map taste bud cells, we used peeled tongue epithelia where many taste bud cells were identified under a microscope with a water immersed objective. The loci of taste bud cells were classified into the central, intermediate, and peripheral concentric circles with the same width, 10 μm that was equal to the doubled diameter of the soma of taste bud cells. Then the mapped taste buds were examined there under tight-seal whole-cell clamp conditions. In the central (five cells) and intermediate concentric circles (14 cells), all taste bud cells examined elicited TTX-sensitive inward currents on depolarization. In contrast, 5/10 taste bud cells elicited no inward currents in the peripheral concentric circle. All taste bud cells examined elicited TEA-sensitive outward currents on depolarization irrespective of their loci. The magnitude of the total outward currents were the intermediate, center, and peripheral concentric circles, in decreasing order. These results indicate that there is a correlation between the loci of taste bud cells and their voltage-gated currents. The mean life span of mammalian taste bud cells are ~10 days and old taste bud cells are replaced with newborn taste bud cells. The differences in their life stages will affect the number or performance of ion channels

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responsible for the inward and outward currents examined. Therefore, it is likely that the newborn taste bud cells shift with ageing from the peripheral to central concentric circle or the central to peripheral one in single taste buds.

215. Influences of the olfactory bulb on the structure of the olfactory epithelium of *Xenopus laevis*

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Retrograde signaling from the brain to the olfactory sensory epithelium is important for neuronal survival, but the role of retrograde signaling in olfactory neuronal plasticity is unclear. We examined the importance of retrograde signaling from the olfactory bulb to the structure of the olfactory epithelium of the African clawed frog, *Xenopus laevis*. Two cavities contain olfactory epithelium in larval *X. laevis*: (i) the principal cavity, used to detect odorants in water, and (ii) the vomeronasal organ, presumably involved in pheromone detection. At metamorphosis the principal cavity is co-opted for detection of air-borne odorants and a new cavity, the middle cavity, develops and senses water-borne odorants. Coincident with the functional changes in the principal cavity are changes in neuronal morphology. To test the role of the olfactory bulb in changes in epithelial structure seen at metamorphosis, we removed the olfactory bulb in larvae at stages 48–50 and allowed larvae to complete metamorphosis (stage 65) before sacrifice. The principal cavity epithelium was substantially reduced in thickness in bulbectomized animals relative to controls. The epithelium of the vomeronasal organ, which undergoes no apparent changes at metamorphosis, appeared to be unaffected in bulbectomized animals. In the middle cavity, the epithelium of two bulbectomized animals apeared no different from controls but the middle cavity epithelium was absent in another bulbectomized animal. While these data are still preliminary, they suggest that target-specific interactions are necessary for normal structure of the principal cavity olfactory epithelium but are not necessary for normal epithelial thickness in the vomeronasal organ. The importance of target-specific interactions on the development of the middle cavity epithelium is unclear from the current analysis. Future experiments will further address these issues as well as the importance of the olfactory bulb on changes in olfactory receptor neuron morphology.

Supported by NIDCD # DC00446 and NIH Training Grant # NS07363.

216. Experience-induced changes in the developing taste system

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During the past 15 years, we have examined how early dietary sodium restriction affects the development of peripheral and central gustatory function and structure in the rat. In addition to characterizing the peripheral and central gustatory functional and structural alterations resulting from early pre- and postnatal sodium restriction, we have begun studies that examine the underlying cellular mechanisms. Converging findings from a variety of our studies strongly indicate that the lack of sodium per se does not affect the development of the taste system. That is, there does not appear to be evidence that lack of sodium contacting developing taste receptors causes our observed effects. Rather, profound influences on circulating factors, such as growth factors and hormones, play a key role in establishing and maintaining normal physiological responses and normal morphological features of the gustatory system. Thus, our results add to an emerging body of literature that shows nutritional factors to be very important in affecting the gustatory system. However, we suggest that the effects in our studies are mediated by at least one other physiological system(s). Therefore, in addition to summarizing the array of findings that we have seen by using this dietary manipulation, information will also be presented concerning the implications of how nutritional factors during very specific periods of development have selective and long-lasting effects on the developing gustatory system.

217. Salt appetite and salt avoidance in an amphibian (*Bufo punctatus*)

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Anuran amphibians take up water by osmotic flow across the ventral skin. Many anurans use a stereotypical behavior called the water absorption response (WR) to maximize skin contact with and water uptake from moist surfaces. When they are given hyperosmotic (250 mM) NaCl, WR behavior of toads is drastically reduced. However, if 1 or 10 mM amiloride is added to the NaCl solutions, WR behavior is partially restored. The effects of 1 and 10 mM amiloride on WR behavior did not differ significantly. These results are consistent with kinetic analyses demonstrating that the inhibition constant for amiloride blockage of Na^+ transport (k_i) is >0.5 mM. Kinetic analyses of the low affinity amiloride analog, 6 chloro-3,5-diaminopyrazine-2-carboxamide (CDPC) showed its k_i to be 40 mM and partial restoration of WR behavior was not seen at CDPC concentrations of >100 mM. Behavioral and kinetic data thus support the hypothesis that the amiloride-sensitive Na^+ channels in toad skin serve a chemosensory function.

In contrast to the avoidance of hyperosmotic NaCl solutions, toads predictably chose dilute (10–50 mM) NaCl over water as hydration sources. Behavioral assays and weight change were used to evaluate the preference for dilute salt solutions in toads dehydrated by ~10% of their standard weight. We found that the duration of WR behavior was greater on substrates saturated with 50 mM NaCl than on deionized water, 100 mM NaCl or higher NaCl concentrations. We found also that toads presented with paired choices of substrates preferred 50 mM NaCl over water and selected 10 mM NaCl over water and all higher NaCl concentrations. Finally, dehydrated toads rehydrating in water or in dilute salt solutions acquired fluid (gained weight) more rapidly in 10–50 mM NaCl than in deionized water or solutions with higher concentrations of NaCl. These results indicate that toads express an appetite for salt that is comparable to the salt appetite expressed in other terrestrial vertebrates and that salt appetite in toads enables them to increase the rate of water uptake.

Supported in part by NSF IBN-9512053.

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218. Cloning of cDNA from rat olfactory epithelium encoding an IP_3 receptor protein

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Inositol 1,4,5-triphosphate (IP_3) has been implicated as second messenger used by olfactory neurons in response to stimulation by selected odorants molecules. Biophysical studies have provided evidence of direct gating of plasma membrane channels by IP_3 in rat olfactory neurons. Immunohistochemical studies, using polyclonal antibodies specific to IP_3R type I localize IP_3R in cilia of olfactory neurons. Western blot has shown at least two polypeptides, 120 and 230 kDa in cilia membrane preparations, consistent with previous photoaffinity labelling studies.

In order to determine molecular identity and organization of IP_3R in the olfactory neurons, a partial cDNA sequence homologous to known IP_3Rs was amplified from ciliary mRNA by RT-PCR. Analysis of purified mRNA by Northern blot, was performed by using a specific radiolabelled probe produced by PCR from this cDNA. Two bands at 10kb and 4kb are detected by this probe. Also, 32 positives clones were obtained in cDNA library screening by using the same probe. Restriction pattern of these clones showed inserts sizes between 250 bp and 3 kb. Sequencing these clones is in progress, further cDNA library screening by probe generated from rat brain IP_3R (Mignery,1990), and 3′ RACE and 5′ RACE PCR reactions are additionally being utilized to generate a full-length cDNA specific to olfactory IP_3R.

Supported by NIH DC-01228 and DC-00566.

219. An approach to a local spatial distribution of olfactory receptor neurons with similar tuning specificities

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Olfactory receptor cells (ORNs) that express the same odorant receptor are randomly distributed in a local epithelial region at least. However the spatial patterning of ORNs with similar odor responsivities has been unclear. In relation to the manifestation mechanism of odor tuning specificities over the olfactory epithelium, it is important to know whether ORNs with very similar tuning specificities distributed within close proximity to each other. To approach this problem, we examined the responsivity to n-aliphatic odorants with a straight chain of 3–9 carbons and the local spatial relationships of individual ORNs of mouse. The ORNs were isolated on a Cell-Tak coated cover glass by the tissue-printing method which was expected to enable to preserve their relative spatial distance in intact tissue within ~200 mm which is two thirds of the width of the epithelial strip. Odor responses of severel ORNs were recorded simultaneously by measuring optically intracellular calcium increases with fura-2. Tuning specificities to *n*-aliphatic odorants were dependent on both the carbon chain length and the functional group (carboxyl and hydroxyl). In most ORNs, the sensitivity was maximal at a single carbon chain length. The similarity of tuning specificities is evaluated by the difference in the carbon chain length of odorant to which the individual ORN is most sensitive. Out of 33 pairs tested, six pairs of which both ORNs were adjacent to each other within 10 mm showed the high similarity where the difference of the most sensitive carbon chain length were less than one carbon. Some pairs with the distance of ~30 mm showed the various similarities of tuning specificity in the carbon chain length. The correlation between the spatial distance and the similarity of tuning specificity in the carbon chain length was weaker than that we reported previously. On the other hand, there existed many ORNs sensitive to other kinds of odorants but not sensitive to n-aliphatic odorants around the ORNs sensitive to a subset of n-aliphatic odorants. Our results indicate that the distribution of ORNs has no strong order in tuning specificity within a distance of 200 mm in a region.

220. Age-related changes in the prevalence of smell/taste problems among the US adult population: results of the 1994 disability supplement to the National Health Interview Survey (NHIS)

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Information about the prevalence of disorders of the chemical senses has been limited. In the late 1970s, a consensus among experts convened by NIH was that more than two million adults in the U.S. had a disorder of smell or taste. A large, nonrandom survey conducted by the National Geographic Society in 1987 found that 1% of their 1.2 million respondents could not smell three or more of six odorants using a 'scratch and sniff' teSt Age was an important factor, with a decline beginning in the second decade of life. No comparable data has been available for taste, although it has been suggested that the sense of taste remains more robust with age. The NIDCD began collaborating with NCHS in 1993 to acquire information on the prevalence of smell/taste problems using the Disability Supplement to the NHIS. This survey was administered to over 45 000 randomly-selected households (80 000 adults) in 1994. Adjusted national estimates show an estimated prevalence of 2.7 million (1.4%) adults with an olfactory problem. Also, 1.1 million (0.6%) reported a gustatory problem. When smell or taste problems were combined, 3.2 million (1.65%) adults indicated a chronic chemosensory problem. The prevalence rates increased logarithmically with age. Almost 40% with a chemosensory problem (1.5 million) were >65 years of age. In a multivariate analysis, we found overall health status, other sensory impairments, functional limitations (including difficulty standing or bending), depression, phobia, and several other health-related characteristics were associated with an increase in the rate of chemosensory disorders.

221. Zinc and copper toxicity of olfactory bulb neurons and the protective effects of carnosine

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Carnosine is an endogeneous neuropeptide which is present in the olfactory bulb (OB) in high concentrations; however, little is known about its function. In an earlier study, we demonstrated that carnosine can modify the neuromodulatory actions of zinc and copper on amino acid receptors. In this study, we found that carnosine also may have a protective role against neurotoxicity mediated by copper, and to a lesser extent, zinc.

The OB contains one of the highest concentrations of zinc

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and copper of any brain region. We have shown previously that zinc and copper can modulate the function of amino acid receptors on OB neurons. In other brain regions, it has been shown that zinc and copper are released by neurons. These data have led us to examine two hypotheses: (i) because zinc released under some conditions (e.g. ischemia) can be neurotoxic, physiological concentrations of copper may also be neurotoxic; and (ii) since carnosine can modify the neuromodulatory actions of zinc and copper, carnosine also may modify the toxicity of zinc and copper.

OB neurons from E21 to P1 rats were dissociated, plated on previously plated OB astrocytes, and cultured for 2–18 days. The culture medium was then replaced with a buffer solution containing the metals (10^{-6}–10^{-4} M), carnosine (10^{-3}–10^{-2} M), or a mixture of metal and carnosine. After exposure for 1 h, the buffer was replaced with Minimum Essential Medium with 4% glucose and neuronal viability was assessed hourly.

We found that within h zinc had a neurotoxic effect on OB neurons similar to its reported effects on hippocampal neurons. Furthermore, we are the first identify similar actions by copper. Both metals had an apparent LD_{50} between 10^{-5} and 10^{-4} μM, well within the range of reported synaptic concentrations. However, when low μM concentrations of copper were applied with 10^{-3} or 10^{-2} M carnosine, the percent of surviving neurons dramatically increased. Carnosine (10^{-2} M) had less of a protective effect on zinc mediated toxicity. This result is consistent with our previous results demonstating that carnosine was more effective in modifing the actions of copper than zinc on amino acid receptors. Although earlier studies have not identified a clear role for carnosine in the olfactory system, our results suggest the carnosine may act as a neuromodulator and a neuroprotective agent.

Supported in part by a grant from the NIDCD.

222. Nasal dilators affect olfactory perceptions

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Although nasal dilators have been shown to decrease nasal resistance as compared to undilated controls, the effect, if any, this decreased resistance has on olfactory ability was heretofore not studied. In the first of thre experiments 10 subjects twice rated the intensity of a series of odorants. During one series, subjects wore nasal dilators whereas for the other series the subjects were undilated. In the second experiment, a loosely applied Band-Aid was used in place of the a nasal dilator in some subjects. In both experiments, the use of the nasal dilator increased the intensity ratings by ~20%. No such effect was found for the Band-Aid. Additionally, as measured by a Fleisch pneumotachograph in a few subjects, the nasal dilator produced no obvious change in the sniff characteristics. These results are consistent with the concept of a perceptual constancy model in olfaction. That is, a decrease in nasal resistance decreases perceived sniff vigor and so produces an increase in perceived odor intensity. The observation in Experiment 2 that the intensity ratings of the distilled water and the CCM solvent were increased when wearing the dilator again seems to argue in favor of a size constancy model. That is, increased intensity ratings of odorless (or nearly odorless) stimuli could likely only happen if the intensity ratings themselves included some component of sniff vigor. In Experiment 3 thresholds were determined with and without nasal dilators. On the average, the presence of a nasal dilator decreased the threshold for phenethyl alcohol by 1.7 $\log_2$ units, suggesting nasal dilators do increase the number of odorant molecules getting to the olfactory receptors. This suggests that one part of size constancy may be a change in the regional airflow.

Supported in part by NIDCD DC00220 and DC00072.

223. Labile short-term memory in conditioned taste aversion learning

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We have recently demonstrated that after an intraoral infusion of sucrose is paired with LiCl, rats express a conditioned taste aversion (CTA) within 15 min of the pairing by rejecting a second intraoral infusion of sucrose. The rapid acquisition of a CTA suggests that CTA has a phase of short-term memory similar to other forms of learning. To determine if short-term CTA memory is labile and decays in the absence of long-term memory, we measured intraoral intake of sucrose after pairing sucrose with low doses of LiCl. Rats were implanted with intraoral catheters, and 17 h food (but not water) deprived. Rats received an intraoral infusion of 5% sucrose (6 ml/6 min); 30 min later, LiCl was injected at three different doses (76, 38, or 19 mg/12 ml/kg i.p.). To control for toxic effects of LiCl, one group of rats received non-contingent (NC) LiCl injections (76 mg/kg). A second intraoral infusion of sucrose was administered 15 min, 1 h, 6 h or 48 h later to different rats in each group (n = 4–6 at each time). Sucrose intake was measured by weighing rats immediately before and after the infusions.

Baseline intake of 5% sucrose was 4.6 ± 0.3 g. At the highest dose of LiCl, intake of sucrose was decreased at all time points after the pairing. At lower doses, decreased intake was observed only at 15 min and 1 h. After NC LiCl, intakes were close to baseline at all points.

LiCl dose	15 min	1 h	6 h	48 h
76 mg/kg	0.0 ± 0.0	0.5 ± 0.3	1.0 ± 0.4	0.8 ± 0.6
38 mg/kg	0.9 ± 0.9	0.4 ± 0.2	4.3 ± 0.7	4.5 ± 0.3
19 mg/kg	1.4 ± 1.2	1.3 ± 0.4	4.8 ± 0.6	3.8 ± 0.9
NC 76 mg/kg	3.3 ± 0.7	4.5 ± 0.7	4.7 ± 0.5	4.7 ± 0.6

Weight gain (g) after intraoral infusion of 5% sucrose (6 ml/6 min).

The formation of long-term CTA memory was dependent on the dose of LiCl paired with sucrose during acquisition. Low doses of LiCl induced a CTA that persisted only for h after pairing. Thus we have demonstrated short-term memory for CTA learning that is rapidly acquired but labile in the absence of long-term memory formation (decaying within 6 h). Because protein synthesis inhibitors can attenuate long-term CTA acquisition, we hypothesize that long-term CTA learning is mediated by gene

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expression but short-term CTA learning is protein-synthesis independent.

Supported by the Whitehall Foundation and NIH DC 03198.

224. Organization of the olfactory bulb (OB) in the larval sea lamprey, *Petromyzon marinus*

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The larval stage of sea lamprey lasts several years, providing a prolonged period to investigate events that occur transiently during early development in other animals. In this study we investigated the organization of the larval OB. As previously observed in the adult lamprey *Lampetra japonica* (Iwahori *et al.*, 1987), lamination was simple. The larval OB is composed of an olfactory nerve layer (ONL), an indistinct glomerular layer (GL), granule cell layer (GRL) and ependymal cell layer. Anterograde tracing by HRP and diO revealed axons extending through the ONL and into the GL, where they terminated either randomly or in small glomerular-like arrangements. The ONL and GL contained a dense matting of tyrosine hydroxylase and synapsin I-IR fibers and vimentin localization. Brain nitric-oxide synthase (bNOS)-IR displayed pert-glomerular organization. Electron microscopy of the GL revealed numerous synaptic contacts. Golgi–Kopsch staining showed mitral cells with unbranched dendrites and granule cells in the central region of the OB. In this GRL, tyrosine hydroxylase-IR fibers were prominent, synapsin I-IR was patchy and bNOS, NCAM and vimentin staining was moderate. The cells of the ependymal region stained intensely for bNOS, NCAM and vimentin and were devoid of synasin I and tyrosine hydroxylase. This study confirms that the larval lamprey is a useful model for investigating the early development of the olfactory system. Minimal glomerular formation, with the exception of distinct periglomerular distribution of bNOS-IR suggests that nitric oxide modulates the organization of olfactory sensory input during glomerular formation.

Supported by NSERC, NIH NS10174 & DC00210. We thank Drs Greengard and Czernik, Rockefeller University for the synap sin I antibody.

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225. Analysis of gene expression in individual taste receptor cells with single cell RT-PCR

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Investigation of the molecular mechanisms underlying taste signal transduction has been hindered by the scarcity of taste receptor cells. We attempted to circumvent this difficulty by using single cell reverse transcription-polymerase chain reaction (RT-PCR) procedure. Mouse lingual epithelium was freed from the rest of tongue by injection of a mixture of dispase II and collagenase B. The circumvallate and foliate papillae were isolated and further dissociated into individual cells by mechanical means or incubation in calcium-free Tyrode's solution. Taste receptor cells were tentatively identified by their characteristic bipolar morphology. This was verified by X-gal staining of taste cells from transgenic mice in which the *lac* Z gene was driven by the mouse gustducin promoter. The single taste receptor cells were either transferred to PCR tubes directly or after electrophysiological recording. The cells were lysed with detergent, then the first strand cDNA was synthesized with oligo dT primers and tailed with dATP. The whole population of single-stranded cDNAs from a single cell was amplified with oligo dT primers that contained restriction enzyme sites. The amplified DNAs were analyzed by PCR with mouse gustducin primers. Among the four electrophysiologically analyzed taste cells, expression of gustducin was detected in one cell which generated both voltage-dependent K^+ and Na^+ currents, and in another cell which produced only voltage-dependent K^+ currents, while gustducin expression was not detected in two other cells, which generated both voltage-dependent currents. Three of eight cells not subjected to electrophysiological analysis expressed gustducin. Single cell RT-PCR should be an effective approach to correlate electrophysiologically determined phenotype with molecular biologically determined pattern of expression.

226. Expression of C-Fos in the olfactory bulbs of newborn rabbits exposed to garlic odor at the time of nursing

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Newborn rabbits can rapidly learn to associate novel odors present on the mother with suckling. For example, 3-day-old pups nursed for just 3 min by a doe who had been scented with garlic odor, will become conditioned and respond to a garlic-scented fur with nipple-search behavior. In order to identify neural changes associated with this rapid odor learning, pups were sacrificed 1.5–2 h after the time of nursing, and the brains examined immunohistochemically for c-Fos expression. Four groups of animals of nine pups each were compared: pups nursed by a garlic-scented female (conditioned), pups nursed by an unscented female (nursed), unnursed pups exposed to garlic odor at the same time (exposed), and unnursed pups exposed to ambient air (baseline).

As expected, only few c-Fos expressing neurones were present in the olfactory bulbs of the baseline group, while significantly more were counted in animals experiencing the odor of garlic while being nursed by their mother. However, unnursed pups exposed to garlic only, and pups nursed only showed similar levels of expression. In all cases, c-Fos expression was unevenly distributed around the circumference of the olfactory bulb and statistical analysis showed this to be non-random. When ratios of cell counts in glomerular, mitral and granular regions were compared, a general correlation between the amount of c-Fos expression was found. To what extent these ratios were affected by treatment is presently under investigation, and results will be presented accordingly.

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227. Experience-induced changes in the developing olfactory system

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The olfactory system is faced already at the receptor level with a problem not encountered in other sensory systems—namely, the high dimensionality and inherent unpredictability of much of the odor world. This raises the question as to how young animals, particularly those receiving little direct parental care, are able to detect relevant odors in their environment, distinguish these from irrelevant background, and evaluate them as positive or negative. Presumably any information they can acquire before being exposed to these tasks would be of advantage.

The European rabbit provides an instructive example. Young rabbits are usually weaned without the opportunity of learning what to eat directly from their mother. However, by feeding pregnant does aromatic juniper berries, which form part of the natural diet, it could be shown that newborn, weanling and even adult animals demonstrate a preference for juniper odor without subsequent postnatal experience, and that this early experience positively influences animals' later food choice. Furthermore, this behavioral preference is associated with enhanced sensitivity at the olfactory receptor level as measured by EOG recording. Together, these neural and behavioral changes suggest that a transfer of information occurs from mother to fetus which may help tune the young to relevant features of the coming olfactory environment.

These findings raise interesting questions regarding the mechanisms supporting such learning: At the peripheral level, is such odor-specific enhancement due to environmental influences in gene expression be it at the level of molecular receptors or neuronal sub-populations, and is the epithelium more readily influenced during development than in the adult? At the behavioral level, does the early acquisition of olfactory preferences represent a distinct form of learning in which mere exposure to an odor is sufficient to induce behavioral change, and how are such preferences to be explained in neural terms given that they must be a product, at least in part, of central processing?

228. Effects of oxymetazoline on olfactory function in acute rhinitis

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The study was performed to investigate dose-related effects of oxymetazoline on both chemosensory event-related potentials (CSERP) and psychophysical measures of olfactory function (intensity ratings, odor discrimination, butanol threshold) during and after an acute rhinits.

The investigation followed a controlled, randomized, double-blind design. Subjects were assigned to groups A, B, or C (six women and six men per group; 19–36 years). Group A subjects always received placebo (solvent), group B subjects received 0.25 mg/l oxymetazoline on the right and placebo on the left side, and group C subjects were treated with 0.5 mg/ml oxymetazoline also on the right side and placebo on the left. Medication was applied to the left or right nostril 15 min before measurements started. After onset of the rhinitis (day 0) measurements were performed on days 2, 4, 6 and 35. Measurements were divided into two sessions. During session 1 CSERP were recorded in response to both olfactory (H2S) and trigeminal (CO2) stimulation of the nasal mucosa; psychophysical testing of olfactory function was performed during session 2.

Oxymetazoline produced an increase of the volume of the anterior nasal cavity. This effect on the congestional state of the nasal mucosa appeared to be independent of the oxymetazoline dosage used. Olfactory perception appeared to improve in a dose-related manner as indicated by changes of CSERP amplitudes N1, overall estimates of odor intensity and the subjects' performance in odor discrimination. In contrast, responses to CO_2 appeared to benefit to a lesser extent from administration of oxymetazoline.

Supported by Bayer AG, Department of Consumer Care, Leverkusen, Germany and NIDCD grant PO1 DC00161:NIH.

229. Analyses of lineal relationships in the mammalian olfactory epithelium

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We have been interested in identifying factors that control neurogenesis and differentiation in the olfactory epithelium. As a first step, we have been examining the lineal relationships among the different cell types in the epithelium, in order to begin to define the processes through which mature olfactory neurons are produced. Here, we have taken advantage of virally-mediated marking techniques to analyze cell types present within populations of cells derived from single marked progenitors.

Using a replication-incompetent retrovirus to mark cells and their offspring in perinatal rats, we have shown that presumptive clones of cells comprise two major types: one, of 1–2 horizontal basal cells, and a second, of variable numbers of globose basal cells and immature and mature sensory neurons. Olfactory nerve lesion, which has been previously shown to increase turnover of sensory neurons, increases both the frequency of, and cell number within, the globose cell-neuron clones. Clusters never contained both horizontal and globose basal cells, and no clusters contained sustentacular cells.

In contrast, using a replication-incompetent adenovirus to mark cells and their offspring, we find presumptive clones composed exclusively of sustentacular cells. This result is consistent with our data generated with retroviruses which suggested that sustentacular cells are not part of horizontal or globose-derived lineages. Together, these data suggest that (i) horizontal basal cells are not precursors of olfactory neurons; (ii) globose basal cells are precursors of olfactory neurons; (iii) sustentacular cells arise from an independent lineage.

Supported by a grant from the NIH and by funds from Tufts University.

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230. Detection of mRNA for nitric oxide synthase, protein inhibitor of neuronal nitric oxide synthase, and heme oxygenase in human fungiform papillae

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Nitric oxide and carbon monoxide are synthesized by nitric oxide synthase (NOS) and heme oxygenase (HO) respectively and are thought to act as cellular messengers in many tissues. Both enzymes occur as constitutive and inducible isoforms. To see if the mRNAs encoding these proteins are present in human fungiform papillae, tissue was obtained under local anesthesia from two adult males and combined. Total RNA was extracted and the polyA+ fraction reverse transcribed to yield cDNA. PCR amplification was performed using primer pairs based on known sequences. Bands of the expected size were gel-isolated, subcloned into pCR2.1 using the TA Cloning Kit (Invitrogen) and their identities confirmed by sequencing. A PCR product (638 bp) for constitutive NOS mRNA was obtained and a BLAST search showed that its sequence had 98% identity with human neuronal NOS. Amplification of inducible NOS was unsuccessful. Recent studies have identified a protein named PIN (Protein Inhibitor of Neuronal NOS) that interacts with NOS. PCR amplification yielded a product (267 bp) whose sequence had 98% identity with rat PIN. Both the constitutive and inducible isoforms of HO mRNA were also detected. The inducible form (270 bp) had 97% identity with a human cDNA. Subcloning of the constitutive isoform (1039 bp) of HO is in progress. The presence in human fungiform papillae of mRNAs encoding these three proteins—NOS, PIN and HO—raises the possibility that both nitric oxide and carbon monoxide may participate in human taste perception.

Supported by NIDCD 1F33DC00219-01, NIDA K21DA-00199-03, NIDR R2910754 and the Department of Veterans Affairs.

231. Insulin and insulin-like growth factor i (IGF-I) in the olfactory system

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The olfactory system is unusual in that higher levels of insulin (INS) and IGF-I are found in the adult rat olfactory bulb than elsewhere in the brain, yet their functions remain to be determined. In peripheral nerves and neuronal cultures, INS and IGF-I acts to promote neuronal growth and differentiation, and it has been suggested that INS in the brain may have neuromodulatory effects. To examine the roles of these neuropeptides in the olfactory system we are first examining the cellular distribution of INS and IGF-I receptors using immunohistochemistry and *in situ* hybridization (ISH). PCR products from olfactory epithelium cDNA were cloned, sequenced to confirm identity and used to generate riboprobes for ISH. Results indicate IGF-I receptor protein and mRNA staining in the supranuclear, supporting cell layer of the epithelium and in nerve bundles. INS receptor staining is very light in normal rat olfactory epithelium, possibly due to internalization in the presence of normally circulating insulin. In diabetic rats, INS receptor-like immunoreactivity is prominent, and is primarily in the olfactory neuron cell body layer. The pattern of staining also differs in the bulb, with INS receptor staining prominent in the glomeruli and olfactory axons, while IGF-I receptor staining is present in fibers surrounding the glomeruli and the mitral cell layer. At one and two weeks after inducing diabetes with streptozotocin, immunostaining for both INS and IGF-I receptors in the epithelium was more intense, suggesting a compensatory response to reduced circulating insulin and IGF-I levels.

Functional assays using calcium imaging of olfactory neurons isolated from normal and diabetic rats are investigating the possibility that INS may modulate neuronal response characteristics.

Supported in part by NIH Grant DC08276.

232. Fractal analysis of EEGs evoked by taste stimulation on the human tongue

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Chaos theory was applied to EEGs measured under resting and taste stimulation conditions and fractal dimensions were computed to quantify the neural dynamics of the brain responsible for taste recognition and discrimination. The seven normal subjects sat on a chair with the chin resting on a frame made of plaster bandage and eyes closed. Distilled water (non-taste stimulus), high concentrated taste (HCT) solution (300 mM NaCl, 500 mM sucrose, 40 mM acetic acid and 1 mM quinine-HCl) and low concentrated taste (LCT) solution (51 mM NaCl, 14 mM sucrose, 3 mM acetic acid and 0.026 mM quinine–HCl) were randomly delivered to the anterior region of the tongue which was protruded slightly out of the mouth. EEGs were recorded from seven positions (Cz, C_3, T_3, T_5, C_4, T_4, T_6) on the scalp. The mean fractal dimension of EEGs in the resting state was 5.42 ± 1.01, and slightly decreased to 4.95 ± 1.01 in the non-taste stimulation state, significantly decreased in the HCT stimulation state to 4.19 ± 1.09; $P < 0.001$, and significantly increased in the LCT stimulation state to 5.76 ± 1.02; $P < 0.001$. These results suggest that information processing of the brain is relatively simple when easily recognized tastes are applied.

233. Inositol 1,4,5-trisphosphate induces responses in rat vomeronasal receptor neurons

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Using the whole-cell mode of the patch-clamp technique, we recorded action potentials, voltage-activated cationic currents and putative second messenger-activated currents from receptor neurons in the vomeronasal sensory epithelium slices of female rats. Current injection of 1–3 pA induced overshooting action potentials. The firing frequency increased with increasing current injections linearly from 1 to 10 pA and reached a plateau at 30 pA, suggesting that rat vomeronasal receptor neurons sensitively elicit

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action potentials in response to a small receptor potential. Under voltage clamp, voltage-dependent Na^+ inward current, inward Ca^{2+} current, sustained outward K^+ current and Ca^{2+}-activated K^+-current were identified. Dialysis of D-inositol 1,4,5-trisphosphate (IP_3) induced inward currents with an increase in membrane conductance in ~54% of the cells and inward current fluctuations in 15% of the cells. L-IP_3 also induced inward currents and current fluctuations in 53 and 13% of the cells, respectively. The mean amplitude of inward currents induced by 100 μM D-IP_3 and L-IP_3 were 84.6 ± 14.0 (SEM, n = 82) and 66.1 ± 9.4 pA (SEM, n = 100), respectively. The IP_3-induced responses were blocked by elimination of Na^+ and Ca^{2+} in the external solution or application of 10 μM ruthenium red. Our results suggested that the IP_3-mediated transduction pathway exists in rat vomeronasal receptor neurons.

234. Immunocytochemical localization of TH and VIP in the fungiform papillae of the frog after denervation

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Sympathetic efferent control of the gustatory system was postulated from electronphysiological evidence, but there is inadequate morphological evidence for such a conclusion.. Thus to further elucidate the relationship between the efferent nerve endings and taste cells in the fungiform papillae of the bullfrog's tongue, we have attempted to determination the localization of tyrosine hydroxylase (TH), as a marker of sympathetic nerve fibers, and vasoactive intestinal peptide (VIP), as a marker of the parasympathetic nerve fibers, by immunocytochemical methods. The GP and hypoglossal nerves on the left side were cut at the level of the angle of the mouth. After surgery, frogs were killed on day 60. In control fungiform papillae of right side, TH- and VIP-immunopositive nerve fibers were found just under the taste disk. In the left fungiform papillae of operative side, TH-immunopositive fibers disappeared, but VIP-immunopositive nerve fibers still remained. These results were in good agreement to our previously observations. These results seem to provide morphological evidence for the existence of an efferent control system in the taste disk.

235. The perceived bitterness of beer and 6-*n*-propylthiouracil (PROP) taste sensitivity

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Pelchat and Danowski found a significantly higher proportion of nontasters of PROP among children of alcoholics than among children of nonalcoholics, suggesting a possible genetic association between alcoholism and PROP tasting ability. They suggested that nontasters may not find alcohol as bitter as tasters do and may be at greater risk for alcoholism because they like the taste of alcoholic beverages more. In the present study we tested this hypothesis by examining how nontasters, medium tasters, and super tasters of PROP judged the taste of two kinds of beer.

Forty-seven males and 53 females between the ages of 21 and 49 rated the perceived bitterness of Budweiser and Pilsner Urquell on the oral Labeled Magnitude Scale (LMS). Subjects also rated their degree of liking or disliking for the two beers on a 9-point Likert scale. PROP papers and the LMS were used to classify subjects into tasting groups.

The results lent some support to the hypothesis in that male nontasters liked the taste of Pilsner Urquell better than male supertasters, when they tasted Pilsner Urquell after Budweiser. (This finding was not replicated for females.) Also, as subjects' ratings of bitterness for the two beers increased, their degree of liking for the taste decreased. Supertasters rated Pilsner Urquell significantly more bitter than medium tasters. Subjects were asked about their drinking habits, and supertasters reported consuming significantly less beer than nontasters when they first started drinking beer on a regular basis. There were no significant differences in current drinking behavior between tasting groups. These results suggest that super tasters are distinct from nontasters with regard to their taste for beer and may to some degree be protected against alcoholism by their dislike for bitter substances.

236. Electrical olfactory evoked potentials for clinical application

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Many pioneers had been reported about the olfactory evoked potential (OEP). But it have not become general clinical tests like ABR. Main reason of hindering clinical application of OEP is the complexity of the olfactory stimulating device. To resolve this problem, we experimented for olfactory evoked potentials produced by electrical stimulation of olfactory mucosa, it was named electrical olfactory evoked potential (EOEP), to apply to clinical use. Aim of this study is to search the possibility of the EOEP as objective olfactometry. Informed consented patients who complained olfactory disturbance were tested. A bipolar stimulation electrode was inserted to the olfactory cleft and the potential change was detected from the frontal sector of the head. Condition of stimulation was the 2 mA, 0.5 ms duration and 300 trials were averaged. Results were compared with T&T olfactometry. EOEPs were well evoked in the smell normal, light hyposmic and also parosmic patients, but nothing was detected from the anosmic patient. The first negative peak ~20 ms after stimulation was recorded clearest through the detectable cases. When some olfactory ability was remained, EOEP was detected. The EOEP is now primary level as olfactometry, but it seemed to be applicable as objective olfactometry. Because this method is easy to perform in the general hospital without special expensive equipment, it seemed to be useful in clinical use.

237. Mdr1 P-glycoprotein in taste buds of rat circumvallate papilla

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Mdr1 P-glycoprotein is a membrane glycoprotein of 150–170 kDa that catalyzes the ATP-driven efflux of various hydrophobic xenobiotics, including fluorescent dyes, from cells. Its location in the apical plasma membrane of secretory epithelia and endothelia

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of the blood-brain barrier suggests that Mdr1 provides a major route of detoxification.

We found that taste cells of the rat circumvallate papilla (VP) had an only slow uptake of hydrophobic fluorescent molecules like Fura-2, BCECF- and calcein-acetoxymethylester. The accumulation measured by fluorescence imaging was accelerated 2- to 4-fold by modulators of Mdr such as cyclosporin A, tamoxifen and verapamil, suggesting that the molecules were prevented from exiting the cells via mdr-mediated transport.

Western blot analysis of VP tissue rich in taste buds revealed the presence of a an immunoreactive protein at 170 kDa, when stained with the monoclonal antibody C219 and C494, which bind to a highly conserved amino acid sequence of mdr1. In contrast, the lingual epithelium surrounding the VP had a much weaker band with these antibodies. Furthermore, using the mdr1-specific antibodies C494 and UIC-2 with tissue sections, mdr1-like immunoreactivity was found in taste cells.

These results show that mdr1 is present and functional in VP cells of rat. Mdr1-related transport may achieve elimination of xenobiotics from sensory cells and thereby protect the peripheral taste organs from a variety of potentially harmful molecules contained in an animal's food.

238. Developmental effects on odor learning and memory in children

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The child version of the California Odor Learning Test (COLT) was designed to assess cognitive functioning in impaired and healthy children. The COLT's rationale was based on two assumptions. First, measures of cognitive functioning are a good index of severity or extent of brain damage if compared between normal and clinical populations. Second, the cognitive-mediated tasks of verbal odor recall, recognition and identification were suitable to evaluate cognitive functioning. The focus of this study was to determine the COLT's ability to detect developmental differences in odor learning and memory. The participants were 51 healthy children who were twice administered the COLT with a weekly delay between tests. They were ascribed to two age-groups (7–10 and 11–15 years old) according to levels of cognitive development. The COLT employed 22 common odors and included two sessions. In the first session, children learned two sets of six odors. The first set was presented three times, the second one once, during a single learning episode. Following learning, children recalled the odors of the first set by name at free-recall and category-cued recall. The second session included the following tasks: long-term free odor recall and category-cued odor recall, odor recognition-memory and verbal odor identification. A series of ANOVAS ($P > 0.05$) with age as between subject variable and repeated measurements on recall revealed significant differences between the two groups on the number of odors correctly recalled by name both at free-recall, category cued-recall, recognition and identification. There were differences in false alarms at odor recognition between the two groups. Children from both groups benefited from a learning effect over odor trials. A gender effect was found for odor free recall at reteSt These results suggest that the COLT has the potential to serve as a useful tool in the assessment of cognitive functioning in children.

Supported by N.I.H. Aging Grant # AG08203 to C.M.

239. Highly specific structure activity relationships among androstanes in the female human vomeronasal organ

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We compared the amplitude of the electrovomerograms of test subjects (20 male, 20 female), in response to picogram quantities of androstanes with different oxygen functionality at the 3-position and with a different number and location of double bonds.

No androstanes showed any activity in the male vomeronasal organ (VNO). However, we found a clear distinction between active and inactive androstanes in the female VNO. Active androstanes may have either a 3-oxo group or a 3b-hydroxy group, but not a 3a-hydroxy group. A double bond in the 4-position appears to be necessary. If such double bond is either absent, or shifted, a complete loss of activity results.

3a-Hydroxy compounds in this series have a distinctive odor whereas the corresponding 3b-hydroxy compounds are odorless. This is the exact opposite of the pattern we observe in the vomeronasal system. Thus androsta-4,16-dien-3b-ol is odorless, but is active in the VNO. By contrast, the stereoisomer androsta-4, 16-dien-3a-ol has an odor, but is inactive in the VNO.

These results indicate that the human vomeronasal system, unlike the olfactory system, is markedly gender specific. Moreover, the putative human vomeronasal receptors themselves are highly specific, and not just responsive to general chemical stimulation.

240. Neurogenesis and migration of the vomeronasal receptor neurons in the opossum, *Monodelphis domestica*

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The sensory epithelium of the vomeronasal organ (VNO) contains primary chemosensory receptor neurons which project to the accessory olfactory bulb (AOB). In the present study, neurogenesis and cell migration were analyzed in the VNO of normal adult, developing young and AOB-ablated opossums (*Monodelphis domestica*) using bromodeoxyuridine (BrdU) labeling. (i) In normal adult opossum VNO, a small number of BrdU-labeled cells were observed which were located in the basal region near the basal lamina of the sensory epithelium 24 h after BrdU injection. The margins of the sensory epithelium where it meets the non-sensory epithelium had similar number of labeled nuclei as in the center of the sensory epithelium. One or two weeks after BrdU injection, the labeled cells appeared in the receptor cell layer and had become receptor neurons as indicated by co-expression of Gia2 or Goa. No evidence of migration from the margins to the center of the sensory epithelium was obtained. (ii) In the developing VNO (at postnatal day 10), there was more BrdU labeling than in normal adult, indicating rapid cell proliferation. Four h after BrdU injection, the labeled cells were located in the basal region near the basal lamina. In the basal region, there were more labeled nuclei near the margins than in the center of the sensory epithelium. Two weeks later the labeled cells were in the receptor cell layer. (ii) In adult animals whose AOB had been removed, BrdU labeling in the VNO was greatly increased

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compared with controls and the labeling was also in the basal region. These observations demonstrate that in the opossum VNO, the process of neuronal turnover is slow in normal adult animals; the proliferating cells are located in the basal region near the basal lamina; these basal cells are the source of new neurons in the normal adults, during development and during reconstitution of the epithelium after retrograde degeneration; and that the newly generated neurons in the basal region migrate vertically into the receptor cell layer.

Supported by NIDCD DC 02745 to M.H.

241. Temporal processing of odour mixtures reveals that identification of components takes precedence over temporal information in olfactory memory

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Temporal processing of binary mixtures results in odorants being perceived in series separated by many hundreds of milliseconds (Laing *et al.*, 1994). Since the odorant perceived first is the main suppressor, knowledge of the order of perception of two odorants can allow prediction of interactions in mixtures. The present study investigated the temporal coding of ternary mixtures composed of carvone, coniferan and triethylamine, and citralva, lillial and triethylamine using a specially constructed air-dilution olfactometer. The results indicated that even though each of the components could be readily identified in ternary mixtures, it was very difficult to indicate which odour was perceived first, with chance scores being recorded. The same outcome occurred even when the 'slowest' odorant was delivered 600 ms after the 'fastest' to the nose. It is proposed that olfactory memory gives precedence to identification of the components of mixtures rather than to their order of perception when more than two odorants are in a mixture.

Supported by a grant from the Australian Research Council to D.G.L.

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242. Associational projections of individual pyramidal cells in posterior piriform cortex in the rat

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Studies of the intrinsic association fiber systems in olfactory cortex with extracellularly injected axonal tracers (Haberly and Price, 1978; Luskin and Price, 1984) have revealed a spatially distributed, non-topographic organization. An important issue with regard to function is the nature of the projections from individual cells that underlie this population-level order. To investigate this question, single pyramidal cells in layer II of posterior piriform cortex (PPC) have been injected intracellularly with biotinylated dextran amine (BDA). Use of survival times of 1 week or more, processing with the Elite ABC kit (Vector), and silver-gold intensification of reaction product has enabled the visualization of what appears to be the full extent of axonal arbors (for the first time).

The results reveal that individual pyramidal cells in PPC have extensive axonal arbors that extend over a large portion of piriform cortex and other olfactory cortical areas. Individual collaterals in these arbors are long, unmyelinated, relatively straight with few branches, and give rise to *en passant* boutons along their entire course. A striking feature is that boutons tend to be spaced at regular intervals rather than clustering at specific locations. Dimensions of the spacing relative to the sizes of dendritic arbors suggest that single pyramidal cells provide synaptic input to a large number of pyramidal cells (>103) distributed over a very large area. The lack of a clustering of axonal branches or boutons further suggests that the strength of input to each postsynaptic cell is weak, and conversely that each pyramidal cell receives a weak input from each one of a large number of other pyramidal cells. This pattern of connectivity is consistent with function as an associative (parallel-distributed) network (Haberly, 1985), although in the absence of functional markers, other principles of operation cannot be ruled out.

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243. Immunolabeling and ultrastructural characteristics of nasal respiratory and olfactory mucosae in newborn and aging macaques

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Two distinct mucosae constitute the mammalian nasal cavity: respiratory and olfactory. Each mucosa has unique functions and epithelial cell types. Nevertheless, little is known about the origin and role of some of the cell types found in these tissues. The present study addresses this issue.

Cells in both epithelia appear to share immunoreactivity to two proteins found in neurons and neuroendocrine cells: protein gene product 9.5 (PGP) and calbindin (CB). In the mammalian olfactory mucosa, including that of man, olfactory receptor neurons (ORNs) are immunoreactive to both proteins. Recently, it has been reported that in perinatal humans, cells in the nasal respiratory epithelium (RE) also show immunoreactivity to PGP and CB. These cells, some of which appeared to overlap into the olfactory mucosa, are similar in morphology and immunolabeling characteristics to neuroendocrine cells of the tracheobronchial respiratory tract.

The present study examines the labeling and ultrastructural characteristics of nasal epithelial cells and fibers from newborn to adult macaques that are immunoreactive for CB, PGP, olfactory marker protein (OMP) and calretinin (CR), all proteins immunolocalized to ORNs in other mammals. Their distribution in the macaque has not been reported.

Light microscopic analysis of tissues from newborns to aged macaques shows PGP immunolocalized to ORNs *and* to

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structurally distinct cells in area of the RE. This demonstrates that at least by postnatal development and into adulthood cells in both mucosae share immunoreactivity to this protein. It also indicates that macaque nasal RE may contain neuroendocrine cells. Thin, ramifying PGP-immunoreactive fibers are seen throughout the nasal mucosa, with some penetrating into both epithelia with *bouton en passant* like varicosities. CB, OMP and CR may be confined to ORNs; this will be confirmed with electron microscopy (EM). Ultrastructural characteristics of immunolabeled cells and fibers in both mucosae are being analyzed and compared using EM.

244. Spatial coding of odorant features within the glomerular layer of the rat olfactory bulb

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Recent studies indicate that there is a large family of individual receptor genes, that each sensory neuron may express only one of these genes, and that projections from sensory neurons expressing the same gene may converge into 2–5 olfactory bulb glomeruli. Based on these findings, it has been hypothesized that receptors might recognize primitive features of odorant molecules, and that each odor then might be represented in the brain by a characteristic spatial pattern of glomerular activation. We have begun to test this model by mapping the metabolic activity of the entire glomerular layer in rats exposed to pure odorants that differ systematically in chemical structure. We exposed 20-day-old rats to air or to an equal vapor concentration (75 ppm) of ethyl acetate, ethyl butyrate, isoamyl acetate, or isoamyl butyrate (5 litters; 1 rat/litter/odor). We sampled uptake of [^{14}C]2-deoxyglucose at 48 fixed angle increments in coronal sections taken throughout the anterior-posterior extent of the glomerular layer and standardized the resulting arrays relative to anatomical hallmarks. Results were displayed as color-coded contour charts. The four esters produced distinct patterns of uptake that were remarkably similar in rats exposed to the same odor. Prior to breaking the code, we attempted to match the rats in different litters that were exposed to the same odorant. We further hypothesized that air would produce the least activity and that patterns would be more complex for the larger odorants due to the greater number of features likely to be present (air < ethyl acetate < ethyl butyrate < isoamyl acetate < isoamyl butyrate). Our assignments of the patterns were 100% accurate. Two small regions (one anterior and midlateral; one midmedial) were stimulated by both isoamyl acetate and isoamyl butyrate, but not by either ethyl acetate or ethyl butyrate, suggesting that the isoamyl esters may share a feature recognized by receptors expressed in sensory neurons projecting to these regions. The location of activity within the posterolateral and posteromedial bulb was more posterior in rats exposed to ethyl acetate and more anterior in rats exposed to isoamyl butyrate, while ethyl butyrate and isoamyl acetate evoked activity in intermediate locations. These responses might be related to overall hydrocarbon content. Our results strongly support the hypothesis that chemical features of odorants are coded spatially within the glomerular layer.

Supported in part by NICHD grant HD24236.

245. Pheromones, the vomeronasal system and communication: from hormonal responses to individual recogntion

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I will review the concept of a 'pheromone' and the degree to which it applies to mammals. A review of previous findings suggests that there may be some chemical substances with special significance for eliciting behavioral or endocrine responses in mammals but that they rarely function as a signal in isolation. Most mammalian secretions are complex mixtures of many compounds, and the resulting odor quality may provide some of the relevant context (for example, species, sex or individual identity). Cues in other modalities may also be important. This view suggests that greater complexity is needed in our models of both the signals and the central processing mechanisms that mediate behavioral or endocrine responses.

The vomeronasal organ (VNO) and accessory olfactory system have often been characterized as specialized in function for (i) reception of large, non-volatile molecues, (ii) reception of specialized chemical signals (pheromones) and (iii) relatively automatic release of biologically important responses. The vomeronasal system clearly has special importance for some responses, but our current knowledge does not fit a simple model. In golden hamsters a specialized signal in vaginal secretions influences male copulatory behavior, and the vomeronasal organ is necessary for these effects. However, the VNO also responds to volatiles in this secretion, and the effects on copulatory behavior only occur in the context of other cues. The main olfactory system is also important for male sexual behavior. In another example, the vomeronasal system in hamsters is also essential for hormonal responses of males to female odors, but it is not essential for such responses to females themselves. Further evidence that the VNO may not be a simple pheromone receptor organ comes from recent data suggesting that the vomeronasal system is essential for discrimination of odors of individuals, an ability that is most likely accomplished by comparing the odour quality of two very similar, complex mixtures that differ only in the proportions of some compounds. If so, this system must be responsive to a wide range of chemical substances and provide the necessary input for a kind of chemical pattern recogntion.

In summary, I suggest that it is necessary to abandon simplistic views of the nature of chemical signals and vomeronasal function in mammals (including humans) if we are to truly understand these processes.

246. Female pheromones modify men's physiology and assessments of women

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Copulins are a mixture of short chained fatty acids found in vaginal secretions of rhesus monkeys, influencing male copulatory behavior. They were also found in 60% of healthy women not taking oral contraceptives. The proportions of the acids change with the menstrual cycle.

To test, if men are able to detect ovulation by these olfactorical

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signals four smell samples were tested. The samples consisted of synthetic fatty acid compositions (copulins) of three different cycle days (menstruation, ovulation, premenstruation) and a water-sample as control. Mixtures followed the descriptions of Michael *et al.* (1975) for five acids (acetic-, propanoic-, butanoic-, methylpropanoic and methylbutanoic acid). Copulins were concentrated at a premeasured threshold of 0.4‰. Subjects were 66 healthy, non smoking male students (mean 23 years). Each subject inhaled one of the samples, heated up to 37°C. During inhalation photographs and voices of women were assessed on attractiveness, dominance and the subject's interest in the assessed woman. Before and after inhalation subjects were required to give a saliva sample. Testosterone levels in the saliva were measured by enzyme immuno assay (EIA).

Salivary testosterone levels changed dependent on olfactory stimulus content. Ovulatory samples produced a distinct increase in testosterone, while the other two copulin-samples caused very little change. The control (water) decreased testosterone, perhaps according to the clinical test-situation. Although the differences are not significant, a replicative study of the effects of the ovulational pheromones on testosterone in four men showed the same increase after inhalation.

The three mixtures did not differ in their effects on assessments, although copulins overall resulted in more positive assessments. Moreover the effect was differentiated in that the more negative an assessment of a woman without smell was, the more the assessment was improved when combined with copulin-smell.

The results suggest a special kind of hidden ovulation, so-called 'facultative ovulation display'. As heightened testosterone levels parallel with selective attention for erotic stimuli, men who smell ovulational copulins will be more sensitive to additional signals of interest of a woman, although their assessments of this woman will not be specifically changed.

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247. The influence of ensheathing cells on olfactory receptor cell neurite outgrowth *in vitro*

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We previously reported that laminin substrates increased primary (1°) neurite outgrowth from olfactory receptor cells (ORCs) *in vitro* (Kafitz and Greer, 1997). To further explore mechanisms underlying the outgrowth of ORC neurites, we have co-cultured ORCs with the ensheathing cells (ENSH) from the olfactory nerve.

ORCs were plated either: (i) directly on monolayers of ENSH [prepared with minor modifications as reported by Doucette (1994)]; or (ii) on coverslips suspended above the ENSH monolayer to investigate diffusible trophic influences of ENSH. In addition, ORCs were co-cultured with either olfactory bulb glia (OBG) or hippocampal astrocytes (HG) or grown on either EHS-laminin (LN) substrates or PLL substrates as controls. The length of ORC neurites was determined after 48 h *in vitro*. Immunocytochemical characterization of the ENSH cultures for p75 NGF receptor and GFAP revealed that those cultures contained more than 80% ENSH. In OBG cultures ~10% and in HG cultures no cells with ENSH characteristics were found. All cells with ENSH characteristics were also LN-immunoreactive. After 48 h in culture ORCs had the longest 1° neurites when they were co-cultured with ENSH. No significant differences in the 1° neurite length were found comparing ORCs grown directly on ENSH and ORCs physically separated from ENSH. ORCs cultured on HG and on EHS-LN showed no significant differences in the ORC 1° neurite length, but on both substrates the ORC 1° neurites were significantly shorter than on ENSH. The length of the ORC secondary neurites did not vary significantly in the different culture conditions.

Our results suggest that while LN appears to contribute to ORC neurite extension, additional diffusible factors released from ENSH are likely to be further determinants of neurite outgrowth. Because the OBG and HG co-cultures did not influence ORC neurite outgrowth as significantly as did the ENSH, it seems plausible to suggest that these diffusible factors may be specific to subpopulations of glial cells.

Supported in part by NIDCD 00210 and NINDS 10174 to C.A.G.

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248. Kinetics of olfactory receptor potentials and a possible mechanism of odor deactivation

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During pheromone stimulation of moth antennae the receptor potential of pheromone receptor cells reaches a constant level while the number of pheromone molecules adsorbed on the olfactory hairs steadily increases. Furthermore, the potential declines after the termination of stimulus exposure even though the pheromone molecules remain adsorbed and chemically unaltered. These observations led to the postulate of a rapid odorant deactivation, which could determine the kinetics of the receptor potential. This is supported by the facts that the time course of the receptor potential differs for various derivatives of the pheromone, and that the deactivation of derivatives apparently saturates at submaximal amplitudes of the receptor potential. Recent *in vitro* experiments (Ziegelberger, 1995) with homogenates of isolated olfactory hairs suggest that the pheromone-binding protein (PBP) is involved not only in pheromone solubilization and transport but also in pheromone deactivation. PBP occurs in a reduced form with one or two intra-molecular disulfide bonds, and in the oxidized form with three disulfide bonds. From kinetic experiments it was concluded that the pheromone is first bound to the reduced form. After activation of the receptor molecules, the complex PBP-pheromone adopts the oxidized form which—hypothetically—is unable to activate further receptor molecules. A quantitative model of receptor activation and pheromone deactivation was developed (with J. Thorson, Oxford) based on biochemical, radiometrical and morphological data, and the kinetics of the receptor potential. The receptor molecules are considered also to serve as enzymes catalyzing the observed redox-shift of the PBP. The advantage of this model is its one-way character, which guarantees that the odorant is deactivated only after it hits a receptor molecule. This would help to avoid the

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deactivation of the pheromone before it had a chance to activate a receptor molecule and, thus, lower the threshold of odor detection.

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249. The effect of ethanol and iso-alpha-acids on taste

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Iso-alpha-acids are the main bitter components in beer. To understand the effect of ethanol and iso-alpha-acids concentration on taste, we prepared 28 solutions varying in ethanol (0, 2, 4, 6% v/v), and tetrahydroiso-alpha-acids (0, 5, 10, 15, 20, 25, 30 mg/l) in deionized water. In a completely randomized design, bitterness and sweetness intensity were rated by 38 subjects using a 13-point category scale. To know the effects of temperature, subjects rated samples at room temperature (RT) and at 8°C on separate days. Each subject rated the samples at each temperature condition twice. Bitterness intensity was increased by raising concentration of tetrahydroiso-alpha acids at both temperatures (8°C and RT). Increasing ethanol concentration from 0 to 4% or 6% also produced significant increases in bitterness. The effect of ethanol on bitterness was reduced as tetrahydroiso-alpha-acid's concentrations was increased. The comparison between samples at two temperature showed that ratings of bitterness were higher at room temperature than at 8°C. Temperature had a greater effect on bitterness ratings for ethanol than tetrahydroiso-alpha-acids. The average rating for sweetness intensity increased slightly as ethanol concentration rose though not all subjects perceived sweetness. These data suggest that ethanol may contribute to taste of beer.

250. Quality discrimination of mixed odors and tastes in elderly people

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The discriminations of odor and taste qualities in 20 young (aged 21–39 years) and 20 elderly (aged 59–74 years) subjects were compared using the triangular test. Four sets of odors, mixtures of suprathreshold concentrations of phenylethyl alcohol and undecalactone, and seven sets of tastes, mixtures of suprathreshold concentrations of sucrose and tartaric acid, were compared. The stimulus solutions were kept at 24°C during the experiments. Each set was randomly presented three times to the subjects. The elderly had a significantly lower discriminability to odors and lower tendency of discriminability to tastes compared to the young. The rates of discrimination showed a correlation between olfaction and taste. There was no evidence that the elderly reporting the decline in their olfactory and gustatory sensitivities showed a lower discriminability and that the elderly not reporting it showed a higher discriminability.

251. Molecular cloning of a voltage-gated potassium channel expressed in taste buds of channel catfish.

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Taste responses to L-arginine in the channel catfish are mediated, in part, by direct gating of nonselective cation channels, resulting in taste cell depolarization. However, in ~50% of the L-arginine sensitive taste cells, L-arginine induces hyperpolarizing responses, consistent with the possibility that K^+ or Cl^- channels also may be involved in the taste responses to L-arginine. To identify K^+ channels potentially involved in taste responses to L-arginine, we isolated cDNA clones encoding voltage-gated K^+ channels using degenerate oligonucleotides with nested PCR and rapid amplification of cDNA ends (RACE). First-stand cDNA synthesized from total RNA, which was isolated from catfish maxillary barbels, was used as a template for the PCR and RACE. The cDNAs were sequenced and the sequence data were compared with available sequences in GenBank using BLAST A full-length cDNA with an open reading frame (ORF) consisting of 2448 nucleotides was identified and the encoded protein (816 amino acids; molecular weight 92.3 kDa) was determined to be a member of Kv2 subfamily of voltage-gated K^+ channels. The ORF is preceded by a 5′ untranslated sequence of 187 bp with in-frame stop codons at positions –6, –111 and –153 and followed by a 3′ untranslated sequence of 178 bp with the poly(A)$^+$ sequence. Sequence analysis indicates that the clone is a full-length cDNA encoding a novel Kv2 potassium channel, thus, designated as fKv2.3. Hydrophilicity analysis of the deduced amino acid sequence predicts six transmembrane domains and a pore region. The corresponding mRNA is expressed specifically in the taste buds of the catfish maxillary barbels. The functions of the cloned K^+ channel cDNA will be determined by expression of the cDNA in *Xenopus* oocytes and pharmacological characterization of the encoded channel.

252. Complex functional attributes of amygdaloid gustatory neurons in the rhesus monkey

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To reveal specific functions of glucose-sensitive (GS) and glucose-insensitive (GIS) amygdaloid neurons in chemical information processing, in the present experiments, extracellular single neuron activity was recorded in the amygdaloid body (AMY) of alert or anesthetized primates during: (i) gustatory stimulations and (ii) microelectrophoretic administration of chemicals. Of the 502 neurons tested, 37 (7.4%) responded to, usually two or more, taste qualities. The hedonically distinct tastants (e.g. orange versus quinine) often elicited opposite firing rate changes of the gustatory cells. More than two thirds of the

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gustatory responses were recorded from GS neurons that represented ~20% of all cells in the AMY. A majority of the taste-responsive neurons exhibited discharge rate changes to iontophoretically applied catecholamines (CAs): the GS gustatory cells were predominantly suppressed by norepinephrine (NA), whereas the GIS taste-responsive neurons were especially facilitated by dopamine (DA). Furthermore, NA- and DA-antagonists were able to attenuate or fully suppress taste elicited responses of a high majority of these neurons. Our findings, along with previous data, indicate a differential functional organization of AMY gustatory cells: The GS and GIS taste neurons display distinct CA sensitivity and appear to differentially integrate feeding-associated humoral-metabolic and motivational information with exogenous chemical signals.

253. Existence of multiple odor receptors in single olfactory neurons of bullfrog and turtle

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The responses of single bullfrog and turtle olfactory neurons to various odorants were measured with the whole-cell patch clamp and with the ciliary recording technique. A large portion of single bullfrog olfactory neurons (~64 and 79% in the whole-cell recording and in the ciliary recording, respectively) responded to many odorants with quite diverse molecular structures, including both odorants previously indicated to be cAMP-increasing and independent odorants. A single odorant elicited a response in many bullfrog olfactory cells; e. g. hedione and citralva elicited responses in 100 and 92% of total neurons examined, respectively. To confirm that a single neuron carries different receptors or transduction pathways, the cross adaptation technique was applied to single neurons of the bullfrog and turtle. For example, application of hedione to single bullfrog neurons after adaptation of an inward current induced by lyral induced a large inward current and vice versa. Ten micromolar hedione induced inward currents in 15 of 26 different olfactory neurons (58%) after application of Ringer's solution and 28 of 49 neurons (57%) after 10 μM lyral. Ten micromolar lyral induced inward currents in 27 of 44 neurons (61%) after Ringer's solution and in 26 of 38 neurons (68%) after 10 μM hedione. These results indicate that pre-application of lyral or hedione did not significantly inhibit the generation of responses to hedione or lyral. It was suggested that most single olfactory neurons of the bullfrog and turtle carry multiple receptors and at least dual transduction pathways.

254. Comparative investigation of formation of behavioral reactions on taste and olfactory stimuli in early ontogenesis in sturgeon fishes

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By application of solutions of different biologically meaningful substances in water (skin extracts of the same species skin extracts of predatory, food substances and 1% solution of NaCl) the food and defensive reflexes were elaborated in juveniles of beluga (*Huso huso*), sturgeon (*Acipenser guldenstadti*), ship (*A. nudiventris*) and sevruga (*A. stelatus*) since their release from caviar to 3 months of age. It was shown that juveniles of all four species failed to acquire any kind of reflexes both on olfactory and taste stimuli. At the age of 8–20 days the conditioned reflexes were elaborated on applied solutions, but were unstable and soon died out. It was possible to form firm conditional reflexes in beluga juveniles at age 23–30 days and sevruga at age 35–45 days. In this occasion the ones on predatory skin extract and significantly slower—the ones on 10% solution of NaCl.

So, the data show that during the first days of postembryonal development (5–8 days) the reactions of sturgeon larvae on food and non-food objects have a generalized character, after which the stage of unstable reactions on chemical stimuli begins. At age 25–40 days these reactions become stabilized and specific both on their character and on their time of appearing.

255. An assay of free amino acids and classical taste substances palatability in grass carp, *Ctenopharyngodon idella*, siblings after long-time rearing on strongly different diets

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The behavioral taste responses of two groups of grass carp siblings 10 months of age and 6–9 cm in body length were compared. Fishes in the first group had blood-worm, *Chironomidae*, as food, fishes in the second group had duckweed, *Lemna minor*, and lettuce, *Lactuca sativa*, as food. Grass carp siblings were separated at 3 months age and were reared on these different diets for 7 months. It was found that vegetarian and carnivorous fishes showed closely similar taste preferences (Spearman rank correlation coefficient, 0.79; $P < 0.001$). Agar-agar pellets, containing sucrose 0.29 M, L-Cys 0.1 M, citric acid 0.26 M, L-Asp 0.01 M and Gly 0.1 M, were the most palatable from 21 common free amino acids and four classical taste substances. Consumption of pellets containing these stimulatory substances was half that of pellets containing blood-worm or lettuce water extract (75g/l). The main part of the free amino acids (L-Asn 0.1 M, Thr 0.1 M, Ile 0.01 M, Pro 0.1 M, Trp 0.01 M, Tyr 0.001 M, His 0.1 M, Nor 0.1 M, Leu 0.01 M, Lys 0.1 M, Met 0.1 M, Phe 0.1 M and some others) were strong deterrent stimuli for grass carp siblings from these two groups. There is a very high positive correlation between the palatability of taste substance and the time of oral processing of pellets by grass carp siblings. The retention time of pellets containing sucrose and L-Cys was 11–13 s, and for all deterrent substances <2 s. These results demonstrate that the taste preferences in fishes are under strong genetical control, or that taste preferences to free amino acids and classical taste substances, at least, do not depend on fish feeding experience.

Supported in part by RFBR (95-04-11754).

256. Comparison of free amino acids and classical taste substances consumption in brown trout, *Salmo trutta* (Salmonidae, Pisces), juveniles from different sea basin populations

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It is know that the taste sensitivity and taste preferences in fishes are highly species specific. The purpose of the present study was to

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assay the intraspecific differences of fish taste responses. The palatability of 21 common free amino acids (L-isomers) and four classical taste substances were behaviorally tested in brown trout juveniles, 5–6 cm body length, belonging to three different geographical population—Terek River (Caspian Sea), Luga River (Baltic Sea) and Vorobjev stream (White Sea). It was shown that fishes from different populations have the same taste preference to various substances: brown trout from all three populations preferred citric acid 0.26 M, and particularly Cys 0.1 M, but ignored sucrose and many free amino acids (Arg 0.1 M, Ala 0.1 M, Leu 0.01 M, The, 0.1 M, Asn 0.1 M, Ser 0.1 M). Some of the free amino acids were deterrent taste stimuli for brown trout (Gly 0.1 M, Gln 0.1 M, Ile 0.01 M, Val 0.1 M, Nor 0.1 M, Met 0.1 M). The ranges of taste substances were rather similar: theSpearman rank correlation coefficient was 0.55 ($P < 0.01$) between Baltic Sea and Caspian Sea brown trout, 0.60 ($P < 0.01$) between Baltic Sea and White Sea brown trout, and 0.76 ($P < 0.001$) between White Sea and Caspian Sea brown trout. The latest, historically very short connection between the Caspian Sea basin and the Baltic Sea and White Sea basins disappeared at least 10–15 thousand years ago. During this period, there were ~300 generations of brown trout, and the fish have kept the taste preferences without strong changes. The obtained results prompted the conclusion that the taste preferences may be considered as species suprapopulational characters.

Supported in part by NSF, MV6300 and RFBR, 95-04-11754.

257. The palatability of sucrose in fishes

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The palatability of sucrose was tested in 22 various species of freshwater and marine fishes from orders Cypriniformes (8), Salmoniformes (6), Acipenseriformes (3), Gadiformes (2) and some others. The tests were performed by offering the fish agar-agar pellets containing sucrose 0.29 M (10%). It was found that sucrose stimulate the pellets consumption (in relation to blank pellets consumption) in three species only which are obligatory (grass carp, *Ctenopharyngodon idella*) or facultative (roach, *Rutilus rutilus*, and dace, *Leuciscus leuciscus*) herbivorous fishes. For grayling, *Thymallus thymallus*, sucrose had a slight deterrent effect; in all other fish species sucrose was an indifferent taste stimulus and did not produce a significant change in fish pellet acceptance. The positive correlation was found between consumption of pellets containing sucrose and sodium chloride 1.73 M (10%) or calcium chloride 0.9 M (10%), and between consumption of pellets containing sucrose and L-Arg 0.1 M, L-Val 0.1 M, Gly 0.1 M, L-Gln 0.1 M, L-Ser 0.1 M or L-Thr 0.1 M. The consumption of pellets containing sucrose and citric acid or other common free amino acids (L-Pro 0.1 M, L-Glu 0.01M, L-Asp 0.01 M, L-Asn 0.1 M) was not connected. It was shown that before the changeover to full exogenous feeding, fish larvae (Siberian sturgeon, *Acipenser baeri*, and stellate sturgeon, *A. stellatus*) already demonstrated ability to discriminate pellets containing sucrose as well as pellets containing sour, bitter and salty substances. The sucrose palatability was the same in specimens having different food experience (grass carp juveniles after long time vegeterian or carnivorous diets) or belonging to different geographical populations (brown trout, *Salmo trutta*, juveniles from Caspian, Baltic and White Sea basins). Our results indicate that taste preference of sucrose demonstrare herbivorous fish species only.

Supported in part by RFBR (95-04-11754) and ISF (MV 6300).

258. Development of masking additive for bitter taste: selective inhibition of bitter taste by phosphatidic acid, phosphatidylinositol and lecithin fraction containing these lipids in high content

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Previously, we demonstrated that lipoprotein composed of phosphatidic acid (PA) and β-lactoglobulin (LG) selectively and reversibly suppressed the frog taste nerve responses and human taste sensation to various bitter substances. Although PA–LG is an ideal bitterness inhibitor as a tool for physiological studies, the proteinious inhibitor presents a number of problems in its practical inhibition of bitterness in drugs and foods. In the present study, we examined the effects of phospholipid such as phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol (PI) and PA on human taste sensation to various substances. PA inhibited the bitterness of various substances dissolved in water without affecting sweetness, saltiness and sourness, although its inhibitory activity was less than that of PA–LG. PI also had the inhibitory activity on bitterness, although its activity was less than that of PA. Hence we prepared a lecithin fraction containing high content of PA and PI from soybean lecithin on an industrial scale and examined its inhibitory effect on bitterness. The lecithin fraction also showed the inhibitory activities for bitterness of various bitter drugs and food. The lecithin fraction belongs to a category of soybean lecithin and can be safely used as an additive to drugs and food. We conclude that the lecithin fraction can be used for practical purposes to inhibit the bitterness of drugs and food.

259. Symposium: Odorant encoding by distributed parallel processing

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An attractive hypothesis, encompassing many experimental data, for how odor stimuli are encoded by vertebrate nervous systems holds that selectivity and sensitivity for odorants arises from the coordinate interactions among several peripheral and central processes in ways that could be referred to as 'distributed specificity'. This idea has arisen from observations (i) that single olfactory sensory neurons (OSNs) appear to be broadly responsive to a number of chemical compounds; (ii) that responses to any one odorant occur over wide regions of the olfactory epithelium. These regions are larger than the regions within which cells hybridizing with a single putative olfactory receptor are found; (iii) that the message for putative molecular receptors is found in OSNs that are widely distributed within circumscribed regions of the olfactory epithelium; (iv) that there is convergence of projections onto a few contiguous glomeruli from OSNs responsive to a

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particular odorant and that express message for one or a few putative receptors and therefore onto single mitral/tufted cells in the bulb; (v) that responses to one odorant are widely distributed across the olfactory bulb; (vi) that mitral tufted cell responses may be maximally responsive to subcomponents of an odorant molecule, implying that many such cells are involved in encoding the entirety of the molecule; and (vii) that the system is strikingly resistant to loss of large amounts of circuitry suggesting that the neural representation for one odorant is not highly localized.

Taken together these data suggest that responses to stimulation by a single odorant in the peripheral olfactory pathway are widely distributed in space and time in a precisely determined manner. These data further suggest that in the olfactory system specific receptors with high binding coefficients for overall molecular structure may not be required for generating highly specific responses. Several artificial olfactory systems have been constructed that are based on a number of these principles. In this symposium distributed mechanisms in another sensory modality—the somatosensory system will be discussed; temporal attributes of odorant encoding in insects will be described: spatial and temporal attributes of odorant encoding in the salamander will be discussed and compared with an artificial system designed on these principles; and general principles of artificial odor recognition derived from olfactory function will be described.

The principles of distributed processing (specificity) in the olfactory system may have relevance for understanding similar modes of processing in other regions of the brain.

260. The *east* gene of *Drosophila melanogaster* is required for normal function of chemosensory neurons of the adult

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Mutations in the *east* gene of *Drosophila melanogaster* affect the firing of a subset of chemosensory neurons of the fly, thereby resulting in abnormal behavioural responses to chemical cues. Extracellular recordings from the taste sensilla on the labellum of the fly show reduced frequency of neuronal firing to various concentrations of salt stimuli, as compared to wildtype. Firing in response to sugars is normal. As expected, gustatory behaviour of mutant flies in response to sucrose is comparable to wildtype, while tolerance to high concentrations of salt is increased.

east expresses in the developing nervous system. In each developing taste sensillum, *east* begins to express in a subset of cells during pupation and continues to express throughout adulthood. Genetic evidence indicates that the dose of the gene product is critical for normal chemosensory responses. Analysis of *east* gynanders indicates that *east* function is required in the gustatory sensilla.

261. The use of affect-toned odours to modulate the human acoustic startle reflex

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The effects of affectively toned odours on the eyeblink component of the acoustic startle reflex elicited by an abrupt auditory stimulus were investigated in 24 normal volunteers. The startle reflex was significantly potentiated during the presentation of unpleasant, and significantly inhibited during the presentation of pleasant odours, relative to a no-odour (neutral) condition. Affective ratings of these odour categories confirmed that emotional state was effectively manipulated by these stimuli in the expected directions. The role of odour hedonics in startle modulation was further confirmed by the finding of a correlation between rated unpleasantness of the odour and the eyeblink amplitude during exposure to that odour. The findings provide support for the theory of Lang and associates that the startle reflex is an objective measure of positive as well as negative affective states in human beings. The results showing a significant bi-directional modulation of the startle reflex indicate that the present experimental paradigm, using olfactory stimuli as the modulating material, may be more powerful than other startle paradigms which have used affectively toned visual stimuli to modulate the startle reflex.

262. Autocorrelational analysis of firing patterns in taste responsive units in the nucleus of the solitary tract of the rat

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Autocorrelational analysis was used to study the temporal organization firing patterns of neurons in the nucleus of the solitary tract (NTS) in the anesthetized rat. Tungsten microelectrodes were used to record single unit responses to gustatory stimuli. These consisted of sapid solutions of 0.1 M NaCl, 0.01 M HCl, 0.01 M QHCl and 0.5 M sucrose. Autocorrelation functions (ACFs) were constructed by measuring the interval between each spike and every other spike in a given trial, regardless of intervening spikes. The number of occurrences of each spike-spike interval was then calculated in 1 ms increment time bins (0–500 ms). For example, an entry of 25 in the 100 ms time bin indicated that there were 25 instances where two spikes occurred 100 ms apart, ignoring intervening spikes. ACFs of spontaneous and evoked activity were of three types. Type 1 ACFs rose to a relatively stable level within 3–5 ms and remained level for at least 500 ms. This type of ACF was most common. Type 2 ACFs were characterized by an initial prominent peak occurring at ~3–8 ms which was twice as large as the remaining, level portion of the ACF. Type 3 ACFs showed a gradual rise over ~40 ms. The remainder of the ACF was then level. The type of ACF shown by a given NTS cell was independent of which stimulus was presented. ACF analysis of spike trains in the gustatory system may offer a method of investigating intrinsic firing patterns in taste-responsive cells. These patterns may then correlate with other functional characteristics of the cell and might be used to predict their role in neural coding for gustation.

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263. Synaptic physiology of rat olfactory bulb revealed by optical imaging of voltage-sensitive dyes

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Synaptic interactions in an *in vitro* slice preparation of the rat main olfactory bulb were studied with high-speed, multiple site imaging of voltage-sensitive dye signals. Horizontal slices of olfactory bulbs from 3- to 4-week-old rats were stained with RH-155 (100 μM), placed in a submerged chamber mounted on an upright microscope and perfused with oxygenated physiological solutions. Optical signals were projected on a 100 element photodiode array. Stimulation in the olfactory nerve (ON) layer evoked a TTX-sensitive compound action potential (half width = 2.8 ± 0.2 ms) that propagated from the stimulation site (0.46 ± 0.03 m/s) into individual glomeruli. Smaller amplitude, longer-lasting depolarizing potentials (half width = 34.3 ± 2.4 ms) were recorded from the glomerular, external plexiform and mitral cell layers. The amplitude of these potentials was largest in the glomerular layer and they were reversibly blocked by removing Ca^{2+} from the bath solution, indicating that they represent postsynaptic potentials evoked in neuronal elements within the glomeruli. The amplitude of these potentials was significantly reduced by the non-NMDA receptor antagonist CNQX (10 μM) and almost completely abolished by further addition of the NMDA receptor antagonist APV (50 μM). In agreement with our previous reports on responses of mitral cells to ON inputs, this suggests that ON inputs to olfactory bulb neurons are mediated by glutamatergic receptors of the NMDA and non-NMDA types. The amplitude of the synaptic potentials was dramatically attenuated (–83.7 ± 19.4%) by application of the $GABA_B$ receptor agonist baclofen (10 μM), and this attenuation was reversed by the $GABA_B$ receptor antagonist CGP 35348 (500 μM). ON stimulation trains consisting of 2–5 pulses, delivered at intervals ranging from 100 to 500 ms, resulted in prominent paired-pulse depression (–84.4 ± 23.9% relative to the conditioning pulse). The paired-pulse depression was significantly attenuated following application of CGP 35348: the amplitudes of the previously depressed responses increased by 273 ± 52%. These findings indicate that ON inputs to the glomerular layer are modulated by $GABA_B$ receptor-mediated *presynaptic* inhibition olfactory nerve axon terminals.

Supported in part by NIH grants NS31078, DC02588, DC03195 and DC00347.

264. Sensory and respiratory responses of normals and anosmics to CO_2 presented by precision olfactometer

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As part of our investigation of olfactory-trigeminal interactions in the perception of nasal irritation we compared normal and anosmic responses to CO_2 in order to evaluate the possibility that this compound could be used to effect trigeminal, but not olfactory, stimulation in normals. We presented 11 normals and five anosmics with analytical grade CO_2 using a precision olfactometer. A range of five CO_2 concentrations from 0.7 to 18.9% (v/v) plus blank controls was presented during 62 trials in each of four identical experimental sessions. Each stimulus lasted 5 s, after which subjects rated odor (O) and nasal irritation (NI) magnitude. Respiratory responses were also measured before and during stimulus presentation. For each session-by-subject combination, we determined the CO_2 concentrations corresponding to 10, 25, 50 and 75% of the 'distance' from the mean response on control trials up to the highest rating recorded.

As a group, normal subjects first began to report some weak odor sensation (elevated relative to that reported on control trials) in the range of 2–3% CO_2. Anosmics reported no odor sensations and, more importantly, were less sensitive in rating NI than normals (except at the highest concentration, to which responses of the two groups were the same). These results suggest that the presence of an intact olfactory nerve increases sensitivity to CO_2. Breathing data were used to calculate inhaled volume (InVol), duration (InDur) and flow rate (InRate) during the first breath after stimulus onset. The results are consistent with the notion that CO_2 sensitivity in the olfactory nerve is at least as great as that for the trigeminal. Taken alone, however, the breathing data do not indicate that the presence of the olfactory nerve increases sensitivity to CO_2. At least one breathing parameter (InRate) appears to be changed by the lowest concentration presented, even though this was undetected by normals and anosmics. Our results support the idea that breathing parameters may rival or even exceed the responsiveness of perceptual endpoints, but call into question the use of CO_2 as a 'trigeminal-only' probe stimulus.

Supported by Center for Indoor Air Research, RJR-UNC Collaborative Olfactory Research Program and NIH Grant DE 06957.

265. Corticosteroid receptors in the olfactory mucosa: demonstration by *in situ* hybridization

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Olfactory mucus bathes the apical receptor surface of the olfactory epithelium and provides the microenvironment necessary for sensory transduction. In mammals, the sources of olfactory mucus are submucosal Bowman's glands although the specific secretory processes are unclear. In other exocrine systems, such as the submandibular gland, secretion occurs via a two step process. Isotonic fluid is released into the glandular lumen via the action of the enzyme Na,K-ATPase. Sodium is then absorbed across the apical membrane of ductal cells resulting in the production of hypotonic secretions. This process again utilizes the energy of Na, K -ATPase. This enzyme, known as the sodium pump, is regulated by the action of corticosteroid hormones. These hormones act by binding to intracellular receptors and subsequently modulating transcription of the Na,K-ATPase gene.

Multiple lines of evidence have demonstrated the presence of high levels of Na,K-ATPase in the olfactory mucosa. Moreover, this enzyme is vital in the maintenance of resting and odorant-stimulated ion flux across this tissue. In order to begin to test the hypothesis that corticosteroids regulate olfactory secretion we have demonstrated the presence of corticosteroid receptors in the olfactory mucosa. Specific RNA probes were generated from linearized plasmids containing short cloned sequences of the rat

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corticosteroid receptor gene, previously amplified by PCR. Using the technique of *in situ* hybridization we have demonstrated the presence of corticosteroid receptor mRNA within the olfactory mucosa. This study supports the concept of systemic regulation of olfactory secretion and suggests that the olfactory mucosa is a target site of corticosteroid action.

266. Cortical olfactory areas identified by fMRI

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The discriminative capacity of the human olfactory system is extraordinary as it can distinguish thousands of odorants at concentrations as low as a few parts per trillion. However, the central processing and cognition of olfaction is still under investigation. In the present study we tried to identify cortical regions activated by olfactory stimulation with fMRI using echo planar imaging (EPI). fMRI data were acquired with a 1.5 T tomograph (Siemens-Vision) using a multisclice EPI sequence (TE 46 ms, 64 × 64 matrix, 27 slices 4 mm, scan time 3 s). Imaging was performed for 70 measurements with a rate of 6 s. Eleven healthy, right handed subjects (four female, seven male; 25–50 years) participated in the experiments. The olfactory stimuli (vanillin and hydrogen sulfide) were delivered within a humidified and temperature controlled constant airflow to the nasal cavity without altering the thermal conditions at the mucosa. The stimulus sequence consisted of 500 ms pulses once every 30 s, with baseline scans at the beginning, in between and at the end of each experiment. fMRI data were evaluated with the z-score method and transformed in Talairach space using the AFNI program. Additional postprocessing was performed with the SPM package. For vanillin significant activation was found in the orbitofrontal region bilaterally with a preponderance to the left side, bilaterally in the parietal region, in the right hippocampus area, and in the left temporal lobe. For hydrogen sulfide activation was found bilaterally but more pronounced to the left orbitofrontal and piriform region, to a lesser extend in the parietal region and the left temporal lobe. The fMRI activation for vanillin and hydrogen sulfide evaluated with different methods revealed consistent activation of the piriform cortex and orbitofrontal areas as well as parts of the parietal and temporal cortices.

267. Molecular development of the olfactory system

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There are, at least, two major questions concerning the molecular development of the olfactory nerve pathway. First, what are the molecular cues responsible for guiding axons from the nasal cavity to the olfactory bulb? Second, what is the molecular basis of axon targeting to specific glomeruli once axons reach the olfactory bulb? Evidence from other systems indicates that axon outgrowth and targeting are mediated by separate mechanisms. Studies in the primary olfactory pathway have therefore focused on the role of the extracellular matrix and ensheathing cells in establishing an initial substrate for growth of pioneer axons between the periphery and brain. Fibroblast growth factor-1 is expressed both by primary olfactory axons and ensheathing cells and appears to stimulate a phenotype in ensheathing cells which is conducive for axon growth. Moreover, ensheathing cells express laminin and heparan sulphate proteoglycans, both of which modulate primary olfactory axon growth. The primary axons also express a multitude of cell adhesion molecules, including N-CAM, L1, TAG-1, N-Cadherin and galectin-1, which regulate fasciculation of axons and hence, may play a role in fascicle formation in the olfactory nerve. Although the olfactory neuroepithelium principally consists of a morphologically homogeneous class of primary olfactory neurons, there are numerous subpopulations of olfactory neurons expressing chemically distinct phenotypes. In particular, subpopulations have been characterized by expression of unique carbohydrate residues and olfactory receptor proteins. Some of these molecules have been recently implicated in the guidance and targeting of axons to specific glomeruli. However, the inter-relationship of these subpopulations and the mechanisms controlling their differentiation remain largely unknown. Very little is known about the molecular development of the second-order olfactory pathway, although axons in this pathway do express unique carbohydrates.

268. Effects of GABA on developing neurons in rat gustatory nucleus of solitary tract

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GABA is an inhibitory neurotransmitter that has differing effects on central neurons at various stages of development. Studies in adult rat brainstem have demonstrated a major influence of GABA in synaptic processing in rostral gustatory nucleus of the solitary tract (NST). To study GABA effects on taste neurons during the period of major functional development, whole cell recordings were made from neurons of the rat gustatory NST in brainstem slices. Rats in three postnatal groups were studied: 0–7, 8–14 and 15–21 days. Rats were anesthetized, the brainstem removed, and horizontal slices containing the gustatory NST were cut at 300 μm. Whole cell recordings were made from 53 neurons in response to a series of hyperpolarizing and depolarizing current pulses. Superfusion with 1 mM GABA resulted in a reduction in input resistance that was significantly greater in the youngest age group. Using GABA concentrations from 1×10^{-4} to 1×10^{-3} molar demonstrated a concentration dependent reduction in input resistance in each group. However, the response function was shifted to the left in the youngest group, indicating greater sensitivity to GABA in early development. More than half of the neurons were hyperpolarized by GABA in all age groups, but depolarizing responses were seen in ~25% of neurons from 0 to 14 days postnatal. The greater sensitivity and more frequent depolarizations in the youngest neurons indicates that GABA responses are not mature in younger animals and suggests that the neurotransmitter may have different functional roles in developing NST than in mature gustatory neurons.

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Supported by Grant DC00456, National Institute on Deafness and Other Communication Disorders, NIH.

269. Change in cytosolic calcium in response to neurotransmitters, observed in Von Ebner salivary acinar cells

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The microenvironments of taste cells in circumvallate and foliate papillae are consist of the saliva secreted from von Ebner's 'washing' glands. The glands are associated with the circumvallate and the foliate papillae only and are known to be the only purely serous salivary gland in mouth. This study is designed to elucidate the change in intracellular calcium activity, evoked by some neurotransmitters, of von Ebner's acinar cells.

Single acinar cells and cell clusters were isolated by a sequential trypsin/collagenase treatment and loaded 2 mM Fura 2-AM for 30 min at room temperature. Fluorescence was excited at 340 and 380 nm. Fluorescence images of resting cells and cells activated by neurotransmitter were recorded and analysed by PTI imaging system controlled by computer.

The intracellular calcium activity was increased rapidly in response to bath application of even 0.1 mM carbachol or 1 mM norepinephrine. In same molar concentration, however, the calcium increase in response to carbachol is much larger than the increase elicited by norepinephrine. The response to carbachol was also observed when extracellular calcium had been removed, indicating release of calcium ions from intracellular stores. These results agree that sympathetic and parasympathetic agonists evoke degranulation of von Ebner acinar cells.

The study of von Ebner gland physiology is expected to aid the understanding of peri-receptor mechanism in circumvallate and foliate papillae, as well as the understanding of excitation-secretion mechanisms in serous salivary gland.

270. Influence of color on flavor perception in soft drinks

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Several sensory studies during the past decades have shown the importance of color in judging food quality. Flavor identification can be confused by inappropriate coloring, and general attributes such as sweetness or flavor strength can be influenced by the choice of color. The foods investigated in most of the studies reported consisted of either commercial products or simple model systems in which color was varied, sometimes in conjunction with one or two other components such as flavor dosage and sucrose concentration.

The present multivariate study uses complex beverages prepared according to designs in which at least five components are varied over two levels. Perceived intensity of basic tastes and specific aroma characteristics of the citrus flavors was measured in order to determine the influence of color on these attributes. The results indicate that color does not affect the judgement made by trained panelists for most flavor attributes. Consumers, on the other hand, are more easily influenced. Quality evaluations (e.g. *natural* as opposed to *artificial* aroma) and impressions of aroma strength can be manipulated by color gradations independent of sensory training.

271. Gustatory nerve transection attenuates quinine-evoked Fos-like immunoreactivity in the rostral nucleus of the solitary tract in rats

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The glossopharyngeal nerve (GL) contains a population of fibers that is uniquely and highly responsive to quinine while single fibers in the chorda tympani nerve (CT) that respond to quinine, do so weakly, and are maximally responsive to other tastants. These results suggest that the GL plays a more substantial role in the quinine-evoked neural signal transmitted centrally. To obtain a picture of the population of neurons in the nucleus of the solitary tract activated by quinine and the roles played by the CT and GL in such activation, we utilized Fos immunohistochemistry in nerve-transected rats. Similar to previous findings, compared with both unstimulated controls (n = 5) and water-infused controls (n = 6), intraoral infusions (7 ml/30 min) of 0.003 M quinine hydrochloride (n = 6) elicited significant increases in the total number of Fos-like immunoreactive (FLI) neurons in the gustatory NST (gNST), most of these in the medial half of the nucleus. Bilateral transection of the GL (n = 6) produced an ~50% decrease in the total number of FLI-neurons following quinine infusion. Surprisingly, the effects of bilateral CT transection (n = 6) were nearly as robust as those of GL transected rats. Combined bilateral transection of CT and GL (n = 6) also produced a significant decrease in the total number of FLI-neurons; the decrease, however, was *not* greater than that found for the single nerve cuts alone. Interestingly, at intermediate levels of the gNST in a dorsomedial subfield, GL transection *was* more effective than CT transection at reducing the number of quinine-elicited FLI-neurons, suggesting the presence of a functional topography within the gNST Collectively, these data support the hypothesis that quinine-evoked FLI-neurons in the gNST result from oral rather than post-ingestive stimulation. The failure for combined transection to further attenuate the *total* number of FLI-neurons suggests that many quinine-induced FLI-neurons in the gNST receive convergent input arising from the CT and GL, perhaps via intranuclear connections.

Supported by NIH grants R01-DC-01628, K04-DC-00104 and DC-00416.

272. Evidence for functional cyclic nucleotide-gated channels in rat olfactory bulb neurons

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Cyclic nucleotides mediate both odor transduction and adaptation in vertebrate olfactory receptor neurons (ORNs) through the direct activation of cyclic-nucleotide-gated (CNG) channels. Recent research has established that CNG channel gene expression is more widespread than originally thought (Kingston *et al.*, 1996),

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and occurs in multiple cell types in the olfactory bulb. We sought to determine whether olfactory bulb neurons possess functional CNG channels that might regulate neuronal activity as they do in ORNs.

Prior experiments using PCR amplification and *in situ* hybridization (ISH) methods detected CNG channel mRNAs in cell bodies of the glomerular, external plexiform, mitral cell, and granule cell layers of the bulb. We have since used bisbenzimide, a non-specific nuclear stain, to counterlabel ISH tissue sections. Cell bodies unlabeled by the CNG channel probes are also present throughout the bulb, suggesting that CNG channel genes are expressed by only a fraction of the cell populations.

Whole-cell patch clamp recordings were obtained from neurons of various morphological classes cultured from embryonic (E17–21) rat olfactory bulbs. Under voltage clamp at holding potentials between –50 and –80 mV, inclusion of 1 mM cGMP in the recording pipette generated sustained inward currents of up to 100 pA in a subset of the neurons tested. These currents exhibited reversal potentials around 0 mV, and were reversibly blocked by application of 3 mM extracellular Cd^{2+}, both features consistent with the pharmacological properties of other CNG channels. Currents with the same properties could also be elicited by bath application of the membrane-permeable 8-Br-cGMP at a concentration of 1 mM. These currents could be blocked with the external application of either 3 mM Cd^{2+} or 40 μM LY83583, a pharmacological agent used to block CNG channel currents in ORNs. These results indicate that olfactory bulb neurons, at least under culture conditions, translate CNG channel mRNA into functional CNG channels. The olfactory bulb is therefore a good model system in which to explore the potential functions of CNG channels in CNS neurons.

Supported in part by NIH grants DC00086 (G.M.S.), NS24803 (C.J.B.) and DC02227 (F.Z.), and an NSF Predoctoral Fellowship (P.A.K.).

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273. Glutamate taste transduction

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Monosodium glutamate (MSG) elicits a unique taste sensation called umami. The umami sensation is potentiated by the presence of 5′ ribonucleotides such as 5′ GMP, and also by the presence of the Na^+ ion. We have used giga-seal whole cell recording to examine glutamate transduction in individual cells of isolated rat fungiform taste buds. Approximately 60% of fungiform taste cells respond to bath application of glutamate (1 mM; holding potential –80 mV). Three types of responses occur: decrease in inward holding current; increase in inward holding current; and a biphasic response, with an increase followed by a decrease in holding current. Similar responses are observed in response to 5′ GMP (0.1 mM). Further, responses to 5′ GMP may occur in cells that are glutamate-insensitive, suggesting that different receptors mediate the transduction of glutamate and 5′ GMP. Simultaneous bath application of glutamate and 5′ GMP results in a synergistic response in some taste cells. Since responses to glutamate are also enhanced by the presence of Na^+, we tested whether amiloride-sensitive Na^+ channels and glutamate receptors are colocalized in the same cells. All glutamate-sensitive cells also respond to amiloride, and all glutamate-insensitive cells are also amiloride-insensitive. These data suggest that Na^+ may interact with the glutamate transduction mechanism. Recently, we have focussed on the identity of the glutamate receptors. L-AP4 (10 μM), an agonist of mGluR4, and NMDA (1 mM), an agonist of the NMDA receptor, induce responses similar to those induced by glutamate. Most cells that respond to L-AP4 show a decrease in inward holding current, while cells that respond to NMDA show an increase in inward holding current. Since activation of mGluR4 in brain decreases intracellular cAMP, we examined the effect of 8-bromo cAMP on the response to glutamate. In most taste cells, 8-bromo cAMP (1 mM) decreases or abolishes the response to glutamate. Taken together, these data suggest that both mGluR4 and NMDA receptors may be involved in the transduction of glutamate.

Supported by NIH grant DC01959.

274. Symposium: Taste transduction: new insights from molecular and physiological studies

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Taste transduction is initiated when taste stimuli interact with receptor sites on the apical microvilli of taste receptor cells. The interaction usually leads to membrane depolarization and transmitter release from taste cells onto gustatory afferent neurons. Ionic stimuli, such as salts and acids, interact directly with apically located ion channels to depolarize taste cells. More complex stimuli, such as sugars, amino acids and most bitter compounds bind to specific membrane receptors which are coupled to G proteins or to apically-located ion channels. This symposium will focus on recent studies which elucidate the role of various signal transducing proteins in taste cells. B. Lindemann will discuss results from molecular cloning studies suggesting that ENaC, the epithelial Na channel that was recently cloned from colon, is also expressed in taste buds. Furthermore, he will present data suggesting that nitric oxide synthase is present in taste buds. T. Miyamoto will discuss physiological studies which support the presence of several different mechanisms for the transduction of salt and acid in different genetic strains of mice. R. Margolskee will discuss new insights into the role of the taste-specific G protein gustducin in sweet and bitter transduction. J. Brand will discuss the purification of the L-arginine taste receptor in channel catfish. These new findings will be discussed in the context of what was previously known about taste transduction.

275. Amiloride does not affect the taste responses of the frog glossopharyngeal nerve to NaCl

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It is well known that amiloride, an epithelial sodium channel blocker, reduces the NaCl taste response of the chorda tympani nerve in rodents. In the frog tongue, amiloride-blockable sodium

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ion channels are found in isolated taste cells. Several researchers reported that the summated response of the frog glossopharyngeal nerve to NaCl was reduced by amiloride. In frogs, however, the sensitivity of the glossopharyngeal nerve to chemical stimuli tends to wane with repeated applications of stimulating solution to the tongue. Thus, the reduction of the response to NaCl in the frog glossopharyngeal nerve by amiloride might not be due to amiloride, but due to fatiguing of receptors. With this in mind, we carefully examined whether amiloride affected the response of the frog glossopharyngeal nerve to NaCl. The frogs were anesthetized with urethane. A single fungiform papilla was drawn into a suction electrode. Unitary discharges from a single fiber, which were recorded with the suction electrode, were analyzed. Each stimulating solution was applied to the surface of the tongue near the suction electrode for 30 s. NaCl at 0.5 M elicited a large response.The stability of each preparation was monitored by application of 0.5 M NaCl before and after each application of stimulating solution. Amiloride at 0.5 mM did not affect the response to NaCl at concentrations of 0.1–0.5 M. In another experiment, a solution of 0.5 M NaCl was applied to the tongue for 20 s, then followed by a mixture of 0.5 M NaCl plus 0.5 mM amiloride. The tonic response to prolonged stimulation with NaCl was not affected by addition of amiloride. We concluded that amiloride-blockable sodium ion channels in the apical membrane of taste cells are not involved in salt taste reception in the frog.

276. Projection and distribution of trigeminal nerve fibers to the facial lobe of fish

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The primary facial taste center of fish is somatotopically organized. This organization is primarily established with mechanosensitive fibers of the facial nerve.Recent anatomical studies also show the somatotopic projection of the trigeminal nerve to the facial lobe in some species of fish. However, the distribution of the trigeminal fibers is not revealed in the facial lobe.

This study was carried out first to reveal whether the direct projection of the trigeminal nerve to the facial lobe is observed among various species of fish and second to reveal the distribution of the trigeminal fibers in the facial lobe. The carbocyanine dye DiI was used as neurotracer in this study. As experimental fish, *Halichoerus poecilopterus*, *Pagrus major*, *Stephanolepis cirrhifer*, *Plotosus lineatus*, *Ictalurus punctatus*, *Parupeneus pleurotaenia*, *Cyprinus carpio* and *Carassius auratus* were used. DiI was applied to the stump of trigeminal root in isolated, paraformaldehyde-fixed brains of these species. After a diffusion period of 10–600 days, the brains were serially sectioned on a vibratome and examined with epifluorescence. In each species the entire trigeminal root was clearly labeled and the whole central projections of the root were easily traced. The direct projections to the facial lobe through the descending trigeminal root were observed in all species examined. The projections were the most prominent in catfishes among fish tested. For example, trigeminal fibers are projected to the entire facial lobe except the trunk-tail lobule in Ictalurus punctatus. These result show that trigeminal fibers contribute to make a sharply defined somatosensory map in the facial lobe of fish as well as the mechanosensory fibers of the facial nerve.

277. Self-inhibition of $CaCl_2$ taste responses: a novel tool for functional dissection of salt-taste transduction mechanisms

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Rat chorda tympani nerve responses to a concentration series of $CaCl_2$ were obtained with the lingual receptive field under current- and voltage-clamp. We found that, unlike other salts, $CaCl_2$ induced a negatively directed change in the transepithelial potential indicating that Ca^{2+} binds to the normally fixed anionic sites of the paracellular pathway rendering it anion selective. Again, unlike other salts, $CaCl_2$ excites taste cells in a dose-dependent manner up to a critical concentration and thereafter, it becomes self-inhibitory. Ca^{2+} has been shown to regulate the degree of 'tightness' of the tight junction in different epithelial cell lines and therefore, modulates the permeability of the paracellular pathways. Evidence presented here suggests that the Ca^{2+} self-inhibition profile is a result of a Ca^{2+}-induced paracellular shunt-mediated blockage. This modulatory effect of Ca^{2+} was tested on three Cl^--salts (Na^+, K^+, and NH_4^+) which are believed to utilize paracellular pathways to gain access to submucosal transduction sites. Ca^{2+} completely inhibited: (i) the amiloride-insensitive component of the NaCl neural response; (ii) the entire KCl response; and (iii) the high concentration domain (>0.3 M) of the NH_4Cl response. The inhibition pattern of Ca^{2+} on the responses of the three salts indicates that their respective transduction sites are accessible through a Ca^{2+}-sensitive transport pathway that they share in common. In conclusion, the results of this study are consistent with the well reported effect of Ca^{2+} on various epithelial cell lines and suggest that Ca^{2+} can be used as a tool to block the paracellular pathway. In addition, they provide further evidence for the involvement of the paracellular pathways in mediating Na^+, K^+ and NH_4^+ taste reception.

Supported by NIH grants DC00122 and DC02422.

278. Performance of surgery-related skills during ambient odor exposure

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Virtual reality is used to train surgeons and to assist in actual surgery. Such surgery is usually performed in environments containing limited olfactory stimuli. We wanted to determine if it was possible to improve the virtual reality performance of surgeons by adding odors to the environment. As a first step in addressing this question, we tested whether ambient odors could improve performance on surgery-related skills. Two studies tested the ability of ambient odors to influence performance on tasks requiring fine-motor skills and visual-spatial cognitive ability. In Experiment 1, 90 individuals worked on five visual-spatial tasks and completed mood, health, and environmental questionnaires during exposure to one of three ambient-odor conditions (no odor, peppermint, or lavender). In Experiment 2, 96 individuals worked on six fine-motor tasks under the same conditions. The results indicate that certain ambient odors can enhance performance on visual-spatial cognitive tasks but have a minimal effect on

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the performance of fine-motor tasks. Ambient odors also can influence perceptions of well-being and performance, often dependent on odor-pleasantness ratings or in interactions with subject gender and stress level. The results suggest that certain surgical skills can be enhanced by exposure to particular ambient odors. Future studies need to investigate the effect of ambient odors in actual virtual reality scenarios and to test odors that might be expected during surgery (i.e. odors of antiseptic, bile or blood).

This research was supported in part by a grant from the Advanced Research Projects Agency (DAMD17-94-J-4113).

279. Cerebral regions sequentially activated after primary gustatory cortex by gustatory stimulation in human

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In a previous study, we applied taste stimulation with an abrupt rise time to the tongue tip of the human subjects and measured very subtle changes of magnetic fields (MFs) from the human brain with a whole head SQUID system. From the short latency of the gustatory MFs and the anatomical location of the dipole estimated, we concluded that the primary gustatory area (area G) in humans was located at the transition between the operculum and insula. In the present study, we atempted to investigate the areas activated after the activation of area G. MFs evoked by stimulation with 1 M NaCl and 3 mM saccharin were recorded by using a 64-channel whole-cortex SQUID system (CTF System Inc., Canada). Stimulus duration was ~400 ms and the inter stimulus interval ~30s. The temperature of the tastant was ~36°C. During the recording session, the subject wore ear plugs, kept his eyes open and watched a fixation point. We sampled MFs at a rate of 250 Hz with a low-pass filter of 40 Hz. Forty trials were presented to a subject per session. After averaging available trials, we estimated current dipoles (ECDs), superimposed the ECDs on MRI-CT, and decided the activated region. We identified these areas in temporal sequence of activation after area G. The superior temporal sulcus, hippocampus, frontal operculum, and parietal lobe were sequentially activated after area G. Several cortical regions, such as the superior temporal gyrus and hippocampus, were recognized as activated areas in PET experiment but the seqeunce of activation was first clarified in the present study. Since the spatial resolution in the inferior part of the brain is not excellent in MEG measurement, activation of hippocampus remains to be studied further.

280. *In vivo* responses of single olfactory receptor neurons of brown bullhead catfish, *Ameiurus nebulosus*, to amino acids

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The olfactory organ of bullhead catfish is extremely sensitive to amino acids. Responses of single, spontaneously active, olfactory receptor neurons (ORNs) to amino acids (0.01–10 mM) were recorded *in vivo* using metal-filled glass microelectrodes tip-plated with platinum. Single-unit discrimination was performed in software (written by T. Amon). Of 48 ORNs sampled, only a suppression of spontaneous activity to amino acids was observed in 25 cells, whereas excitation was seen in five cells and suppression and excitation to different amino acids occurred in two cells. Eight of 17 ORNs tested with 2–6 amino acids (L-norval, L-ala, L-arg HCl, L-lys HCl, L-cys, L-met) responded to more than one stimulus. ORNs responded to different amino acids with a change in firing rate in 17/21 tests (81%) with 10mM amino acids (n = 14 ORNs) and in 14/51 tests (27%; n = 36 ORNs) to 0.01 mM stimuli. These latter results indicate that amino acid thresholds for the same amino acids are different for different ORNs. ORNs (n = 14) that were suppressed by a low concentration of an amino acid showed increased suppression when tested with higher stimulus concentrations.

Supported by MZT J1-7127-0487 (T.V.) and MZT S16-0487-014/15235 (A.K.).

281. Effect of coffee aroma on regional cerebral blood flow duing an auditory oddball task

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To investigate changes in cognitive function induced by coffee aroma, rCBF during an auditory oddball task was measured in nine healthy males smelling the follwing odors; coffee, lavender oil and distilled water. rCBF was measured in 24 region of interests (ROIs) by using positron emission tomography (PET). The stimuli of the odball task consisted of two tone bursts of different frequencies. No significant differences were found in the value of rCBF among the odors in any ROI. However, the rCBF value under coffee aroma was the highest in the majority of ROIs of the right hemisphere (piriform cortex, hippocampus, nucleus accumbens septi, orbitofrontal cortex, hypothalamus, thalamus, transverse gyrus of Heschl and anterior part of superior temporal gyrus). In contrast, in the left hemisphere, it was lowest in seven ROIs. Furthermore, the rCBF value under coffee aroma was higher in the right hemisphere than in the left hemisphere for any pair of the 12 areas. These results suggest that coffee aroma mainly activates the limbic system of the right hemisphere which is closely related to emotional function.

282. The role of G protein-coupled IP_3 transduction cascade in taste reception of the fleshfly

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We previously suggested that the response of labellar taste receptor cells of fleshfly *Boettcherisca peregrina* to sugars, amino acids and a nucleotide is mediated by G protein-coupled transduction cascade (Koganezawa and Shimada, 1997). In the

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this study, the effects of IP_3, neomycin (an inhibitor of IP_3 production) and ruthenium red (a blocker of the IP_3-gated channel) were investigated on the taste receptor cell of the fly. To introduce IP_3 or neomycin into the receptor cells, the tip of a chemosensory hair was treated with IP_3 or neomycin in 0.03% deoxycholate. During treatment with IP_3 + deoxycholate, spikes of sugar receptor cell were elicited which were not observed during treatment with deoxycholate alone. This IP_3 induced response was increased in dose dependent manner. After treatment with neomycin + deoxycholate, the responses of the sugar receptor cell to sugars and amino acids were depressed compared with those after treatment with deoxycholate alone. The depression was remarkable in the response to D-glucose. However, such depressive effect was not observed in the response of salt receptor cell to cAMP. When the taste stimuli mixed with ruthenium red, the responses to the sugars and amino acids were inhibited. Ruthenium red was especially effective on the response to D-glucose. On the contrary, the response of salt receptor cell to cAMP was not depressed but enhanced. These results suggested that the response of sugar receptor cell of fleshfly was mediated by IP_3, while the response of salt receptor cell to cAMP was transduced by some other second messengers.

283. Pharmacological experiments on goldfish demonstrate significant differences between bulbar mitral and ruffed cells

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Potentials from mitral cells (MC) and ruffed cells (RC) were simultaneously recorded. Epithelial application of TTX had no recordable effect on MC and RC. Anesthesia (Tricain, 30 mg/ml) of the epithlium, however, always resulted in a rapid and total blockade of MC activity. The number of RC potentials simultaneously increased, and remained on a constant and regular level of activity. In agreement with anatomical investigations, RC have no synaptic input from the olfactory epithelium. The frequently burst-like activity of MC obviously is triggered by the activity of epithelial receptor neurons, and MC via granule cells (GC) laterally inhibit RC. After removal of anesthesia eventual bursts of MC potentials resulted in a simultaneous blockade of regular RC potentials.

The regular spontaneous activity recorded from RC during epithelial anesthesia obviously is directly or indirectly triggered by bulbopetal inputs from telencephalic nuclei: blockade of glutamate receptors following bulbar application of 1 mM AP5 (DL-2-amino-5-phosphono valeric acid), an NMDA (*N*-methyl-D-aspartate) antagonist) resulted in total suppression of MC activity, and frequently in a delayed suppression of RC activity. In contrast to epithelial anesthesia by Tricain, application of olfactory stimuli eliciting an excitation of spiking activity of MC (and a simultaneous suppression of RC activity) before AP5 induced glutamate blockade, resulted in similar effects during epithelial application after glutamate blockade. In other words, bulbar application of AP5 blocked glutamate receptors during the interstimulus interval, not, however, when great amounts of glutamate were released during epithelial application of a MC exciting stimulus.

Supported by DFG 112/7-1.

284. Sodium detection during the water absorption response in *Rana pipiens*

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While taste in vertebrates is typically associated with specialized receptors in the lingual epithelium, Hoff and Hilyard in 1993 reported that the toad *Bufo punctatus* is able to 'taste' sodium with the abdominal skin. This was reflected in a differential behavioral escape response to hypertonic NaCl. The present study tests for the presence of such abdominal chemoreceptors in the frog *Rana pipiens*.

The experiment was a five-condition design in which frogs were placed on filter paper saturated with: deionized water, 250 mM NaCl, 350 mM NaCl, 12.9 μM amiloride and 350 mM NaCl + 12.9 μM amiloride. The time that the frogs remained on the test substrate before escaping to a surface of deionized water was recorded as the latency to escape. It was necessary to dehydrate the frogs to 80% of their body weight to elicit a behavioral response to the NaCl whereas dehydration to only 90% has been reported effective in *B. punctatus*.

The frogs displayed significantly shorter latencies of escape response to both concentrations of NaCl as compared to deionized water with the shortest latencies occurring when 350 mM NaCl was used. Amiloride alone did not have an effect upon escape latencies but did successfully block the escape response to the 350 mM NaCl. The results thus indicate that *R. pipiens*, like *B. punctatus* have epithelial chemoreceptors for the detection of NaCl on hydrated surfaces and that these receptors, like those of mammals are amiloride sensitive.

285. Evolutionary ecomorphology of chemosenses in fish

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Fish chemosensory equipment, olfaction and taste, may vary greatly between species. In a broadly comparative approach we ask for the evolutionary and ecological conditions of this diversification. With an estimated total of 25 000 species, teleosts represent the largest radiation of vertebrates, occupying virtually all aquatic habitats. This offers an opportunity to relate ecology with brains and sensory systems. By comparing closely related species from different habitats, cods, cyprinids and African cichlids, we scanned for patterns of divergence and, by comparing distantly related species from similar habitats, intertidal and deep-sea, we examined convergence. We specifically ask, how habitats relate to sensory equipment, particularly chemosenses. In most fishes, visual orientation dominates. In addition, lateral line and hearing are highly developed in epi- and mesopelagic species. In bathypelagics, brain size and the lobes for vision and taste are greatly reduced. Towards shallow water and deep sea benthic habitats, chemosenses increase in importance and vision may be reduced, particularly in turbid environments. Shallow tropical marine and freshwater reefs (African lakes) further visual predominance and seen to excert a considerable selection pressure towards increased size of the (non-olfactory) Telencephalon. The development of cognitive skills (spatial learning, problem solving)

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in fish seems to be associated with visual orientation and well-structured habitats, but not with well developed chemosenses.

Supported by the Verein der Forderer KLF.

286. Gamma-aminobutyric acid (GABA) and baclofen alter ion fluxes in axons of the garfish, *Lepisosteus platostomus*, olfactory nerve

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Receptors for GABA, the major inhibitory transmitter at synapses in mammalian brain, have been identified in the glomerular layer and mitral cell layer of the vertebrate olfactory bulb. In other parts of the nervous system, GABA receptors have been also found extrasynaptically in thin, unmyelinated, sensory nerve axons, similar in structure to olfactory nerve axons. Therefore, we tested the hypothesis that GABA receptors are located in olfactory receptor neurons by measuring potassium efflux from olfactory nerve axons in the presence of GABA and baclofen, a selective agonist at $GABA_B$ receptors. Potassium efflux is a sensitive measure of changes in membrane potential. The garfish olfactory nerve is useful for these studies since it contains an easily accessible, homogeneous population of 10^7 unmyelinated axons. Olfactory nerves were removed from animals of either sex according to APS/NIH guidelines and attached to wire frames, incubated in a bicarbonate-buffered Ringers solution containing ^{42}K. A flow-through system was used to determine the rate coefficient of ^{42}K efflux at 27°C in isotope-free perfusate. In control experiments in the absence of GABA agonists, K efflux was ~0.02 min^{-1} over the 30 min efflux period. In the presence of 100 mM GABA, K efflux increased maximally by 22 ± 9% (±SEM, $n = 5$, $P < 0.05$). Baclofen, at 100 mM, also increased K efflux by a maximum of 22 ± 7% ($n = 5$, $P < 0.05$). Since increasing the extracellular potassium concentration also increased K efflux from this nerve, the results are consistent with GABA- and baclofen-induced depolarizations of the membrane potential. From these studies we conclude that the olfactory nerve axons of the garfish contain $GABA_B$ receptors and possibly $GABA_A$ receptors.

Supported by NIH GM50317.

287. Receptor cell density and olfactory sensitivity in senile rats (*Rattus norvegicus*)

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Earlier studies suggested that in the rat a correlation may exist between receptor cell density in the olfactory epithelium and olfactory sensitivity on one side and age of the animal on the other side. Receptor cell density for up to 33 months old Charles River rats was measured and was found to decline in animals older than 18 months. In another study using Wistar rats, however, only up to the age of 7 months, a similar age dependent change in cell density was shown; no information on senile Wistar rats was available. Since ontogenetic changes in e.g. the olfactory bulb differ in different rat strains, it seemed inappropriate to transfer data from one strain to another. Therefore, to correlate the olfactory sensitivity of 31 months old Wistar rats, which is significantly below that of young-adult rats, receptor cell density in senile Wistar rats was estimated.

Male Wistar rats 31 months old served. They were perfused under deep anesthesia with saline followed by formaline/glutardialdehyde fixation (Karnovsky). The right olfactory mucosa was dissected of the cartilaginous septum, cut in pieces of ~2 mm^2 and embedded in Araldite. From different areas of the epithelium 1 µm thin sections were cut in the horizontal plane and stained with toluidine blue. To estimate the number of receptor cells, olfactory knobs were counted. For each animal, knobs were counted at a total length of at least 1.5 mm of the epithelium and mean density of receptors per 1 mm^2 surface area was calculated. The data reveal a significant age dependent decrease in receptor cell density in senile rats. Mean density in e.g. 7 months old rats was: $9.38 \times 10^4/mm^2$; 31 months old rats: $6.18 \times 10^4/mm^2$ (significance of difference: $P < 0.05$). The data confirm that in senile Wistar rats the proposed relationship between olfactory sensitivity and receptor cell density still exists. Besides receptor density the convergence ratio between receptor neurons and mitral cells has to be taken into account when comparing the sensitivity of different age groups and strains.

288. GABA-immunoreactive neurons in the medial amygdala project to the salamander olfactory bulb

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Centrifugal projections to the main olfactory bulb, mediated by excitatory transmitters, are characteristic of the vertebrate olfactory system. In addition, GABA was found to be involved in centrifugal innervation of the mammalian olfactory bulb. Using retrograde axonal tracing, we previously demonstrated that, in the tiger salamander, *Ambystoma tigrinum*, some telencephalic neurons project to the olfactory bulb. In the current study, the sources of bulbopetal projections in the salamander were further investigated to determine whether they contain a GABAergic component. GABA-immunoreactive (GABA-IR) neurons were observed in telencephalic structures which contained bulbopetal cells. A combination of GABA immunohistochemistry with retrograde axonal tracing showed double labeled neurons, which were mainly located in the pars medialis of the amygdala (APM). These data suggest that the salamander olfactory bulb receives GABAergic afferent fibers, and their main locus of origin is the APM.

GABA-IR telencephalic projections, terminating on granule cells, were identified in the frog olfactory bulb. Furthermore, spontaneous GABAergic synaptic input to granule cells has been recently reported in the salamander olfactory bulb. Such an inhibitory action may be provided, at least partly, by axons of GABA-IR neurons found in the APM. Owing to reciprocal connections between the APM and olfactory bulb, the activity of this input is likely proportional to the intensity of bulbar output. Finally, these findings indicate that GABAergic centrifugal influences on the olfactory bulb are present not only in mammals, but also occur in evolutionary divergent lines of tailed and anuran amphibians, which are widely used for investigating the vertebrate olfactory system.

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Supported by NIH research grant 5 PO1 DC 00161 from the National Institute on Deafness and Other Communication Disorders.

289. Central nervous correlates of chemical communication in humans

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The recording of chemosensory event related potentials (CSERPs) has been established as an objective method in the assessment of central odor processing in humans. In the present study CSERPs were used to investigate whether the human body odor is genetically determined by the major histocompatibility complex (MHC), referred to in humans as the human leukocyte antigen (HLA). The immunological function of the MHC is the discrimination of self/non-self within the immune system. In rodents it has been shown that the body odor is significantly influenced by the MHC and that it can be discriminated by members of different species.

To create a sufficiently large subject pool, 144 subjects were screened for their HLA class I loci A and B. During the EEG-session the subjects (n = 40/20 women) were confronted with the body odor (axillary hair) of three different donors. Two donors (d1 and d2) were HLA-similar but had a different HLA-type than the third donor (d3) and the perceiving subject. The third donor and the perceiver shared a similar HLA-type. Half of the perceivers received odors from donors of the same sex, the other half smelt odors from donors of the opposite sex. In the EEG-session subjects were presented with 200 trials. The odors were delivered through a constant flow olfactometer non-synchronously to breathing. The odor of d1 appeared frequently (P = 0.6) whereas the odors of d2 and d3 appeared each at a rate of P = 0.2. During half of the trials the subjects were instructed to respond to the odor of d2, during the other half to the odor of d3. The EEG was recorded from Fz, Cz, Pz, F3, P3, F4 and P4 in reference to linked mastoids. First results show that male perceivers show enhanced potentials in response to male donors of a similar HLA-type (d3). The CSERP results of the other groups as well as valence and attractiveness ratings will be discussed.

The study was supported by the Stiftung Volkswagenwerk (I/656 14).

290. Quantitative relationships between taste bud development and gustatory ganglion cells

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To determine whether patterns of taste bud innervation change during postnatal rat development, the number of geniculate ganglion cells that innervate single taste bud were quantified for the tip- and mid-regions of the tongue in adult and developing rats. While there was a large variation in numbers of ganglion cells that innervate individual taste buds, there was a high degree of organization in the system. Namely, the number of labeled geniculate ganglion cells innervating a taste bud was highly correlated with the size of the taste bud. This relationship between taste bud size and number of innervating ganglion cells develops over a prolonged postnatal period. Specifically, there was no correlation between taste bud size and number of innervating geniculate ganglion cells, in 10-, 20- or 30-day-old rats. However, there was a correlation between taste bud size and the number of innervating ganglion cells in 40-day-old rats. Interestingly, taste buds did not reach their adult size until postnatal day 40. Therefore, the relationship between taste bud size and innervation does not develop until taste buds reach their adult size.

In a second series of experiments, we sought to determine whether neural rearrangement of chorda tympani neurons is responsible for the development of this relationship by double-labeling single taste buds at different ages. We found that the number of ganglion cells innervating an individual taste bud on postnatal day 10 was correlated with the size that the taste bud becomes by postnatal day 40 and that most of the ganglion cells labeled at 10 days were also labeled at 40 days (i.e. double labeled). This finding suggests that neural rearrangement is not responsible for establishing the relationship between taste bud size and the number of innervating ganglion cells during development. More importantly, it strongly suggests that the 'neural template' for the mature innervation pattern is determined during early postnatal development. Accordingly, the number of ganglion cells that innervate taste buds during early development may determine their mature size. Thus, the developing taste system could provide a unique opportunity to examine an alternative mechanism responsible for the coordination of peripheral morphology and innervation during postnatal development.

Supported in part by #R01 DC00407.

291. Development of a regional taste sensitivity test using isointense and isoviscid stimuli

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We sought to develop a highly reliable, suprathreshold, regional taste sensitivity test using isointense and isoviscid stimuli. Subjects (n = 10) used magnitude matching to equate the intensities of five stimuli based on a.3095 M NaCl solution. Cellulose gum was added to stimuli and an Ubbelohde kinematic viscometer used to equate viscosities. Viscosities were equated to reduce bias arising from tactile cues as well as to increase control of localization of stimuli on the tongue. Test design included a method for alleviating sour/bitter confusion by administering presentations in modules of sweet, sour and salty, versus sweet, bitter and salty. Compounds representing sweet were sucrose and aspartame; those representing salty, sodium chloride and potassium chloride; and those representing sour and bitter, citric acid and caffeine, respectively. Stimuli are presented in 15 µl amounts using an Eppendorf pipette, in randomized order, to the right and left anterior tongue [innervated by the chorda tympani nerve (CNVII)], and to the right and left posterior tongue [innervated by the glossopharyngeal nerve (CNIX)]. Presentations of individual stimuli within modules are made once in each tongue region before moving to the next module. Modules are used six times, generating a total of 72 trials. Subjects are required to rinse their mouths with distilled water between presentations. Responses are forced-choice

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and subjects are asked to provide confidence ratings (0–5) for answers as well as intensity ratings for stimuli on a nine-level, log-based, scale of shades of gray. Normative data are being collected.

Supported by NIDCD grant PO1 00161, NIH.

292. Second messenger signaling in olfaction

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Biochemical and physiological studies support the notion that the olfactory signaling cascade is mediated by heterotrimeric GTP-binding proteins. Towards an identification of the G-proteins functionally involved in the transduction of odor stimuli, a novel approach was employed: photoaffinity labelling of receptor-activated G-proteins with [α-^{32}P]GTP–azidoanilide followed by immunoprecipitation with subtype specific G-protein antisera. It was found that odorants, which elicit a cAMP-response induced the labelling of a Gαs-protein, whereas odorants which elicit an IP_3-signal caused labelling of Gαo-proteins. These observations were extended by immunological approaches, demonstrating that PLCβ2, which is activated by βg-subunits of the $G_{i/o}$, family is enriched in olfactory cilia. In order to approach the question if the two seond messenger cascades may interact, the level of cAMP was elevated either by pretreatement with cAMP-odors or appropriate agents, like forskolin, prior to stimulation with IP_3-odorants. The results indicate that at higher cAMP-concentrations, the PPI-hydrolysis was inhibited; in contrast, reduced cAMP-levels, by inhibiting adenylyl cyclase (MDL, ddAdo) led to augmented odor-induced IP_3-responses. Similarly, pretreating cilia with IP_3-odorants prior to stimulation with cAMP-odors caused a reduce cAMP-response, whereas inhibition of the PPI-pathway (U73122) led to amplified odor-induced cAMP-responses. These results are indicative for a cross talk between the two second messenger systems. In the attempt to explore the mechanism how e.g. cAMP may affect the IP_3 pathway first results indicate that protein kinase A mediates the attenuating effect. This observation is in line with recent studies in COS cells demonstrating that cAMP-dependent protein kinase directly phosphorylates and thereby inhibits Gβg-activated PLCβ2, but not Gαq-activated PLCβ1 (Liu and Simon, Nature, 1996); such a mechanisms could also be active in olfactory neurons governing the efficiency of the phosphatidylinositol pathway.

Supported by the Deutsche Forschungsgemeinschaft.

293. Molecular cloning of *smell-impaired* genes that affect odor-guided behavior in *Drosophila melanogaster*

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We have used single *P*-element *P[lArB]* insertional mutagenesis to identify autosomal loci that affect odor-guided behavior in *Drosophila melanogaster*. The avoidance response to benzaldehyde was quantified in 379 *P*-element insertion lines, and 14 lines were identified in which the insertion of the *P*-element caused aberrant olfactory behavior. *P*-element insertion sites were mapped by *in situ* hybridization to larval polytene salivary gland chromosomes. They mapped to different cytological locations on the second and third chromosomes and were named '*smell-impaired*' (*smi*) followed by their chromosomal band designations. *LacZ* reporter gene expression was evident in 10 of the *smi* lines in olfactory tissues, (antennae and/or maxillary palps). *P[lArB]*-tagged DNA sequences were rescued as inserts in pBluescript after digestion with *Hin*dIII and used to screen a genomic DNA library. Genomic clones were isolated for five of the *smi* lines. Partial sequence analysis and the lack of candidate loci at the cytological locations of our *smi* genes indicate that the *smi* genes we identified are novel. Complementation studies show that the *smi* loci are recessive and double heterozygotes reveal epistatic interactions between several but not all loci, e.g. *smi26D* and *smi60E*. To provide evidence that the *P*-element indeed is responsible for the observed olfactory impairment, the phenotypes of *smi* mutants can be restored through excision of the *P*-element. Revertants have been obtained for lines *smi26D*, *smi60E* and *smi97B* with phenotypes ranging from homozygous lethal to partial or complete reversion. The gene products encoded by these *smi* genes will enable us to link the expression of specific ensembles of genes directly to odor-guided behavior.

Supported by NIH grants DC 02485, GM 45344 and GM 45146.

294. The cytoskeleton and the membrane of the olfactory dendrites in the silkmoth *Antheraea* sp.

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We identified three major cytoskeletal proteins: tubulin, actin and intermediate filament-like protein, two motor proteins: kinesin and unconventional myosin and the membrane anchoring, actin-binding-protein (fodrin) in the olfactory dendrites by the use of immunofluorescence microscopy. We have also found filamentous connections between the microtubules and between the microtubules and the membrane. Isolated whole olfactory dendrites extruded onto 'Thermanox' plastic coverslips and formvar coated grids were investigated in the scanning and transmission electron microscope respectively. They show swellings or 'beads', either being splayed or forming a bundle of microtubules passing through the centre. We also observed parts of the same dendrite exhibiting differential staining when treated with one of the two fluorescent dyes: acridine orange (AO) and/or ethidium bromide (EtBr). The identification of the proteins described above within the dendrites may provide valuable information concerning their role in transduction, transport and motility. In fact, we have already got some indirect evidence that the cytoskeleton of the dendrites may be involved in transduction based on a study wherein over-stimulation of the olfactory dendrites with a particular component of the pheromone damages the microtubular cytoskeleton. The results obtained with respect to differential staining of the same dendrite with AO or EtBr may probably reflect local differences in acidity/alkalinity and damaged/undamaged membranes respectively at different regions along the dendrite. The membrane of the dendrite was investigated with the atomic force microscope. We found 'pores' in the membrane, 3 nm in depth and ~15 nm in diameter, with a density of ~10 000 for the whole surface area of the thicker dendrite (426

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mm^2). Based on a number of indirect evidences, we think that these 'pores' might represent ion channels. The relationship between 'pores' on the membrane and how they affect the underlying cytoskeleton is yet to be investigated.

Supported by DFG grant Ke-398/2-1 (G.L.K.) and SFB 266-B4 (T.A.K.).

295. Changes of taste responses in bullfrog during development

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The integrated taste nerve responses were recorded from bullfrogs (*Rana catesbeiana*) aged 1–2 weeks and over several months after the metamorphosis of tadpoles into frogs. Although both the phasic and the tonic components were induced by salts in the mature frogs, the only phasic components were observed in the immature frogs. The only phasic components were induced by bitter substances in both stages of the frogs. No responses were observed by sugars in the immature frogs.

On the other hand, neural impulses were recorded in both stages of the frogs by suction electrode method. The magnitude of impulses were multiple. The discharges of the large and the small impulses were induced by salts in the mature frogs. However, the only small impulses were observed in the immature frogs. The only small impulses were observed by bitter substances in the mature frogs and the immature frogs. Whereas the discharges of the small impulses were transient in both developing stages of the frogs after applying taste substances, those of the large impulses lasted during the taste stimulation in the mature frogs.

These results are suggesting that the phasic and the tonic components of the integrated responses to salts were caused by the generation of the small impulses and large impulses, respectively.

296. Odorant adaptation and its Ca-dependence in the olfactory receptor cell

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Olfactory sensation expresses remarkable adaptation and fatigue to the applied odorants. In the present talk, we describe odorant adaptation of the single, dissociated olfactory cells under the whole-cell recording, and the experimental evidence showing that odorant adaptation is almost solely regulated by the influx of Ca ions through the transduction channel. When solitary olfactory cells were stimulated by the long-step of odorant, current response showed a rapid and strong decay. Similar response decay was also observed when cells were stimulated by double pulse protocols. Using double pulse stimulation, we confirmed that the current reduction was actually the reduction of cell sensitivity due to adaptation. When the external Ca was removed, this adaptation was almost completely abolished; prolonged stimulation caused current response with long-lasting wave form, and double pulse stimulation caused responses with the same amplitude and time course. In addition, at +100 mV where no Ca influx is expected, double pulse stimulation caused outward currents with the same wave form and amplitude. EGTA loading of the cell also inhibited adaptation. Ca permeation through odorant-activated cationic channel was confirmed by the permeablility ratio in bi-ionic condition (P_{Ca}/P_{Na} = 6.5). We therefore conclude that Ca influx through cAMP-gated channel into ciliary cytoplasm is responsible for the regulation of odorant adaptation.

Supported by JSPS, HFSP,EC, MESSC.

297. Introductory remarks on umami

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Umami substances such as monosodium glutamate (MSG), disodium 5′-inosinate (IMP) and disodium 5′-guanylate (GMP) are sodium salts. Hence it has been argued whether the umami substances stimulate only the salt receptor or an umami receptor as well as the salt receptor. In rat chorda tympani responses, there was no direct evidence that the umami substances stimulate a umami receptor. On the other hand, in dogs, mice and monkeys, it has been clarified that the responses to umami substances are independent of salt responses.

An idea that the umami substances are enhancers for the four primary tastes has been widely distributed without any scientific data supporting this idea. However, systematic psychophysical and electrophysiological studies indicated that the umami substances have no enhancing activity on other primary tastes. Single fiber analysis using mice and monkeys showed that the responses to umami substances are independent of other primary tastes. Hence we can say that the umami taste is one of the primary tastes.

In humans, there is a large synergism between MSG and the nucleotides, but the synergism was very small in the rat. Remarkable synergism was found in dogs. The synergism was explained in terms of an allosteric model. In humans, MSG induces a large umami taste, but IMP itself imparts only a weak umami taste. In this sense, MSG acts as an agonist and IMP is an enhancer or modulator in humans. This is also true in monkeys. In the dog, GMP and IMP alone induce a large response, suggesting that the nucleotides are the main agonists and MSG is a modulator. Thus we should note that there are large species differences in the response to umami substances.

298. Symposium: Umami: taste perception, nutrition and central mechanisms

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A growing body of evidence suggests that certain amino acids—the most prominent being glutamate—and ribonucleotides elicit a distinctive taste quality which has been called by the Japanese word, umami. The closest English words to describe this sensation are savory or meaty. This Symposium provides a timely opportunity to update research into the mechanisms and function of umami taste. Progress in understanding the molecular and cellular basis for umami taste and the remarkable synergism between glutamate and nucleotides will be discussed by several participants. Central representation and processing of umami taste will be a focus of other presentations. The effects of umami substances on flavor and food preferences in mice and in humans will also be described. In sum, these presentations and the discussions that accompany them should demonstrate both the

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remarkable progress that has been made in understanding umami taste and what aspects of this phenomenon remain a mystery.

299. Metrics of odorant dissimilarity: labeled magnitude scale versus magnitude estimation

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Magnitude Estimation (ME) has long been the standard rating tool for stimulus intensity because of the simplicity and generalizability of the resultant power function relationships. However, the choice of a scaling procedure for rating complex stimuli (odorant) dissimilarity must be based on a different set of criteria due to an inability in evaluating relationships against a physical continuum. Recently, chemosensory Labeled Magnitude Scales (LMS) have been developed which produce ratings with many of the same properties as ME. The current study sought compare LMS to ME as metrics for describing the magnitude of perceived odorant dissimilarity. Forty subjects rated pair-wise odorant dissimilarity with either a LMS or ME. Each subject evaluated two sets of odorants with differing ranges of dissimilarity (high contrast and low contrast) in order to compare the sensitivity of the two measures to possible contrast convergence. The ME ratings were normalized such that the two metrics produced equal means. The scales produced similar ratings of odorant dissimilarity (r = 0.92) and showed small but similar effects of contrast convergence. However, the coefficient of variation of dissimilarities rated with ME were 2.8 times those produced with the LMS. Subjects accepted the LMS without the usual anxiety which accompanies first time users of magnitude estimation. LMS provide stable ratings of odorant dissimilarity while preserving the inferred ratio scale properties of ME.

Supported by NIH grant number 9-PO1 DC00220.

300. A longitudinal study of the olfactory loss that accompanies an HIV infection

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A number of studies have shown that HIV infection is associated with decreased olfactory ability. Additionally, reduced odorant identification may precede the advent of AIDS Dementia Complex (ADC). However, it is not known whether changes in olfactory ability are a manifestation of neurocognitive decline which may precede the appearance of ADC or damage to the peripheral olfactory system from opportunistic infection or whether olfactory structures have a particular sensitivity to HIV. These issues were addressed in a longitudinal study examining changes in neuropsychological, neurological, otolaryngological, auditory and olfactory status in HIV positive subjects. When possible, subjects were evaluated every 6 months. Sixty subjects were tested at least once, 28 of these subjects were tested more than once. A stepwise regression provided evidence that the ability to identify odorants was influenced by age, nasal structure and pathology, neurocognitive ability, and level of ADC. On the other hand, only nasal pathologies accounted for the variability in olfactory thresholds. On average, the ability to identify odorants decreased over time, whereas the ability to detect odorants remained unchanged. These data suggest that identification and thresholds tests may reflect different olfactory pathologies. Because of their rapidly changing neurocognitive status, HIV positive patients represent an excellent group in which to study the determinants of olfactory ability.

Supported by NIH grant 9-PO1 DC00220.

301. Modification of fragrance release and perception by product matrix

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Fragrance character is a key distinguishing attribute of most consumer products and fragrance release, from the initial product and from the product during use, are important considerations for the consumer. Surfactants, the major ingredient in cleaning products, form micelles which solubilize fragrance molecules, altering their volatilization from a detergent solution and affecting the perception of the intensity and possibly the character of the fragrance itself. The partitioning behavior of fragrance molecules between surfactant micelles and the aqueous solution has been investigated using headspace/GC methodology which provides direct sampling and the simultaneous analysis of many components. Physical values derived include the solubilization site within the micelle for a solute and the solute partition coefficient or its equivalent value for a detergent solution, which we have defined as the Differential Solubility Number. This approach provides a comparison of fragrance raw materials independent of surfactant nature or concentration. The interaction of fragrance and flavor chemicals with inclusion complexes such as cyclodextrins and with liposomes, which are similar in structure to cell membranes, can also be studied by this approach.

302. A multi-adsorption model with different types of olfactory receptor sites

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In previous studies (Ben Lamine and Bouazra, 1996, 1997), we have established an analytical expression for the olfactory response $\Re$ by using a statistical thermodynamics treatment of the adsorption of olfactory stimulus on neuro-receptor sites. This allowed us to understand some empirical relations established in the literature and to relate the used empirical parameters to more basic physico-chemical parameters. These results were obtained with the assumption of single type receptor sites.

In this study, we generalize our model to adsorption by different types of receptor sites, as established by many authors. In effect, the observed partial saturations of $\Re$ (Duchamp-Viret, 1988) can be obtained in our model by using different values for the half saturation parameter $N_{g1/2}$.We get: $\Re = \alpha \mathrm{Si}(N_M)_i\{1 + [N_{g1/2})_i/N_g]^{ni}\}$. Using this expression of $\Re$, we have been able to get good fit to the experimentally obtained response in the case of frog (Duchamp-Viret, 1988) and estimates for the different model parameters: $(N_M)_i$, n_i and $(N_{g1/2})_i$. From established in our previous work, we were lead to the conclusion that different receptor sites are mainly determined by different energy values $(e_m)_i$. This allowed us to determine an energy band De_m which characterizes all the receptor types activated as the concentration

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of a given stimulus is varied. Numerical results of De_m, $(N_M)_i$, n_i and $(N_{g1/2})_i$ for the experimental results in (Duchamp-Viret, 1988) will be discussed.

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303. Genome dynamics, polymorphisms and protein modelling in the human olfactory receptor superfamily

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The human olfactory subgenome represents several hundred olfactory receptor (OR) genes in a dozen or more clusters on several chromosomes. One OR gene cluster on human chromosome 17 has been characterized by us in detail. Based on large scale DNA sequence analysis, we have identified events of gene duplication as well as the generation of pseudogenes. The latter instances of 'gene death' could underlie the widespread phenomenon of human specific anosmias. Comparative analysis of the duplicated genes revealed a conserved gene structure: a long (5–6 kb) intron separates the intronless ~1 kb coding region from an upstream, short non-coding exon. Furthermore, a conserved putative genecontrol region could be discerned, which could be meaningful for OR clonal exclusion. Aiming to assess the polymorphism level among OR genes in this cluster, genes as well as pseudogenes were subjected to mutation detection analysis using a modified restriction endonuclease fingerprinting (REF) method. Several genes were found to have two or more alleles with about equal frequency in the population. These polymorphism occur in functionally significant regions of the OR protein, indicating a potential for explaining sensory variability. By utilizing the rich information available on several dozen OR sequences, we could construct a model of the odorant binding site, containing 14 hypervariable residues on three of the seven transmembrane helices. Such a model could be useful in analyzing additional OR gene sequences in the human genome, in terms of odorant binding.

Supported in part by NIDCD 00305.

304. Afferent and efferent connections of the nucleus sphericus in the snake *Thamnophis sirtalis*: convergence of olfactory and vomeronasal information in the lateral cortex and the amygdala

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The afferent and efferent projections of the nucleus sphericus (NS), which is the major secondary vomeronasal structure in the brain of the snake *Thamnophis sirtalis*, were studied by means of iontophoretic injections of biotinylated dextranamine into the NS. There are four major efferent pathways from the NS: (i) a bilateral projection that surrounds the accessory olfactory tract and innervates several amygdaloid nuclei (nucleus of the accessory olfactory tract, dorsolateral amygdala, external amygdala and ventral anterior amygdala), the rostral parts of the dorsal and lateral cortices and the accessory olfactory bulb. (ii) A bilateral projection (previously undescribed) that courses through the medial forebrain bundle and innervates the olfactostriatum (rostral and ventral striatum). (iii) A commissural projection that courses through the anterior commissure and innervates, mainly, the contralateral NS. (iv) A meager bilateral projection to the lateral hypothalamus. On the other hand, the only important afferent projections to the NS arise in the accessory olfactory bulb, nucleus of the accessory olfactory tract and contralateral NS.

This pattern of connections has three important implications: firstly, the lateral cortex is in a position to integrate olfactory and vomeronasal information. Secondly, as the NS projection to the hypothalamus is meager, and does not reach the ventromedial hypothamic nucleus, vomeronasal information is not directly relayed to that nucleus, as previously reported. And finally, a structure located in the rostral and ventral telencephalon, the olfactostriatum, stands as the major tertiary vomeronasal center in the snake brain. These three conclusions change, to an important extent, our previous picture of how vomeronasal information is processed in the brain of snakes.

This research was supported in part by a NIH grant (DC00104). E.L. was supported by a grant of 'la Caixa'.

305. Semantic mediation of age-related deficits in episodic recognition of common odors

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In two studies, we addressed the relationship between semantic memory and episodic recognition of common odors across the adult life span. Young, young-old and old women were presented with 16 odors and memory was assessed immediately and 48 h after study. Subjects made familiarity ratings at study and were asked to name the odors in conjunction with the delayed recognition teSt In the first experiment, odors were encoded following three different formats: name-only, odor-only or odor-name. The results indicated that the younger subjects outperformed the two older age groups in both recognition tests, although the two older age groups did not differ. Memory decreased reliably from immediate to delayed recognition and all age groups exhibited similar forgetting rates. Across age groups, odor memory was higher in the odor-name condition, as compared with performance following the single-format conditions. This outcome suggests that activation of prior knowledge improves episodic retention of olfactory information equally across the adult life span. Both rated familiarity and odor naming were positively related to memory performance in all age groups. Most important however, was the finding that when naming ability was statistically controlled, age differences in odor recognition disappeared suggesting that access to verbal labels largely determined age differences in recognition of common odors. In a second experiment, we replicated the findings of age deficits in odor recognition and significant forgetting. Furthermore, general semantic knowledge known to be related to proficiency in episodic memory for verbal and visual information (e.g. vocabulary, verbal fluency) proved unrelated to episodic odor memory. Instead, specific semantic knowledge about the odors were related to memory performance. Perceived familiarity and the

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quality of the emitted odor naming predicted subsequent odor recognition performance at both times of testing. Regression analyses indicated that age and odor naming were the most potent variables in predicting performance and controlling for odor naming resulted in the effect of age disappearing. Taken together, these experiments indicate the pivotal role of accessibility of odor names for successful episodic odor recognition, and for age-related differences in odor recognition.

306. Discrimination ability of squirrel monkeys for structurally related fatty acids

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Studies of structure–activity relationships suggest that within a group of structurally related substances molecular features like carbon chain length or steric conformation may determine odor quality and thus perceived similarity between members of a given class of chemicals. One useful means to assess possible correlations between odor quality and molecular properties which is also applicable to nonhuman species is to test the discriminability of structurally related odorants.

Using a behavioral paradigm designed to simulate olfactory-guided foraging behavior and based on the multiple discrimination of simultaneously presented odor stimuli in a manipulation task, we investigated the ability of squirrel monkeys to discriminate between aliphatic fatty acids. This group of substances comprises odorous compounds predominant in vaginal secretions and skin-borne body odors and thus is presumably of biological significance for these primates.

Four squirrel monkeys were trained to distinguish *n*-valeric acid (S+) from *n*- and iso-forms of other fatty acids (acetic acid to octanoic acid) as S–. Their performance in these tasks was compared with their discrimination ability in a control task (*n*-valeric acid versus anethole) which was interspersed between the critical trials.

We found that all four animals were clearly able to discriminate between all odor pairs tested. However, three of the odors used as S– presented significantly more difficulty for the monkeys compared to the control and all other tasks: two of these (*n*-butyric acid and *n*-hexanoic acid) are direct neighbours of *n*-valeric acid in the corresponding homologous series suggesting that carbon chain length may be correlated with perceived odor similarity, whereas the third odor (iso-valeric acid) is an isomeric form of the S+ suggesting that the two odorants were perceived as qualitatively similar despite different steric conformation.

Using a triple-forced choice procedure human subjects were tested on the same tasks in parallel and showed an almost identical pattern of discrimination ability compared to the squirrel monkeys.

The results of this study provide evidence of well-developed olfactory discrimination ability in squirrel monkeys for aliphatic fatty acids and support the assumption that human and nonhuman primates may share common principles of odor quality perception.

Supported by the Deutsche Forschungsgemeinschaft (La635/6-1).

307. Putative pheromone binding proteins of scarab beetles do not bind pheromones

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Scarab beetles from the subfamily Rutelinae utilize an unusual group of g-lactones (5-alkenyl-dihydrofuranones) as their sex pheromones. These compounds differ primarily in length of the alkenyl side chain and the stereochemistry at the chiral center. In two species, *Anomala osakana* and *Popillia japonica*, the opposite enantiomers have opposite behavioral effects: the unnatural isomer inhibits male responses.

In our attempt to disclose the molecular basis of discrimination of stereoisomers we have characterized putative odorant/pheromone binding proteins present in these and several other species of scarab beetles. In most cases there was only one class of antennae-specific proteins which was present in both sexes. In *A. cuprea* we also analyzed grubs and detected the same protein in the last larval instar. The N-terminal sequences of these proteins were highly conserved within the family and showed moderate homology to putative OBPs from *Drosophila melanogaster* (47%), *Lygus lineolaris* (45–50%) and the ABPX protein from *Bombyx mori* (30–35%). We purified these proteins by gel filtration and ion exchange chromatography. The proteins did not bind radiolabeled pheromones in native PAGE assay, neither in crude extracts nor in the pure form. Unexpectedly, these ligands were bound by a PBP from a moth, *Samia cynthia ricini*, which utilizes (*Z,Z*)-6,11-hexadecadienal as a pheromone. This fact suggests that the ligand specificity in this class of proteins may not be as high as it has been postulated. Pheromone receptors and general odorant receptors are clearly separated on lamellae of these beetles. We analyzed protein extracts from each part and found the same protein present in both of them in the same proportion.

308. Neurite outgrowth of olfactory bulb neurons *in vitro*: do glial cells provide an avenue for outgrowth?

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Studies of neurons *in vivo* and *in vitro* have indicated that neuron–glia interactions influence neurite initiation and extension. However, studies about the effects of an *in vitro* glial environment on neurons intrinsic to the olfactory bulb are generally lacking. We are co-culturing fetal (embryonic day 15) olfactory bulb neurons from rat with cortical astrocytes and fibroblasts (meninges-derived) in order to assess neurite initiation and outgrowth in short-term cultures. Astrocyte and fibroblast monolayers plated on poly-D-lysine (PDL) coated coverslips are infected with a replication-deficient adenovirus/Lac-Z construct for labeling (courtesy of Dr Mark Kay) and subsequently adapted to Neuobasal® media over several days. Neurons are harvested using standard methods, and a single-cell suspension of neurons is plated onto the confluent monolayers. The co-cultures are fixed after 1, 3, and 5 days *in vitro* (DIV) and identified using double-label immunofluorescence with antibodies against

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neurofilament-200 (NF) and microtubule associated protein-2 (MAP-2) or non-phosphorylated neurofilaments (SMI311) and glutamate. Parallel cultures consisting exclusively of the monolayers are maintained identically to the co-cultures for verification of phenotype. Olfactory bulb neurons plated onto PDL coated coverslips serve as a control. Cell bodies as well as the proximal neurites of all neurons stain positively for NF, MAP-2, SMI311 and glutamate. However, glutamate staining (co-localized with SMI311 versus NF/MAP-2) reveals a greater extent of the neurites, displaying networks of fibers on astrocytes and what appear to be shorter, fasciculated fibers on fibroblasts. NF and MAP-2 staining shows a dispersal of neurons over astrocyte monolayers, similar to controls, whereas neurons co-cultured with fibroblasts tend to adhere to one-another. In conclusion, it appears that (i) astrocytes support greater neurite outgrowth of olfactory bulb neurons *in vitro* than fibroblasts and (ii) anti-glutamate may provide a better label for neurites *in vitro* than some other standard labeling methods.

This research is supported by NIH grants NS09678, DC02370 and T32NS-07144. L.E.W. is an affiliate of the CHDD.

309. Visualizing odor detection in olfactory cilia by calcium imaging

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Signal transduction in vertebrate olfactory receptor neurons (ORNs) begins in the olfactory cilia when odor molecules bind to specific receptors and initiate a cascade of events leading to rapid cAMP formation followed by the opening of Ca^{2+} permeable cyclic nucleotide-gated (CNG) cation channels. As transient elevations of intracellular Ca^{2+} levels play important roles in the regulation of the sensitivity of olfactory transduction, it would be useful to monitor the activity of single cilia on ORNs. We have developed methods by which odor detection can be visualized in individual cilia enabling us to determine at anatomically distinct sites the spatio-temporal dynamics and origin of local Ca^{2+} signals evoked by natural odor stimuli. For this approach we used freshly isolated salamander ORNs which contain relatively large cilia (diameter: 0.3 μm; length: 50–100 μm). ORNs were immobilized on the substrate, loaded with the Ca^{2+} indicator dye fluo-3/AM, and Ca^{2+}-mediated fluorescence changes were measured with a laser scanning confocal microscope. In the presence of TTX, brief micropulses of single odor ligands produced detectable fluorescence increases in the cilia. These signals reached their peak within ~1 s and decayed on a monoexponential time course with a time constant of a few s. Odor-stimulated fluorescence changes in the cilia were dependent on the influx of extracellular Ca^{2+} through activated CNG channels. Fluorescence signals were abolished reversibly by decreasing external Ca^{2+} to levels ≤0.6 μM or by applying a pharmacological blocker of the CNG channel pore. The phosphodiesterase inhibitor IBMX mimicked odor-dependent fluorescence changes. Membrane depolarization through elevated K^+ solutions was not sufficient to increase Ca^{2+} in the cilia. Analysis of the odor sensitivity of different cilia of a given ORN suggests a model in which all cilia of a cell contain the same odor receptor or receptor subset. Other experiments, in which localized stimuli were applied onto single cilia, suggested that olfactory cilia are semi-independent functional units for odor transduction. The detection of Ca^{2+} entry through CNG channels in single olfactory cilia provides a new approach for real-time monitoring olfactory function, and is a first step toward monitoring quantal interactions of odor ligands with odor receptors in the ciliary membrane.

This research was supported in part by NIH grants DC02227, DC00086, DC00210 and NS10174.

310. Ca^{2+} store depletion does not affect olfactory signal transduction

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To begin to understand the role of Ca^{2+} stores in olfactory signal transduction, we have used electrophysiological and Ca^{2+} imaging techniques to examine the effect of store depletion on cellular events in salamander olfactory receptor neurons (ORNs). Thapsigargin, a Ca^{2+}-ATPase inhibitor that promotes Ca^{2+} release from intracellular compartments, was employed to study intracellular Ca^{2+} release and depletion. Using confocal imaging, thapsigargin increased the Ca^{2+}-fluorescence in the dendrite and soma, where Ca^{2+} stores (mitochondria and endoplasmatic reticulum) were localized by EM. No increase in Ca^{2+} fluorescence was observed at the site of primary olfactory transduction, the individual olfactory cilia. It is known that Ca^{2+} store depletion sometimes can cause the activation of sustained inward currents due to store-operated ion channels: I_{CRAC} and/or the trp channels. In our experiments, however, thapsigargin-stimulated Ca^{2+} release did not induce any inward current using the perforated patch-clamp technique. Instead, thapsigargin increased the amplitude of a component of the outward K^+ currents and induced a hyperpolarisation of the ORN membrane potential. In addition, thapsigargin seemed not to alter the amplitude or kinetics of IBMX- or cineole-induced currents and receptor potentials. Using imaging techniques, IBMX or cineole-evoked Ca^{2+} responses in cilia and knob were unaffected by the thapsigargin treatment. In contrast, the fluorescence signals in the dendrite and soma, which display complicated kinetics in the control situation, were significantly reduced after thapsigargin treatment. This suggests that several distinct mechanisms contribute to the total fluorescence changes in the dendrite and soma, reflecting the involvement of thapsigargin-sensitive Ca^{2+} stores. We conclude that thapsigargin-sensitive Ca^{2+} pools are present in the dendrite and soma of ORNs, that Ca^{2+} released from these stores appears not to activate a CRAC- or trp-like channel, and that the primary transduction mechanism is not influenced by these stores.

This research was supported by NIH grants DC02227, DC00086, DC00210 and NS10174.

311. Autocorrelational analysis of spike trains in the parabrachial nucleus of the pons following gustatory adaptation

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Previous studies have suggested that inhibitory input to taste cells

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in the parabrachial nucleus of the pons (PbN) may be involved in modification of response profiles following adaptation to some taste stimuli. Further evidence of this inhibitory influence may be evident through analysis of the temporal organization of individual spike trains in response to taste stimuli. Taste evoked spike trains were recorded from PbN units following adaptation of the tongue to one of the following tastants: NaCl (0.1 M), sucrose (0.5 M), quinine HCl (0.01 M), and HCl (0.01 M). Responses to each of these tastants, presented individually, were recorded initially. A target stimulus was then chosen from among these four stimuli and applied to the tongue repeatedly until the PbN response was eliminated. A test stimulus chosen from the remaining three tastants was then applied to the tongue, followed by a distilled water rinse. This adaptation-test procedure was repeated until each of the tastants served as both target and test stimulus. Autocorrelation functions (ACFs) were constructed by measuring the interval between each spike and every other spike in a given trial, regardless of intervening spikes. The number of occurrences of each spike-spike interval was then calculated in 1 ms increment time bins (0–500 ms). For example, an entry of 25 in the 100 ms time bin indicated that there were 25 instances where two spikes occurred 100 ms apart, ignoring intervening spikes. ACFs of PbN spike trains have shown that, in general, prior to adaptation, spike-spike intervals from ~3 to 500 ms are equally probable. However, following adaptation to some taste stimuli, ACFs sometimes showed a suppression of spike-spike intervals up to ~40 ms. ACFs associated with other test stimuli showed a sharp attenuation of 5–10 ms spike-spike intervals followed by a gradual 'recovery' up to 50 ms. These effects have been observed both with and without a concomitant change in overall response magnitude. These data provide further evidence that adaptation may invoke inhibitory influences on taste responsive cells in the PbN. Furthermore, present results demonstrate the time course of this influence.

Sponsored by NSF grant IBN9630326 to P.M.D.

312. Coding of pulsatile olfactory stimuli by cockroach antennal lobe projection neurons

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Behavioral evidence indicates that insects preferentially orient toward pulses of odorants as they occur down wind from a point source. Our recent results have shown that cockroach olfactory receptor neurons are able to reliably resolve 10 Hz pulses of the general 'green' odorant 1-hexanol, but it is unknown to what extent the central olfactory pathway is able to resolve temporal aspects of a general odor stimulus. In the present study, temporal response characteristics were measured in antennal lobe projection neurons of female American cockroaches *Periplaneta americana* in response to series of short odor pulses (2.5–20 Hz). Odor pulses were delivered to olfactory sensilla in a moving airstream controlled by electromagnetic valves and quantified by replacing the odorant with oil smoke and measuring the concentration of smoke passing through a light beam. The responses of projection neurons were recorded with an intracellular microelectrode placed in the projection neuron cell body. A variety of time courses of responses were recorded. Response patterns were consistent among identical stimuli within a neuron and varied among neurons. Some neurons increased spike frequency with stimulus onset while others decreased spike frequency. The latency to the change in spike frequency and the duration of the response also varied among neurons. Regardless of the temporal characteristics of the responses, nearly all projection neurons were able to resolve pulses of 1-hexanol presented at 5 Hz and some could resolve 10 Hz pulses. Thus, responses of antennal lobe projection neurons can reflect fine structures of non-uniform distributions of general odorants in a turbulent odor plume. In addition, the variety of temporal response characteristics to identical stimuli suggests that odor quality is coded by a temporal code expressed across a population of projection neurons.

313. The role of early experience in olfactory bulb development

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Unilateral naris occlusion at birth decreases bulbar activity, reduces the size of the bulb and decreases the number of olfactory bulb neurons. In addition, there are mechanisms that may compensate for early sensory restriction. For example, there is an increase in both glomerular-layer dopamine D2 receptors and nerve growth factor receptors in the deprived bulb. Early olfactory deprivation also increases the responsiveness of olfactory bulb neurons to odors. Specifically, reopening of the sealed naris induces both increased glomerular 2-DG uptake and an increased incidence of odor-responsive mitral/tufted cells when deprived bulbs are exposed to olfactory stimuli. It is possible that these changes contribute to the saving of most cells in the bulb from an early death after olfactory deprivation.

Cell death is a normal aspect of brain development and we have found that both the main and accessory olfactory bulb have many cells that undergo apoptotic cell death over the course of development. The number of apoptotic cells increases sharply when the perinatal olfactory system is subjected to olfactory restriction, but the majority of the cells survive this radical change in experience. We found that a gene, bcl-2, which has been shown to protect other kinds of cells from apoptosis, has an increased expression following early olfactory restriction. It seems possible that an increase in bcl-2 expression could compensate for the loss of olfactory stimulation and thereby could protect cells from an early death.

Supported in part by NICHD grant HD24236.

314. Distribution and synaptic arrangements involving GABAergic terminals in the rNST of the rat using postembedment immunohistochemistry

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GABA and GABA-mediated inhibition is thought to play an important role in the processing of taste stimuli in the rostral nucleus solitarius (rNST). We have specifically labelled and thus identified GABAergic terminals and somata in the rNST using postembedment immunohistochemistry. More than 25% of all terminals in the rNST are GABAergic. GABAergic terminals

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synapse with dendrites, dendritic spines, other terminals and occasionally with somata as well. Synapses onto other terminals likely represent a mode of presynaptic inhibition. There are two types of GABAergic synapses in the rNST as defined by their vesicular density. Sometimes they converge onto the same post-synaptic target. The origin of these two types of terminals is unknown, however one appears to be dendritic (low density) while the other may be axon terminals (high density). Some of the latter may arise from extrinsic projections to the rNST As in other parts of the brain, one type of GABAergic terminal may be associated with $GABA_A$ receptors while the other may be associated with $GABA_B$ receptors. Both receptor types are present in the rNST We have determined that when GABAergic synapses are present on somata, they tend to be low density terminals. Conversely, high density terminals tend to synapse with dendrites. Thus, the two types of terminals have different postsynaptic targets. The synaptic arrangement of the two subtypes of GABAergic terminals onto physiologically-characterized gustatory neurons filled with neurobiotin is being investigated.

Supported by NIH Grant RO1 DC01074-05.

315. Chemosensory impact of pallidotomy

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It is well established that patients with Parkinson's disease have decreased olfactory and possibly gustatory abilities. Recently pallidotomy has been used to treat this disease, and several of our patients who have had this have indicated that they noticed an improvement in their smell and/or taste ability. Fibers from the olfactory bulb and cortex terminate in the medial dorsal nucleus of thalamus which is under inhibitory control from the ventral pallidal area. Surgical inactivation of pallidum may allow return of chemosensory ability by blocking inhibitory fibers. Fifteen patients who have had this procedure had an average preoperative UPSIT score of 15/40 correct, and had taste losses between total ageusia and ~50% decrease on identification tests. Their post operative results will be presented along with a discussion of the results.

316. Modulation of taste responses in the hamster solitary nucleus by GABAergic mechanisms

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The rostral portion of the nucleus of the solitary tract (NST) receives gustatory afferent fibers from the VIIth, IXth and Xth nerves. Neurons in this area form an obligatory relay in the afferent processing of gustatory information. Immunohistochemical studies have shown that cells in both caudal and rostral portions of the NST contain γ-aminobutyric acid (GABA) or its principal degradative enzymes, suggesting the possibility of a GABAergic network within the gustatory region of the NST Previous *in vitro* studies in both hamsters and rats have demonstrated that GABA inhibits many cells within the gustatory region of the NST, where there appears to be a tonic GABAergic inhibitory network. Although *in vitro* studies provide valuable information about the biophysical properties of NST neurons, it is not possible to determine their gustatory properties. In the present study we investigated the mechanisms of tonic inhibition on taste-evoked responses in the NST *in vivo*. A three-barrel glass pipette assembly was used to apply GABA or the $GABA_A$ antagonist bicuculline methiodide (BICM) into the vicinity of an NST cell and to record single-unit activity in response to gustatory stimulation. The taste stimuli were 0.1 M sucrose, 0.1 M quinine hydrochloride, 0.1 M NaCl and 0.01 M citric acid, presented to the anterior tongue before and after application of GABA or BICM into the NST GABA decreased taste-evoked responses in a dose-dependent fashion, whereas BICM produced a dose-related increase in the responses of NST cells to tastants. Thus, these cells are under tonic GABAergic inhibitory control; the gustatory responses of these cells can be increased or decreased by GABAergic mechanisms. Responses to all classes of tastants appear to be equally subject to GABAergic modulation. Additional studies are investigating sources of this control, including descending corticofugal inputs to the NST.

Supported in part by NIDCD DC00066 (D.V.S.).

317. Chorda tympani disorders and spatial testing in humans

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Understanding of the outcome of taste nerve injury in humans is limited. Because the tongue is supplied with four cranial nerves (two on each side), patient reports and whole mouth testing are inadequate for assessing the integrity of the peripheral taste system when individual nerves may be damaged. The present study uses a spatial test (Abrahams *et al.*, 1993) to evaluate the function of the chorda tympani and glossopharyngeal nerves, right and left, in five patients with suspected chorda damage. Patients 1, 2 and 3 had sustained chorda section during middle ear surgery but had no taste complaint. Patient 4 presented with dysgeusia and trigeminal dysaesthesia following mandibular molar extraction. Patient 5 complained of taste loss consequent to ear pain. Four normal subjects served as controls. For testing, NaCl and Sucrose (both at 0.1 and 0.32 M) were applied with cotton swabs, in repeated separate trials, to the anterolateral tongue (R and L) and to the lateral-most vallate papilla (R and L), and the subject rated the taste intensity. Patients' intensity ratings of anterior tongue stimulation in most cases were low; in all cases, on both sides, ratings differed markedly from posterior tongue ratings. The anterior–posterior difference averaged 66%, and included 100% bilateral lower ratings (anterior ageusia) in patient 1 and 61% higher anterior rating (contralateral to a 67% reduction) for patient 5. In contrast, controls rated intensities of anterior and posterior stimuli similarly (20% mean difference, range: 32% lower to 31% higher anterior rating). R-L differences in mean intensity ratings for the anterior tongue were pronounced for patients 2, 3 and 4 (69, 106 and 285%). This measure was not meaningful for patient 1 (bilateral ageusia), while patient 4 showed little difference (21%). R-L anterior differences for controls were slight (12%, range 7–20%). R-L differences for the posterior tongue were greater for patients (66%, range 21–203%) than controls (23%, range 3–46%). These results demonstrate that disorders of the chorda tympani, which differ in etiology, are detectable with

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topical testing as alterations in sensitivity between the R and L anterior tongue, the anterior and posterior tongue, or both.

Supported by NIH grant DC02045.

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318. Longitudinal change in olfactory function and olfactory cortex plaque numbers in multiple sclerosis: a pilot study

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We have conclusively demonstrated that a number of persons with multiple sclerosis (MS) exhibit odor identification deficits, as measured by the University of Pennsylvania Smell Identification Test (UPSIT). Furthermore, as described by Doty *et al.* elsewhere, we have shown that a strong association exists between UPSIT scores and MRI-determined plaque numbers in sectors of the central nervous system associated with olfactory processing. In this study, we evaluated, longitudinally, change in UPSIT scores over time in 10 MS patients, and established associations between UPSIT scores and MRI-determined plaque numbers in olfactory eloquent brain regions. Each patient was evaluated 3–4 times across periods ranging from 18 to 30 months. In seven cases, no meaningful changes in either plaque number or olfactory test scores were observed. In two cases, olfactory function declined during the study period, commensurate with increases in the number of plaques within the inferior temporal and frontal lobes. The remaining case, a 43-year-old woman, exhibited an increase in UPSIT scores from 30 to 35 and a concomitant decrease in plaque numbers from 14 to 8. These pilot data suggest that at least some MS patients may exhibit improvement in odor identification as plaque numbers decrease in brain regions associated with central olfactory processing. Additional work is needed to ascertain the proportion of MS patients who improve over time and whether other measures of olfactory and cognitive function exhibit similar time-related alterations.

Supported by NIDCD Grant PO 00161, NIH.

319. Assessment of odor annoyance from combustion exhausts and its relationship to stimulus concentration and odor intensity from pyridine

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The commonality of odor annoyance as an environmental complaint calls for evaluation of quantitative assessment of this complex concept. Therefore, by comparing the outcome with that of odor intensity (pyridine), the present study addressed the issues of (i) reliability of judgments of odor annoyance with the method of magnitude estimation; (ii) whether annoyance can be described as a power function of stimulus concentration; and (iii) the extent of interindividual differences in exponents of power functions for annoyance. Furthermore, a master-scale equation (psychophysical group power function) for odor annoyance was used to calibrate judgments of odor annoyance for context effects and individual differences in scaling behavior. This master-scale equation was used to (iv) calibrate judgments of annoyance from combustion exhausts at a heavily trafficated road and at a less polluted backyard. The results showed that reliability of estimates and the extent of interindividual differences in exponents are similar for annoyance and intensity of pyridine odor, and that subjects in general generate stable power functions for annoyance. After the master-scale calibration it was in addition to expressing perceived annoyance in master-scale units, also possible to express the annoyance from the target stimuli in terms of pyridine equivalents. The results encourage assessment of odor annoyance with magnitude estimation and show that prerequisites for master scaling are met and that it is possible to calibrate for individual scaling behavior in an experimental field setting.

Supported by the Örebro Environmental County Fund, Örebro.

320. Molecular and experimental analysis of olfactory neuro-differentiation and plasticity in bird embryos

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Two steps of olfactory neurogenesis are particularly critical for odor discrimination capabilities: individual neuroreceptor differentiation and orderly organization of central synapses.To study these processes, we cloned several subfamilies of putative olfactory receptor genes in the chick (COR) and used their expression as markers of olfactory neuron differentiation. During embryonic development COR expression starts early. From the beginning and before any functional synaptogenesis in the bulb, each individual neuron expressed only one COR. COR expression was also transiently observed in placodal derived cells along the nerve. It may be involved in axon guidance and specification of areas on the bulb primordium. A study of OR expression in developing interspecific chimeras allowed further insight in to the implications of tissue interactions in olfactory neurogenesis.

COR genes, their organization into clusters and their chromosomal location was analyzed in several bird species. These results provide some clues for a better understanding of OR gene evolution and possible regulatory mechanisms.

Finally, an experimental model is presently being screened for the expression of diverse genes, in order to compare neurogenesis under normal developmental and regenerative conditions.

321. Host plant volatiles synergistically enhance the capture of males in commercial sex pheromone traps for various moth species

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Since female moths call and release their sex pheromones while perched on their host plants, we isolated and identified host plant volatiles (HPVs), and tested various HPVs for their ability to

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attract moths and/or modulate the attraction of males to synthetic commercial sex pheromones in both field and orchard crops. To test their inherent atttractancy, individual HPVs were placed alone in traps and were found to be nonattractive to both male and female moths. To test the effects of HPVs on sex pheromone attraction, pairs of pheromone-baited traps were placed in close competitive positions, with one of the pheromone traps augmented with a HPV, the other without. When co-emitted with sex pheromone from baited traps, various HPVs were found to synergistically enhance the male attraction and capture efficacy of the pheromones by between 20 and >300%. The HPVs acted as sex pheromone-synergists, increasing the capture of exclusively male moths and not causing attraction/capture of female moths. Various HPVs were found to synergistically enhance the attraction of commercial pheromones of a wide range of moth species, in the families: Noctuidae, Gelechiidae, Tortricidae, Olethreutidae, Pterophoridae and Plutellidae. The HPVs identified as pheromone synergists were of various chemical structure, ranging from hydrocarbon monoterpenes and sesquiterpenes to aliphatic alcohols, aldehydes, acetates and acids. A number of different compounds were identified as pheromone-synergists for various species. Night-vision assisted observations in the field suggest that the HPV-enhanced pheromone trap capture of males was due to increased attractancy of pheromone traps and not increased alightment/ensnarement in traps. HPV pheromone-synergists significantly lowered the amount (~50 fold) of synthetic pheromone required to elicit a threshold attractive response in the field.

322. Ionic currents and pheromone responses in hamster vomeronasal neurons

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We have previously characterized the ionic currents of mouse vomeronasal (VN) neurons and described several fundamental differences between these sensory neurons and main olfactory neurons. Most significantly, we found no evidence for a cyclic-nucleotide gated current in mouse VN neurons, which along with molecular data suggest the presence of a novel transduction pathway. In order to elucidate mechanisms of transduction, we have now recorded responses in dissociated VN neurons to known pheromones. For these experiments we used cells from the golden hamster, an animal in which behavioral responses to pheromones have been well characterized and for which a pheromone that elicit mounting behavior, aphrodisin, has been isolated.

Vomeronasal neurons from mature male golden hamster were singly dissociated as described for mouse VN neurons. Most properties of hamster VN neurons were like those of mouse. For example, like mouse VN neurons, hamster VN neurons could fire sustained trains of action potentials in response to current injections of 1–3 pA. However, in the hamster, a larger proportion of VN neurons (75%) fired only a transient train of action potentials to steady current injection. Voltage-clamp recordings revealed that this population of cells had a reduced component of a slowly inactivating inward current. As with the mouse, we found no evidence for the opening of channels by cyclic-nucleotides.

To detect a pheromone response we applied aphrodisin to hamster VN neurons and measured action potential generation in cell-attached recording. Forty percent of cells (n = 75) fired action potentials at low frequency at rest or in response to a capacitive current across the patch membrane. Aphrodisin (0.25 mM) applied for 0.5–1.0 s elicited trains of action potentials in 4% of cells. In the most robust examples, the response occurred after a 0.5–2 s delay and lasted for 2–4 s.

Supported by NIDCD R03 DC02889-2.

323. Immunocytochemical localization of epithelial Na^+ channel subunits in rat taste cells

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Amiloride-sensitive Na^+ channels play an important role in Na^+ salt transduction in rat. Electrophysiological recording has revealed that the channel is present and functional in rat fungiform taste cells. In contrast, Na^+ currents in vallate taste cells are not amiloride-sensitive (Doolin and Gilbertson, 1996). Amiloride-sensitive Na^+ channels in fungiform taste cells share properties with an epithelial Na^+ channel (rENAC), which was cloned recently from rat colon (Canessa *et al.*, 1994). Functional channels consist of three homologous subunits, α, β, γ. To determine if rENAC is the basis of the amiloride-sensitive Na^+ current in taste cells, and to determine if subunit expression is different in the different taste papillae, we used affinity-purified antibodies directed against the three subunits of rENAC. Thirty micron frozen sections of circumvallate, foliate and fungiform papillae, as well as colon and kidney were obtained from rats fixed with buffered 0.075M L-lysine, 4% paraformaldehyde and 0.23% periodate. Immunoreactivity for all three subunits was observed in a subset of taste cells in fungiform, foliate and circumvallate papillae, as well as in the kidney and colon. Immunostaining was largely cytoplasmic, but in some cells heavier staining was observed in the apical region of the cell. The reactivity for a subunit was strongest, with reactivity for β and γ subunits somewhat weaker. These results suggest that the subunits of rENAC are present in rat taste cells. Further studies will be needed to determine if all three subunits are present in the same taste cells.

Supported by NIH grant DC 00766 and NIDCD 00244.

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324. Salty and sweet transduction

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The transduction pathways of peripheral taste involve membrane receptors, G-proteins, second messengers and ion channels, and for a given taste quality two or more pathways are typically used in parallel. In rodents, the Na^+ specific component of salt taste is mediated by apical, amiloride-sensitive Na^+ channels. However, less specific and not or weakly amiloride-sensitive taste

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components exist in addition. We used RT-PCR to ascertain the presence of the amiloride-blockable Na^+ channel ENaC in isolated CV-taste buds. mRNA of α-, β- and γ-subunits was detected. In addition, mRNA of a different cation channel, MDEG (a novel member of the ENaC family), was found in isolated CV-taste buds. MDEG requires high amiloride concentrations for blockage and may serve for salt taste components of reduced Na^+ selectivity. We used antibodies against ENaC subunits to show the Na^+ channel distribution in taste buds. The α-ENaC signal coincided fairly well with the distribution of amiloride binding sites (RA6.3) and occurred in cells which contained tryptophan hydroxylase-like reactivity (type III cells?). ENaC-like immunoreactivity was less evident in cells which contained α-gustducin (type II cells?).

Sweet-sensitive taste cells of the rat respond to sucrose with activation of adenylyl cyclase, followed by cAMP-dependent membrane events and Ca^{2+} uptake. The same cells respond differently to some artificial sweeteners, i.e. with IP_3-dependent Ca^{2+} release from intracellular stores. We probed for additional transduction pathways and found that CV-taste buds have NADPH-diaphorase activity under conditions which should indicate NO-synthase (NOS). However, NOS-I like immunoreactivity was localized merely to a few basal cells or basal aspects of elongated cells of the CV taste buds. The significance of NO signaling in taste remains to be determined.

325. Test–retest reliability of chemosensory thresholds determined with an adaptive maximum-likelihood staircase procedure

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Last year we reported that the adaptive maximum-likelihood staircase procedure, a method new to the chemical senses, is a good alternative for measuring chemosensory thresholds. Compared to the ascending method of limits and the up-and-down staircase procedures, the adaptive maximum-likelihood staircase procedure yields threshold estimates with small confidence intervals, while requiring relatively few trials to reach the threshold criterion.

To investigate whether the adaptive maximum-likelihood method yields threshold estimates which are stable over time, a test-retest protocol was used to test 28 subjects on four different occasions with respect to their sensitivity for butanol and NaCl. Half of the subjects were tested four times on four consecutive days and the other half was tested on four days in a three week period. Traditional measures of test-retest reliability (correlations, distribution of difference scores) show greater stability for NaCl thresholds than for butanol thresholds. Mean Pearson r_{xy} values were 0.876 and 0.575 for taste and smell thresholds respectively.

326. Characterization of glomeruli differentially labeled with the lectin *Dolichos biflorus*

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Lectins are complex glycoproteins that bind to cell surface carbohydrates. Although the significance of lectin binding reamins to be established, one possibility is that they reveal cell surface markers that may be involved in axon guidance and/or adhesion. In the olfactory system several lectins, including *Lotus* and *Dolichos biflorus* (DBA), label subsets of olfactory receptor neurons (ORNs), including their axons. In the present study we have sought to further our understanding of the glomerular distribution of DBA immunoreactive (DBA^+) ORN axons. In particular, we have been interested in examining the organization of glomeruli that are homogeneously innervated by DBA^+ axons versus glomeruli that are heterogeneously innervated by both DBA^+ and DBA^- axons. C57/BL mice were perfused with 4% paraformaldehyde and 50 μm olfactory bulb sections were incubated in 200 μg/ml biotinylated DBA for 24–48 h. Single DBA^+ axons were visible coursing through the olfactory nerve layer, forming fascicles and arborizing in glomeruli. A total of 30–40 glomeruli (~2%) contained DBA^+ axons. Some glomeruli showed intense DBA^+ innervation with dozens of individual axons visible, while others appeared to contain only one or two labeled axons. The intraglomerular distribution of single axons appeared similar to that previously described from Golgi studies. Double labeling to olfactory marker protein (1:600) demonstrated that DBA^+ axons were also OMP^+ and therefore from ORNs. Also, double labeling demonstrated that glomeruli sparsely innervated by DBA+ axons were also innervated by OMP^+/DBA^- axons. The glomerular distribution of DBA^+ and DBA^- axons did not suggest a stereotyped pattern. Because some glomeruli are heterogeneously innervated by both DBA^+ and DBA^- axons it is reasonable to conclude that axons in single glomeruli can differ in their molecular phenotype. The significance of these observations relative to the observation that ORN axons containing specific odor receptor mRNA converge onto only one or two glomeruli remains to be established, however one plausible hypothesis is that DBA binding sites might act in concert with other surface markers to influence intraglomerular target selection.

Supported in part by NIDCD 00210 and NINDS 10174.

327. The 'sugar' receptor cell in *Protophormia terraenovae* bears both amiloride-sensitive and insensitive receptor sites

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An amiloride-sensitive cation conductance has been shown to exist in insects: however, unlike the case of most vertebrates, it appears to be involved in the sugar reception mechanism rather than the salt one. As the sugar receptor cell of labellar chemosensilla in the blowfly *Protophormia terraenovae* also seems to mediate reception of amino acids and proteins, we have studied the effects of amiloride on the responses to sugars as well as to bovine serum albumin (BSA) and L-alanine (its C-terminal amino acid). The spike responses of the four chemoreceptor cells present in each chemosensillum were simultaneously recorded extracellularly and then sorted out by a spike waveform analysis. Results show that: (i) amiloride both at 100 and 500 μM does not influence the spike firing frequency of the 'sugar' receptor cell in response to sucrose, but depresses the response to fructose and, at 500 μM, decreases the 'sugar' cell response to BSA and L-alanine; (ii) spike firing frequency of the 'salt' cell in response to sucrose, fructose, BSA

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and L-alanine is not influenced by amiloride 100 μM, while at 500 μM the responses to both BSA and alanine are decreased; and (iii) amiloride at both concentrations does not affect the spike activity of the 'water' and '5th' cell to any of the stimuli tested, except for the response of the former cell to L-alanine, at 500 μM. According to these results the 'sugar' cell apparently bears both amiloride-sensitive and insensitive receptor sites corresponding to the furanose and the pyranose site types respectively. The latter also appears to mediate, in part at least, the stimulatory effectiveness of BSA and L-alanine on this cell.

Funded by Italian Board of University (M.U.R.S.T.; 40% and 60% Funds).

328. Chemosignal transduction in the vomeronasal organ of garter snakes: chemoattractant-receptor-mediated protein phosphorylation

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We previously found that the binding of ES20, a chemoattractive protein, to its receptors in the vomeronasal (VN) sensory epithelium of garter snakes activates G-proteins resulting in an increase in the level of second messengers, IP_3 and presumably DAG. The former mobilizes intracellularly sequestered calcium, whereas the latter presumably activates protein kinase C. In VN sensory epithelial preparations, when ES20 binds to its receptors, phosphorylation of three proteins [one membrane bound 44 kDa protein (p44) and two cytosolic 55 kDa (p55) and 99 kDa (p99) proteins] is remarkably affected. The membrane-bound p44 is particularly interesting, as it is the most prominently phosporylated membrane-bound protein on membrane and is the first such protein to become phosphorylated following ES20-receptor binding. The degree of p44 phosphorylation is affected by Ca^{2+} concentration, DAG and GTP-gamma-S. These results suggest that p44 may play a role in VN chemo-signal transduction pathway of garter snakes. This protein, p44, can be phosphorylated by two protein kinases, a membrane bound enzyme and a cytosolic enzyme and these kinases are affected differently by Ca^{2+} concentration, DAG or GTP-gama-S. This protein appears as a single labeled band on SDS–PAGE when 32P-gama-ATP is used as a phosphate donor and appears as two distinct.

Supported by NIDCD grant DC# 00104.

329. Chemosignal transduction in vomeronasal organ of garter snakes: identification of two types of adenylate cyclase

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We previously reported that ES20 receptor binding activates phosphoinositide (PI) turnover resulting in an increase in IP_3 which in turn mobilizes intracellularly stored calcium in the vomeronasal sensory epithelium. We also found that the activity of adenylate cyclase (AC) in the VN organ of garter snakes is very sensitive to Ca^{2+} regulation. Thus, AC is likely to constitute an important component in the signal transduction in VN system of garter snakes. We proceeded to clone the gene(s) encoding AC from VN organ of garter snakes. mRNAs were isolated from garter snake brain and RT PCR was performed using a pair of dengerate primers (5′ ATH AAR ACN ATH GGN WSN ACN TAY ATG CG and 5′ TTN ACN GTR TTN CCC CAD ATR TCR TA). The products of 250 bp fragments were amplifled and cloned. Sequence analysis revealed two types of clones: one shows 81% homology to human AC type V and 81% homology to rat AC type VIII mRNA and the other shows 81% homology to AC type VI of rat or mouse. Using RNase protection assays AC type V was found in mRNAs from brain and olfactory epithelium but not in those from VN epithelium. The mRNAs from VN epithelium contained the highest abundance of AC type VI, whereas mRNAs from olfactory epithelium and brain showed only very faint bands. In addition, a 1×10^6 VN cDNA library was screened with 250 bp probes derived.

Supported by NIH grant DC 00104.

330. Chemoreception for fat: fatty acids modulate K channels in taste receptor cells

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We used patch clamp recording on isolated rat taste receptor cells (TRCs) to determine if there were gustatory cues provided by fats. Because free fatty acids (FFAs) are known to function as extracellular messengers and rats contains significant amounts of lingual lipase and oral lipophilic carrier molecules which bind fatty acids, we focused on investigating the effects of FFAs on rat fungiform TRCs. *Cis*-polyunsaturated fatty acids (PUFAs) inhibit the delayed rectifying K channel (K_{DR}) in all TRCs and enhance inwardly rectifying K channels (K_{ir}) in a subset of TRCs. PUFAs have similar effects in TRCs isolated from the vallate and foliate papillae, as well as from the soft palate. Saturated, monounsaturated, *trans*-polyunsaturated FFAs as well as FFA methyl esters were ineffective in modulating these K channels. Triglycerides containing *cis*-PUFAs tested under the same conditions were not able to significantly inhibit K_{DR} channels. The inhibition of K_{DR} by arachidonic acid was concentration-dependent with an inhibition constant of 1.04 μM. Pharmacological studies have ruled out the involvement of G protein-mediated pathways, protein kinase C and protein kinase A, the lipoxygenase, cyclooxygenase and cytochrome P450 pathways in the PUFA response. PUFAs applied intracellularly were ineffective in modulating K_{DR} activity. These results are consistent with PUFAs acting directly on the extracellular face of the K_{DR} ion channel or a closely associated regulatory protein. Taken together, this inhibition of K_{DR} and enhancement of K_{ir} would have the net effect of depolarizing TRCs at rest and prolonging stimulus-induced activity. These results demonstrate that fats, in the form of FFAs, may provide direct cues to the gustatory system and may profoundly influence TRC responses to other taste stimuli.

Supported in part by NIH NIDCD grant DC02507 to T.A.G.

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331. Early developmental stages of cells and axonal tracts in the olfactory system

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Olfactory axons extend from the olfactory receptor neurons localized outside the brain, in the olfactory epithelium of the nasal cavity. These fibers grow and enter into the glomerular layer of the olfactory bulbs where they make synaptic connections with periglomerular, tufted and mitral cells forming the first relay and integrative synaptic station in the olfactory system: the olfactory glomerulus. During early embryonic development, before the anatomical formation of the olfactory bulb, olfactory receptor neurons originate in the olfactory placode, which develops as a local thickening of the epithelial tissue in the rostral tip of the embryonic head. From the very early stages of development a fibrillar and cellular continuity persists between the olfactory placode and the central nervous system. Thus, some cells originating in the olfactory placode leave this structure and migrate toward the telencephalic vesicle. Among these migrating cells, four cell populations have been described by different authors, interacting with the telencephalic vesicle LHRH-secreting-cells, ensheathing cells, olfactory marker protein (OMP)-immunoreactive cells and a novel population of cells recently described by our group which interact with the neocortical preplate cells at the beginning of the layer formation. Those populations of cells use different migratory mechanisms to reach their targets in the telencephalic vesicle. This communication addresses the development of the projection of primary olfactory axons, the migratory patterns of different cell populations derived from the olfactory placode and the olfactory epithelium and finally the early developmental stages of axonal tracts in the olfactory system, including the sequence of events during the early growth of the main fiber tracts emanating from the olfactory bulb output neurons.

Research grant PB91-0066 from the Ministerio de Educación y Ciencia (MEC) of Spain.

332. Chemosensory alteration of information processing

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Odor effects on cognitive processing are varied. Previous research has found both facilitation and interference when odors are introduced during task performance. Additionally, because of the highly suggestible nature of odor effects, rigorous controls are needed to evaluate alterations in cognition purportedly due to odors. The present experiment sought to control a variety of confounding factors in the evaluation of odor effects on task performance.

Eleven undergraduate subjects were asked to make judgements on three different types of visual stimuli while event-related potentials were measured from 30 sites on the scalp. Subjects button pressed to indicate whether the current visual stimulus was larger than the previous stimulus. The stimuli they compared were the referents of nouns (car, house, etc.), numbers (125, 301, etc.) and circles (o, O, etc.) in different phases of the experiment. Two seconds prior to 66% of these stimuli, a tone or odor (butanol or peppermint extract) was presented via a constant flow olfactometer. Both tones and odors were presented during the inspiratory phase of the breathing cycle. In the remaining 33% of the trials, no stimulus was presented (blank).

After correction for eye movements and digital smoothing, the P300 data to the visual stimuli were compared with determine the effects of the odors, tones, and blanks. These data indicated a significant ($P < 0.05$) interaction of the class of visual stimulus being processed with the modality of the preceeding stimulus. P300 amplitude was attenuated following the odor stimuli for symbolic (nouns and numbers) but not orthographic (circle) stimuli. The opposite was true for following the auditory stimuli and blanks.

These data and the results of other experiments will be discussed in terms of potential sources of intrahemispheric interference during olfactory and symbolic information processing.

333. Characterization of unsteady flow at a chemosensory surface by the dimensionless Womersley number

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Chemosensory surfaces of many organisms experience unsteady flow, either because the signal-laden fluid (such as air or water) has an unsteady component to its velocity as it approaches a receptive surface, or because the chemosensory organ is moved in an unsteady manner through otherwise steady fluid. The flow profile in the vicinity of a chemosensory surface is one factor that determines the rate at which the surface will intercept chemical signals from its surroundings. An unsteady component to the flow is a complication that is often ignored due to the difficulty of its explicit consideration. However, the thickness of the velocity boundary layer will be a function of the unsteadiness of the flow, and therefore an unsteady component may be significant in influencing chemical signal uptake. There is a relatively simple way to characterize the unsteady behavior of a fluid, and that is by the magnitude of a dimensionless number, the Womersley number (*Wo*). For flow adjacent to chemosensory surfaces that may be approximated as flow inside a cylinder or flow between parallel flat plates, a dichotomy in fluid behavior is seen for values of $Wo < 1$ and $Wo > 1$. When $Wo < 1$, the flow is predicted to faithfully track an oscillating pressure gradient ('quasi-steady' behavior). If the oscillation frequency of the driving pressure gradient increases such that $Wo >> 1$, the oscillations in the flow are predicted to decline precipitously in amplitude, and the flow profile no longer exhibits the parabolic Hagen–Poiseuille shape.

334. Psychophysical measurement of 6-*n*-propylthiouracil (PROP) taste perception

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The ability to taste PROP is genetically determined. PROP

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tastesmoderately bitter to 'medium tasters' (MT), intensely bitter to'supertasters' (ST), and tasteless to 'nontasters' (NT). The psychophysical method used to characterize PROP status should capture the entire range of perception, while minimizing context, ceiling and other effects. Magnitude estimation successfully captures the variability in PROP perception, but requires normalization and may be difficult to conduct in industrial settings. Two labeled scales were tested as part of three separate studies (S1, S2 and S3) to measure perceived intensity of PROP and sweeteners. All studies included reportedly healthy volunteers aged 21–62 years recruited at Cultor Food Science in Groton, CT. In S1 [n = 163 (55 males, 108 females)], subjects rated perceived intensity of PROP-saturated paper and sucrose (1.0 M) on the Labeled Magnitude (Green) Scale (LMS) [labeled line with descriptors (no taste—strongest imaginable)]. In S2 [n = 152 (49 males, 103 females)], subjects rated perceived intensity of sucrose (1.0 M) and PROP solutions (0.001 M, 0.0032 M) on the LMS. In S3 [n = 136 (48 males, 88 females)], subjects rated perceived intensity of sucrose (1.0 M) and PROP solutions (0.001 M, 0.0032 M) on a 9-point category scale (1 = not at all; 9 = extremely). In all experiments, water rinses were included between each tastant and PROP was the final stimulus. Statistical analyses included descriptive statistics, regression analysis, and ANOVA. In S1 and S2, those with higher PROP perception perceived sucrose more intensely [(S1:r = 0.32; $P < 0.001$); (S2: r = 0.25; $P < 0.01$)]. A higher frequency of females were ST than males. Also, the PROP effect on sweet perception was most evident in female ST. This apparent sex difference may be the result of hormonal variation associated with menstruation. As well, in S1 and S2 subjects aged 20–40 years, females had significantly greater variance among sucrose intensity ratings than males (F = 3.66; $P < 0.01$), which may be due to hormonal changes with menses. The S3 results failed to show either the positive correlation between PROP and sucrose perception or the sex difference. Thus of the two labeled scales, the LMS appears to be better for assessing PROP perception, as it is continuous and also minimizes ceiling effects. Future research will extend these studies by including sucrose and high intensity sweetener concentration series.

Research supported by NIH Grant DC00283 (Bartoshuk) and Cultor Food Science.

335. Activation of rat trigeminal ganglion neurons by capsaicin and two non-pungent analogues

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Capsaicin, the pungent ingredient in chili pepper, activates and subsequently desensitizes a subset of polymodal nociceptors. Capsaicin is a analgesic and anti-inflammatory compound that is used clinically to treat a variety of aliments. However, because its initial application to skin produces nociception, non-pungent analogues have been synthesized to overcome these problems. Two such analogues are olvanil and glycerol nonivamide. To determine how these non-pungent compounds differ from capsaicin, whole clamp recordings were performed on rat trigeminal ganglion neurons.

In neurons held at –60 mV, capsaicin, olvanil, and GLNVA can activate one or two kinetically distinct inward currents. For these compounds the current-voltage curves rectified and had reversal potentials of 28 and 5 mV for the more rapidly and slowly activating currents, respectively. These data provide evidence for subtypes of vanilloid receptors. One major difference between these compounds is that, on average, the activation kinetics of the currents evoked by 1 μM olvanil and 30 μM GLNVA were considerably slower than those evoked by 1 μM capsaicin. Measurements of the peak current I_p versus agonist concentration were fit to the Hill equation to yield values of the maximum current (I_{pmax}), the dissociation constant ($K_{1/2}$) and the Hill coefficient (n). For capsaicin, olvanil and GLNVA I_{pmax} = 7.10 nA, 7.19 nA, and 5.86 nA; $K_{1/2}$ = 0.68 μM, 0.59 μM and 27.0 μM; and n = 1.38, 1.32 and 1.24, respectively.

We propose that olvanil and GLNVA are non-pungent because their activation kinetics are relatively slow compared with the rate these compounds inhibit the generation of action potentials.

336. Taste receptor cell distribution of membrane proteins involved in NaCl, KCl, and NH_4Cl taste

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It is thought that NaCl and KCl stimulate taste receptor cells, in part, by cations entering through specific ion channels. In peripheral taste receptor cells of the rat, amiloride-sensitive epithelial Na^+ channels are implicated in taste stimulation by sodium salts, while 4-aminopyridine-sensitive K^+ channels (4-AP K^+ channel) are implicated in taste stimulation by KCl and NH_4Cl. Other studies indicate that epithelial Na^+ channels may also play a role in taste cell stimulation by KCl and NH_4Cl tastants. In a recent study, we measured the integrated responses of the chorda tympani nerve to 30, 75, 150, 300 and 500 mM concentrations of NaCl, KCl and NH_4Cl mixed with and without amiloride (1 and 25 μM), benzamil (1 and 25 μM) and DMA (1 μM) at 35°C. The low concentrations of amiloride (1 μM) and benzamil (1 and 25 μM) should exhibit selective antagonism of the epithelial Na^+ channel pathway. The low concentration of DMA (1 μM) should exhibit selective antagonism of the Na^+/H^+ exchanger pathway. Our results showed that the addition of amiloride, benzamil, and DMA to stimulating solutions of NaCl, KCl and NH_4Cl significantly inhibited chorda tympani nerve responses to all three salts. These results support the idea that different monovalent salts may utilize the same mechanisms to transduce their chemical signal into electrical impulses in the afferent chorda tympani nerve. A crucial next step in this analysis is to determine the contribution of epithelial Na^+ channels, 4-AP K^+ channels, and Na^+/H^+ exchangers on taste cells innervated by individual fibers of the chorda tympani nerve. Previous single fiber studies in rats and hamsters indicate that epithelial Na^+ channels may be present exclusively on taste receptor cells innervated by salt-best N-units. The contribution of 4-AP K^+ channels and Na^+/H^+ exchangers on the responses of different fiber types of the chorda tympani nerve is unknown. The present study examines the inhibitory efficacy of 1 μM concentrations of amiloride, benzamil, DMA, and 4-AP on responses of individual chorda tympani neurons to NaCl, KCl and NH_4Cl solutions at 35°C. The results will be presented in relation to neuronal type (i.e. salt-best, sweet-best, acid-best and quinine-best). This study will help clarify the roles of four transmembrane mechanisms in salt taste processing and coding of sodium and nonsodium salts.

This research was support by NIH grant DC 02641.

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337. Measurement of taste receptor cell (TRC) intracellular pH (pH_i) by microfluorometric and imaging techniques: effects of external pH, HCO_3^- and PCO_2

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The changes in TRC pH_i induced by varying external pH (pH_o), PCO_2 and $[HCO_3^-]_o$ were studied in taste bud fragments (TBFs) from rat and hamster fungiform papillae, with the pH-sensitive fluoroprobe, BCECF, using microfluorometric and imaging techniques. In microfluorometric studies, changing pH_o of HEPES-buffered solutions between 6.5 and 8.0 (22 ± 1°X) induced rapid and monotonic changes in TRC pH_i. The relationship between pH_i and pH_o was steep with slopes varying between 0.8 and 1.2. Similarly, in HCO_3^--buffered solutions varying pH_o by changing PCO_2 (by equilibrating Ringer's solutions with 1.7, 5.0, 10.0 and 19.5% CO_2–O_2 mixtures) at constant $[HCO_3^-]_o$ (36 mM) or changing $[HCO_3^-]_o$ (9, 36 and 90 mM; $NaHCO_3$ replacing equivalent amount of NaCl) at constant PCO_2 (5% CO_2) led to rapid monotonic changes in pH_i. The relationship between pH_i and pH_o was once again steep with slopes varying between 0.8 and 1.2. However, simultaneous changes in $[HCO_3^-]_o$ and PCO_2 at constant pH_o of 7.40 (12.2 mM $[HCO_3^-]_o$/1.7% CO_2 or 72 mM $[HCO_3^-]_o$/10% CO_2) did not cause any significant changes in steady-state pH_i. In imaging studies single isolated TRCs responded to changes in pH_o with parallel changes in pH_i in the soma and apical process. In addition, changes in pH_o induced parallel changes in pH_i throughout the TBFs. These data suggest that the steady-state TRC pH_i appears to be a function of pH_o and independent of PCO_2 and $[HCO_3^-]_o$ over the range of pH_o, PCO_2 and $[HCO_3^-]_o$ tested. Changes in TRC pH_i may be involved in acid sensing, and salivary $[HCO_3^-]$ may play a role in maintaining steady-state TRC pH_i and in the neutralization of acid-induced changes in pH_i.

Supported by NIH grant DC00122 and Department of Veterans Affairs.

338. Ruthenium red and neomycin block the zebrafish olfactory cyclic nucleotide-gated channel

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The basic biophysical and pharmacological properties of the cyclic nucleotide-gated (CNG) channel of zebrafish olfactory receptor neurons (ORNs) were functionally characterized using excised patch recording methods. In general, the cAMP sensitivity, ionic selectivity and pharmacology of the zebrafish CNG channel are similar to other olfactory CNG channels. Drugs reported to block other olfactory CNG channels (amiloride, diltiazem, W-7 and trifluoterazine) blocked the zebrafish CNG channel. We also investigated the effects of two drugs previously reported to affect olfactory PLC/IP3 mediated transduction on the zebrafish olfactory CNG channel. The ruthenium red block of the olfactory CNG channel was voltage dependent, reversible and effective at sub-micromolar concentrations. The neomycin block was also voltage dependent and reversible, but required higher concentrations (50–200 μM) and only blocked 50% of the maximum outward current (at +60 mV). Our findings indicate tha the properties of the zebrafish olfactory CNG channel are similar to other vertebrate CNG channels and reveal that neither ruthenium red nor neomycin should be considered specific blockers of olfactory transduction cascades.

Supported by NIH DC-01418.

339. Puberty acceleration and estrus induction in the house mouse (*Mus domesticus*) by the male-originated, synthetic analogs of natural pheromones

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Primer pheromones play a critical role in the reproductive physiology of the house mouse and other rodents. Since the 1960s, urine has been investigated as the main source of primer pheromones that are responsible for puberty acceleration, estrus induction, puberty delay and pregnancy block in mice. The subsequent chemical characterization of some of these pheromones in our laboratory now provides the opportunity for a better understanding of the relevant physiological/biochemical processes pertaining to reproduction. A unique urinary constituent, 6-hydroxy-6-methyl-3-heptanone, demonstrates clearly the puberty-accelerating activity through the standard uterine weight assays. It has been tested alongside 2-*s*-butyl-4,5-dihydrothiazole and 3,4-dehydro-*exo*-brevicomin (previously implicated in the estrus synchronization effect), using the natural stimuli as the positive control. Additionally, two synthetic farnesene isomers (found as the most characteristic constituents of the male preputial glands) are shown to cause estrus induction in grouped female mice. The occurrence of these primer pheromones as ligands of the major urinary protein suggests the protein's role as a natural slow-release agent, or a transporter.

Supported in part by PHS DC 02418.

340. Cerebrovascular responses to odorants: a possible mechanism for odor-induced headache

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The pain associated with vascular headaches is believed to be caused by stimulation of cerebrovascular nociceptors. The majority of these nociceptors originate from the nasociliary nerve, a branch of the trigeminal nerve (Suzuki, 1989). Electrical stimulation of nasociliary fibers causes an increase in cortical blood flow. This increase in cortical blood flow is associated with vascular headache. Since odorants may elicit vascular headaches such as migraine or those reported in sick building syndrome, we decided to examine the effects of odorants on cortical blood flow.

Blood flow was monitored in rats using a laser-Doppler flowmeter. The flowmeter probe was placed over the left cortex of an anesthetized rat while several concentrations of propionic acid (PA), cyclohexanone (CH), amyl acetate (AA) and butanol (BU) were individually presented. Odorants were delivered via a computer-controlled, air-dilution olfactometer. Blood flow was recorded for 90 s prior to odorant presentation, during the 90 second presentation, and for 90 s post-presentation.

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Results showed that cortical blood flow increased as the concentrations increased for three of the odorants tested. The greatest increase in blood flow occurred to PA, followed by CH, and then AA. There was no response to BU.

Previously, our lab had obtained electrophysiological responses from the rat ethmoid nerve (a branch of the nasociliary nerve) to a variety of chemical stimuli. The concentrations of odorants used in the current study were similar to those which stimulated the ethmoid nerve. In addition, the relative effectiveness for the odorants PA > CH > AA > BU was the same for the neural responses and the cerebrovascular responses. We hypothesize that the mechanism for odor-induced headache may involve the stimulation of trigeminal fibers innervating the nasal mucosa resulting in activation of cerebrovascular nociceptors.

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341. The effects of hue saturation and absorbance on perceived odor intensity

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Interaction of the olfactory sense with visual processes may be an inherent part of odor perception. The purpose of this experiment was to determine whether there was a perceived difference in odor concentration when hue saturation (absorbance) was varied along one dimension. Twenty-seven subjects judged the perceived odor intensities of amyl acetate (an aroma chemical reminiscent of banana) and lime oil (a natural extraction from the lime peel), ranging in five geometric dilutions, each one varying in saturation and absorbance along four levels of hue: one colorless and three intensities of green. Visual magnitude estimations were also taken for a set of five tinted solutions, three of which were identical to the three intensities of green fragrance solutions. The findings for amyl acetate indicate that subjects tended to give higher estimates for darker tinted odorants at lower levels of concentrations. This was not true for lime oil as there were no significant correlations between the estimate of concentration and the tint. It is speculated that it might be because people associate a green color with lime. Further studies are necessary to determine whether these reports were due to differences in sensitivity or response bias. Studies could be useful for pursuing correlation's of subjective and objective influences in olfactory perception, and even more valuable, for the detection and measurement of pollutants in the environment.

342. Family-specific topography in patterns of odorant receptor expression in salamander epithelium

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Odorant receptor distribution within the olfactory epithelia of rodents is topographically ordered, whereas in fish olfactory neurons expressing individual odorant receptors appear more homogeneously distributed across the epithelial sheet. To characterize the distribution of odorant receptors in the epithelium of an evolutionarily intermediate species which is a useful physiological model, we have performed *in situ* hybridization histochemistry (ISHH) using probes specific for odorant receptors cloned from the salamander, *Ambystoma tigrinum*. Using PCR cloning based on degenerate oligo primers derived from conserved regions of rodent odorant receptors, 22 unique odorant receptors were cloned and sequenced. Alignment of the predicted protein sequences, followed by homology analysis, identified at least 6 gene families (<40% homology) and 12 subfamilies (>60% homology).

ISHH probes from clones representing 5 families were used to generate topographic maps of cells expressing each of these odorant receptors. Patterns of expression of odorant receptors associated with each family are restricted to loosely circumscribed zones which are roughly mirrored between the dorsal and ventral epithelium. Each family exhibits a different zonal pattern, primarily organized along the rostro-caudal axis, such that one family occupies a more rostral zone, another a more caudal zone, with the remaining families occupying intermediate positions. These 'family' zones, although distinct in their distribution patterns, overlap along their rostral or caudal borders.

These data indicate that salamander odorant receptors exhibit zonal distribution patterns somewhat similar to those described in the rodent. The association of zonal patterns of odorant expression with genetic relatedness may reflect a functional relationship between gene clustering (characteristic of highly homologous genes) and topographical patterns of expression.

Supported by NIDCD (J.S.K.).

343. Influence of genetic background on phenotype of mice with an olfactory marker protein (OMP) gene deletion

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Functional characterization of the role of genes in mammals has been greatly facilitated by targeted gene deletion There is growing recognition of the importance of the genetic background against which a gene deletion is studied. We report that the peripheral manifestations of the OMP null phenotype are conserved while the central (bulbar) phenotype depends on genetic background.

OMP is a phylogenetically conserved gene product of unknown function primarily expressed by mature olfactory receptor neurons (ORNs). The OMP gene was deleted by homologous recombination in ES cells derived from129sv/J mice. The chimeric males were mated with 129sv/J and C57BL6/J females. Those matings resulted in lines containing the gene deletion on 129sv/J and mixed, 129sv/J × C57BL6/J, backgrounds respectively. There were no obvious differences in anatomy or grossly assessed olfactory guided behaviors such as mating or maternal behavior in null mice from either of these lineages. However, clear influences of the genetic background were observed on the biochemical phenotype of the olfactory bulb of the OMP-nulls. The low levels of bulbar TH and

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CCK observed in the null mice on the mixed background (compared with normal littermates) were not seen in the mice on the inbred background. These results indicate a novel regulatory influence of genetic background on TH gene expression. By contrast, the different genetic backgrounds exerted a minimal influence on the electrophysiological phenotype. In both strains the ability of the primary olfactory neurons to respond to odor stimuli was modified. EOGs measured *in vivo* on null mice of mixed genetic background and *in vitro* on null mice on the inbred background showed similarly reduced response parameters to an initial stimulus. The magnitude of the response to a second stimulus application also was decreased in mice from both backgrounds and was dependent on interstimulus interval. These observations demonstrate that the altered electrophysiological phenotype of OMP nulls is conserved in different genetic backgrounds.

These results support our hypothesis that the OMP is a participant in ORN stimulus transduction and illustrates the importance of genetic background when analyzing gene function by targeted deletion.

344. Focused attention and the detectability of weak gustatory stimuli: empirical measurement and Monte Carlo computer simulations

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Attentional processes can modulate the detectability of weak stimuli; for example, the detectability of visual or auditory signals can depend on whether attention is allocated to the appropriate spatial location (vision) or acoustic frequency (hearing). Earlier attempts in the first author's laboratory to find analogous effects of focused attention on the detectability of taste stimuli were equivocal, in part it seems because human gustatory sensitivity can fluctuate substantially over time, a serious problem when using procedures that track sensitivity (d') to a constant stimulus concentration. To circumvent this problem, we adopted an adaptive psychophysical procedure, the transformed up-down method, using a 3-down/1-up rule to determine how the threshold to detect weak concentrations of sucrose and citric acid depended on whether the stimulus presented in a given two-alternative, forced-choice trial was expected or unexpected. The results showed threshold sensitivity to be slightly but consistently poorer when the test stimulus was unexpected (e.g. sucrose presented when citric acid was expected) than it was when the test stimulus was expected (e.g. sucrose presented when sucrose was expected).

In this attentional paradigm, the unexpected stimulus must perforce be presented on only a small fraction of the trials. In selecting a procedure, we chose a 3-down/1-up adaptive rule rather than the more popular 2-down/1-up rule, a choice that turned out to be in line with results of Monte Carlo computer simulations. These simulations suggest that across a wide range of conditions (starting stimulus concentrations, step sizes), the variability in threshold measurements can be smaller with a 3-down/1-up rule than with a 2-down/1-up rule, even when the total number of trials is the same and not very great.

Supported by NIH grant 5 RO1 DC00271-13.

345. Abscence of neuronal reorganization in the olfactory bulb of the mutant mice staggerer

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Staggerer mutation, which concerns a ROR-alpha gene, mapped on chromosome 9, and was used as a model of cerebellum neurodegeneration but we have recently demonstrated anomalies extended to all the olfactory system. This disorder is probably due to secondary alteration or expression defect of an adhesion molecules gene of N-CAM type. Besides atrophic cerebellum and locomotor troubles, we have observed severe olfactory alteration and abnormal olfactory bulb organization. Using olfactory cells necrosis induced by $ZnSO_4$, we have observed the following neuronal reorganization at two different ages in these mutants: 30 and 90 days. Though recovery of olfactory sensitivity was noticeable 15 days after necrosis, 30 days old animals presented lower scores in discrimination tests (two odors: amyl acetate and naphthol) than normal mice did. At 90 days old, mice remained unable to disciminate between the odorants 15 days after $ZnSO_4$ application. Histological observations indicated dramatic decrease in granular and periglomerular cells number and high density of degenerated mitral cells(MC). Moreover, the more old mutant mice were exposed to odorant the more cell death occured making impossible any reorganization including a participation of tufted cells.These results are on the opposite of observations on olfactory bulb of mice suffering Purkinje Cell Degeneration, in which tufted cells seemed to replace the degenerated MC. It is supposed NCAMs defect are the primary responsible of olfactory system disorganization since these molecules play a major role in olfactory nerve synaptic targetting leads. If we consider staggerer mice as a model of neurodegeneratiion in human disease, our results lead to conclude that repeated odorant tests directed to sensory re-education in Rett, Huntington or Tourette's syndrome can worsen the cognitive deficit of the patient.

346. Comparison of insect sex pheromone concentration in a field disruption treatment with that expected downwind of an emitting female

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The effect of inundative release of sex pheromone components on mating of the beet armyworm, *Spodoptera exigua* (Hübner), was examined in cotton fields in north Florida. We asked what is the relationship between the average airborne concentration at plant height in the field and the average airborne concentration expected immediately downwind of a 'calling' female? One measure and one estimate based on the female emission rate is required to answer this question. In the field airborne (*Z*,*E*)-9,12-tetradecadienyl acetate (zeta) and (*Z*)-9-tetradecanol (*Z*9–14:OH) was trapped on a filter through which a known volume of air had been aspirated. The filter was eluted and the two pheromone components measured by gas-liquid chromatography. Simple calculation provides the comparison of the airborne concentration in the field to the estimate of that expected downwind of a 'calling' female.

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The highest concentration measured in the field was about one female equivalent. A concurrent measure of the effectiveness of the treatment was obtained by comparing the percentage mating of clipped-wing females to females in pheromone-free cotton fields.

347. Chemoreception mediates gregarious settlement of the barnacle *Balanus amphitrite amphitrite* (Darwin)

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Permanent attachment of barnacle larvae to a surface must occur near conspecifics to enable reproduction of sessile adults. Barnacle larvae locate conspecifics by detecting peptide pheromones. Pheromones are mimicked by peptides that have arginine or lysine at the carboxyl terminus preceded by several neutral amino acids. The nanopeptide bradykinin and bradykinin analogs, settlement pheromone, and settlement factors induce larval settlement and metamorphosis. Cyprid larvae were incubated in increasing concentrations of peptides and pheromones with [^{3}H]bradykinin for 1 h at 6°C. All peptides and pheromones tested compete with [^{3}H]bradykinin except settlement factors. Larvae bind [^{3}H]bradykinin in a specific and saturable manner. There are two binding locations. One has a K_D of 2.5×10^{-8} mol/larva and the other 1.2×10^{-7} mol/larva. The number of binding sites for one location is 4.0×10^{-15} mol/μg/ml protein and the number for the other is 5.8×10^{-15} mol/μg/ml protein. Separate counting of antennules and bodies of cyprids suggests antennules are the site of chemoreceptors.

348. Partially purified cultures of the newborn rat olfactory epithelium support survival of immature and mature olfactory neurons

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Previously, we described dissociated cell cultures that supported survival and differentiation of olfactory receptor neurons (ORNs) (Pixley, 1992). These cultures involve disaggregation of the olfactory epithelium, the underlying lamina propria, and the perichondrial layer and plating onto an astrocyte bed layer. The presence of both epithelial and lamina propria components in these cultures complicated experimental analysis. Here, we describe a preparation in which olfactory epithelial cells were partially purified from the underlying lamina propria of the newborn Sprague Dawley rat with the enzyme thermolysin. These partially purified cells were plated onto laminin coated glass coverslips in keratinocyte basal medium (Clonetics) with 33% fibroblast (from lamina propria) conditioned medium. At 2 days *in vitro* (DIV), flat epithelioid cells formed islands surrounded by fibroblast-like cells. After five DIV, these cultures contained NST-positive (NST$^+$), OMP$^+$ and olf-1$^+$ cells. Anti-olf-1 stains ORNs and cells at the base of the epithelium that are thought to be globose basal cells i.e. the precursors to neurons (Reed, 1996). In the partially purified cultures, olf-1$^+$ cells were more numerous than NST$^+$ cells suggesting the presence of olf-1$^+$/NST-neuronal precursors. Some epithelioid islands contained GLA-2$^+$ cells indicating the presence of Bowman's gland cells. This preparation contained fewer glial and fibroblast-like cells than our original preparation. This, together with the absence of an astrocyte bed layer, will allow better characterization of cell–cell interactions and factors that influence olfactory neurogenesis and differentiation.

Supported in part by NIDCD 00347.

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349. Design principles of fragrances to cover axilla malodor

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The development of methodology to enable fragrances to be created that both smell good and cover axilla malodor is outlined. The design principle uses two factors: (i) the intrisic ability of fragrance materials to cover axilla malodor and (ii) the substantivity of these materials in the axilla. The paper focuses on how this is applied to mixtures and experiments exploring additivity and synergy in malodor coverage are discussed and related to the development of effective deodorant fragrances.

350. Identification of functional neural systems supporting human learning and memory: normal operation and age-related changes

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Neuroimaging methods such as positron emission tomography (PET), functional magnetic resonance imaging (fMRI), provides an unique opportunity to explore activity across the entire human brain in many different behaviors. An important additional feature of these methods is that we can now map functions both in terms of regional activity, and interacting neural systems using methods that focus on the covariances of activity. This presentation focuses on advances in the characterization of brain functions through the measurement of neural system interactions. Specific examples (from PET) include identification of shifting frontal and limbic interactions across increasing demands in a working memory task, and exploration of learning-related changes in feedback effects to primary sensory cortices. A second extension explores how these interactions change with age, possibly reflecting both age-related declines in neural operation and the attempt to compensate through the recruitement of other regions into the functional network.

351. Lateral detection thresholds on left and right anterior tongue for four stimuli

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The human tongue is a relatively symmetrical anatomical structure and is generally assumed to be functionally equivalent on both sides. Previous studies exploring functional asymmetries found that there were no sensitivity differences between the two sides either by electrogustometry or by aqueous taste stimulation. These studies were based on the statistical analysis of the threshold

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values for both sides, obtained from many subjects, and presented as an average across individuals. However, since asymmetries in sensitivity may be randomly distributed across individuals, a within-subject analysis is more relevant to investigate the (a)symmetry in sensitivity. We tested twelve subjects for their detection thresholds on the left and right tongue tip. Thresholds were determined for Na saccharin (Sac), NaCl, citric acid (CA), and quinine HCl by presenting them in solution with cotton swabs to the tongue surface. A two-interval forced-choice staircase procedure which employed a three-down-one-up protocol with a six-reversal criterion was followed. Threshold measurements were performed five separate times for each subject, for each compound, and for each lateral side of the tongue in order to enable within-subject statistical analysis (n = 480 thresholds). Our results showed no overall significant differences in threshold between left and right sides of the tongue for all but one individual. Positive correlations among all five thresholds within a compound were generally very high, except with saccharin for which the correlations were inexplicably much lower. Threshold values ranged between subjects by approximately one order of magnitude for Sac, NaCl and CA, and two orders of magnitude for quinine–HCl. Our results demonstrate that although there are large reliable threshold differences between subjects and between compounds, threshold values did not reveal significant differences between the left and right sides of the tongue within most individuals.

Paul Breslin is a Morley R. Kare Fellow. This work was also funded by NIH grant # R29 DC02995 (P.A.S.B.).

352. G-Protein-coupled receptors expressed in olfactory receptor neurons

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It is well known that olfactory neurons are often responsive to more than one odorant and biochemical data suggest that odorant receptors are relatively specific in their ligand binding. These observations suggest that olfactory neurons may express multiple odorant receptors. Single olfactory receptor neurons were isolated from channel catfish (*Ictalurus punctatus*) and individually analyzed by RT–PCR to determine the expression of the putative olfactory receptors. Receptor PCR products were analyzed from 19 separate cells. Restriction digestion with frequent cutting enzymes, genomic Southern blotting and sequence analyses indicated that in some olfactory neurons, a single odorant receptor was expressed. In other instances, sequence analysis and genomic Southern blotting indicated that multiple receptors are present in a single olfactory neuron. As our molecular data do not always agree with the physiology data, we investigated if other types of G-protein-coupled receptors may be expressed in olfactory neurons. Since glutamate is a known odorant for catfish, we investigated the expression of metabotropic glutamate receptors in olfactory tissue by RT–PCR. Two glutamate receptor subtypes were found that shared significant sequence similarity to the mGluR1, mGluR3 subtypes. PCR analysis of cDNA from single olfactory receptor neurons showed that metabotropic glutamate receptors were coexpressed with each other and with the odorant receptors in the same cells. Immunohistochemical analyses indicated that mGluR1 and m GluR3 subtypes are expressed in the dendritic knobs and cilia of the receptor cells. These results are consistent with the conclusion that mGluRs may represent another group of candidate olfactory receptors in catfish.

Supported by NIH DC01500 (to R.C.B.).

353. Olfactory neuron responses of malaria mosquitoes to host attractants and human emanations (Diptera; Culicidae)

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In the process of odour-guided host-finding mosquitoes perceive volatiles by different kinds of sensory receptors, situated on the antennae and palps. Sensitivity and specificity of the olfactory receptors of the mosquito *Anopheles gambiae* s.s., an important vector of human malaria, were studied by using electrophysiological methods.

Responses of single olfactory neurons were recorded to mixtures of fatty acids known to be attractive in windtunnel bio-assays as well as to individual fatty acids. Sensitive neurons for short chain fatty acids were found in characteristically shaped medium sized sensilla trichodea, a type abundant on the female antenna. Most neurons responded by an inhibition-type of reaction. In order to compare responses of the olfactory neurons to the individual fatty acids, corrections were made for differences in volatility between the compounds.

Furthermore, sensory responses were used to assist the identification of potential host attractants originating from human emanations, by using electrophysiological set-ups for electro-antennogram (EAG) and single cell recording.

Scanning electron microscopy studies on the antennae of *A. gambiae* were performed to supplement electrophysiological studies with morphological information. Preliminary observations indicate that the sensilla trichodea (known to house olfactory receptors) differ from those of other well-studied *Anopheles* species, like *A. stephensi*.

354. Observations on glomeruli and mitral cell numbers and accessory olfactory bulb in adult and aging human olfactory bulbs

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Complete anterior–posterior serial sections of the olfactory bulbs (OB) from human females aged 25102 years, collected and histologically prepared at 10 μm and Nissl stained (Bhatnagar *et al.*, 1987), were studied for aging changes in distribution and number of mitral cells and glomeruli, by various morphometric procedures, as well as for localization of the accessory olfactory bulb (AOB). The results showed that the numbers of mitral cells and glomeruli were both at their highest in the 25–30 year age group (~40 000 mitral cells and 8000 glomeruli per OB); these numbers declined markedly and significantly with age, so that average numbers in the oldest groups (90–100 years) had decreased

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to ~10 000 mitral cells and 2000 glomeruli per OB. The data indicated a loss of ~10% per decade in each of these categories. Interestingly the ratio of mitral cells to glomeruli remained relatively unchanged in the various age groups, at ~5 mitral cells per glomerulus. The aging decline in mitral cells and glomeruli population occurred in all regions of the OB so that the distribution of the mitral cells and glomeruli along the OB's anterior–posterior extent were similar in the various age groups. This distribution consisted of increasing numbers along the anterior regions, a peak around 1/3 to 1/2 of OB's length and decreasing numbers toward the posterior end. Interestingly, this regional distribution conforms to the general patterns seen in other mammals such as the rat and rabbit, as found in our other studies. In addition to these quantitative observations in the main OB we searched for any evidence of the AOB in our various olfactory bulb series. Our focus included both the dorso-medial region where the AOB has been found in the human fetus as well as in other bulbar regions. Our investigation revealed no evidence of an organized structure resembling the AOB. The results indicate that gradual degenerative changes in mitral cells and glomeruli number occur in the human OB during aging and also support previous claims that the adult humans do not possess a well-defined accessory olfactory bulb.

Supported by University of Illinois Research Funds.

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355. The human infant's ingestive and hedonic responses during spoon feeding following repeated exposure to novel vanilla flavoring in bananas

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The ability to acquire flavor preferences at weaning age may facilitate the human infant's transition from a relatively uniform diet of mother's milk or milk formula to the many new flavors in a varied diet of cereals, fruits and vegetables. However, there has been very little study of changes in weaning infants' flavor preferences as a function of spoon feeding experiences. In the present study, 4- to 6-month-old infants were spoon fed pureed bananas by their mothers once on each of three days usually every other day. All infants were spoon fed pureed bananas on the first day (baseline) to establish basic measures of intake and feeding behavior with the test food. On the second day (treatment) infants were matched for baseline intake and offered the bananas with either novel vanilla flavoring added or without the flavoring. On the third day (test) both infant groups were spoon fed the vanilla-flavored bananas. Thus, infants with 1 day of vanilla exposure prior to the test day were compared with control infants naive to the flavor. The results of several measures of intake and behavior converged to show that infants increased preference for the vanilla-flavored food. First, the vanilla-exposed infants increased intake on the test day relative to controls. Second, vanilla exposure produced a greater increase in feeding speed on the test day compared controls without such exposure. Finally, observation of infants' behavior revealed that vanilla exposure produced a greater percentage of positive responses to spoon feeding relative to controls on the test day. These results indicate that human infants can learn a preference for a novel flavor in their food based on limited spoon feeding experience. The present protocol should be useful in analyses of the learning mechanisms that might be involved in formation of the flavor preferences by human infants at weaning.

This research was supported by grant RO3 DC02474-02 from NIH/NIDCD to the first author.

356. Membrane potential oscillations in crayfish protocerebral neurons are organized presynaptically

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In decapod crustaceans, antennular olfactory receptor neurons impinge upon interneuronal targets in the midbrain; processed olfactory information is then relayed by projection neurons to targets in the lateral protocerebrum that reside in bilateral regions termed the hemi-ellipsoid bodies.I have recorded from local interneurons (LPIs) in the hemi-ellipsoid bodies of the crayfish *Procambarus clarki*, with the eventual goal of determining the role these neurons may play in olfactory-mediated behaviors. In isolated, cannulated head preparations, crayfish LPIs exibit ongoing depolarizing membrane potential oscillations with periods of 0.5–1.0 Hz at 18°C. I have used electrophysiological and pharmacological techniques in investigating the origins of these periodic potentials, as well as how this spontaneous activity is modified by odorants applied to the antennules. We found that the amplitude of the depolarizations increases when the LPI membrane is hyperpolarized by injected current. We have found, moreover, that the sodium channel blocker TTX is effective in reversibly eliminating the periodic membrane potential depolarizations, presumably by inhibition of presynaptic impulse pathways. Furthermore, bathing the preparations in zero calcium, high magnesium saline also reversibly eliminates the oscillations.Both treatments also blocked the excitatory postsynaptic potentials in LPIs generated by electrical stimulation of the antennules.From all of these results I conclude that the periodic depolarizations are imposed upon the the LPIs by activity in presynaptic pathways. The most likely pathways for this action are axons of the projection neurons, in the olfactory-globular tract (OGT), and this assumption is currently being investigated by reversible block of OGT activity. We are also investigating the question of whether synchronous activity in a subset of projection neurons is responsible for the LPI depolarization. An answer to this question is being sought by obtaining simultaneous recordings from LPI pairs.

Supported by grant # IBN-9319406 from the National Science Foundation.

357. Structure and organization of chemosensory neurons in the scorpion pectine

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The primary chemosensory organs of scorpions are the pectines, two ventral appendages that survey the substrate intermittently during locomotion. Morphologically these organs appear to detect both spatial structure and composition of chemical signals

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deposited on substrates. The pectinal appendages are sexually dimorphic, comb-like structures composed of up to 40 'teeth' each with several hundred to more than a thousand truncated (<5 μm) setaform sensilla ('pegs'). These peg sensilla are arranged in a 2D array facing the substrate. Each peg sensillum is innervated by a bundle of 9–15 sensory neurons, all but one (a mechanoreceptor) being chemoreceptors by ultrastructure. Electrophysiological studies indicate that chemoreceptive neurons within one peg may interact synaptically, thus forming a local circuit for peripheral information processing. We are now using confocal laser scanning microscopy to describe the 3D structure and arrangement of pectinal neurons in the sand scorpion, *Paruroctonus mesaensis*, males of which are calculated to have > 10^6 pectinal afferents. Histological preparations of metal-impregnated (golgi, bodian, cobalt backfilling) tissues indicate the sensory neurons within each tooth are arranged in two laminae—an outer layer composed of many small bipolar neurons (1–2 μm) and an inner (proximal) layer of larger (3–7 μm) monopolar neurons. Application of nerve fiber tracers (Neurobiotin), fluorescent markers (Strepavidin-Cy3) and fluorescent nuclear dyes (DAPI) support these findings. We are presently using correlative confocal/TEM studies with immuno-gold to trace the path of primary sensory cells through the pectinal circuitry, and antibodies to synaptic vesicle proteins to localize their sites of synaptic interaction. Given the simple and iterative cytoarchitecture of neurons in the pectine, it should be possible to describe quantitatively the entire population of sensory elements there. Ultimately, this sensory network can be related to topographically organized glomerular neuropil contained within the scorpion's brain.

Supported by NSF (IBN-9320362).

358. Ultrastructural localization of signaling proteins in microvilli of the rat's vomeronasal organ

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In the rat the main olfactory organ (MO) makes use of cilia as signal-capturing organelles, while the vomeronasal olfactory organ (VNO) uses specialized microvilli for this. Light microscopy suggested that the VNO also uses different proteins, e.g. $G_{o\alpha}$ and $G_{i\alpha 2}$ (Berghard and Buck, 1996; Jia and Halpern, 1996) for signal-processing than the MO which uses $G_{olf\alpha}$. To pursue this ultrastructurally rat nasal specimens were used, fixed with paraformaldehyde or not, and freeze-substituted. Immunocytochemistry was applied on the sections with colloidal gold conjugates as secondary probe. The studies were complicated by the fact that labeling in sensory cells could only be detected when affinity purified antibodies were used. Most unpurified antisera, including those to signaling proteins, olfactory marker protein, and also normal rabbit and normal goat sera labeled abundantly microvilli of VNO supporting cells. Microvilli of olfactory supporting cells and microvilli and cilia of the non-sensory region of the VNO did not display this behavior. This suggests that VNO supporting cell microvilli are very different from those of the MO. In contrast to not purified antisera, affinity purified antibodies to both $G_{c\alpha}$ and $G_{i\alpha 1\text{-}3}$ (recognizes $G_{i\alpha 2}$) abundantly labeled microvilli of some but not all VNO sensory cells. Apical structures of VNO supporting cells, cells in the VNO non-sensory epithelium, and olfactory epithelial cells labeled considerably less or not at all. Anti-$G_{i\alpha 1\text{-}3}$, but not anti-$G_{o\alpha}$, did label microvilli of a different cell in the transition zone to the non-sensory epithelium. This zone also had sparse ciliated cells with apices that look like those of olfactory receptor cells, but their cilia did not bind antibodies to MO or VNO signaling proteins. Concluding, VNO and MO signaling regions differ in many respects, ultrastructurally as well as cytochemically.

Supported by NIH-NIDCD Grant # RO1 DC02491.

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359. Odorant adaptation in the olfactory receptor cell: targeting of calcium effect

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The major mechanisms underlying olfactory adaptation in intact isolated receptor cells of the newt *Cynops phyrrhogaster* has been investigated by using a combination of odorant stimulation and caged cAMP photolysis. Currents were measured with the whole-cell patch-clamp technique and caged cAMP was loaded in the ciliary cytoplasm through diffusion from the patch pipette. Application of ultraviolet-light flashes to the cilia causes the photolysis of caged cAMP producing rapid and repeatable increments in cAMP concentration and subsequent opening of the cAMP-gated channels. Therefore photolysis of caged cAMP bypasses the early stages of signal transduction (receptor, G-protein and adenylyl cyclase). By stimulation of the cell with double flashes of UV light, we show that the adaptation properties of the photolysis-induced responses are very similar to those of odorant-induced responses, indicating that the adaptation process occurs entirely at downstream of the cyclase. As cAMP directly gates cationic channels other possible mechanisms of olfactory adaptation could involve an enhanced activity of the phosphodiesterase that hydrolyses cAMP or an altered ligand affinity of cAMP-gated channels. We show that our results are consistent with the idea that phosphodiesterase activity remains constant during adaptation and that the principal means of adaptation to odorants is by modulation of the cAMP-gated channel.

Supported by JSPS, HFSP, EC and the Japanese Ministry of Education.

360. The infants' exploration of scented toys

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To evaluate the breast-fed infants' responses to scented objects, we videotaped the facial and bodily reactions of 63 infants (median age = 7 months) as they explored, in succession, three toys that were identical in appearance, but different in their characteristic odor. Two of the toys were scented with odors known to be transmitted to human milk, ethanol and vanilla, whereas the third toy was unscented. Each videotape was subjected to frame-by-frame analysis by means of an IBM-based event

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recorder program called *Observer* (Noldus, Inc., Wagenigen, The Netherlands) to measure a variety of behaviors that are considered either to be exploratory in nature in that they lead to perceptual information about the object or to reflect the infants' hedonic reaction. Such analyses revealed that the infants spent significant more time attending to (e.g. mouthing, manipulating) the scented objects when compared with the unscented object. Moreover, differential exposure to the odors of ethanol and vanilla, as determined by consumption of alcohol or vanilla-flavored foods by a parent, was related to differential responses to these odors. These and other preliminary findings suggest distinctive responses to scented objects which may be determined in part by experiential factors.

This research was supported by NIH Grant AA 09523 and the Gerber Companies Foundation.

361. Effect of GABA on the Cl^- activated Mg-ATPase in fish olfactory mucosa

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We had previously shown the existence of the Cl^- activated Mg-ATPase in the plasma membranes, which operates as the Cl^- channel in the formation of Cl concentration gradient in fish olfactory epithelium and participates in the transduction of olfactory signal. Moreover, the transduction of olfactory; signal in fish is sensitive to GABA-ergic drugs. It's still unclear which way enzyme activity (MgATP-hydrolysis) may be coupled to activation regulation of the intrinsic Cl^- channel activity. The objective goal of this study is to investigate the effect of GABA on the Mg-ATPase activity in common breams (Abramis brama) olfactory mucosa in the presence and in the absence of Cl^-. The increase GABA concentrations in preincubation medium from 10^{-10} to 10^{-6} M caused the twofold increase of the Mg-ATPase activity whereas effect of Cl on the Mg-ATPase resulted in the decrease one was absent. The maximum activation of MG-ATPase was observed at, 10^{-9}–10^{-7} M of GABA and was effective for Cl^- activated Mg-ATPase reducing the enzyme activity by 100%. The decrease and then disappearance of activating effect of GABA on the enzyme activity was observed when increasing GABA concentration from 10^{-6}–10^{-4} M. This confirms results obtained earlier and shows that the Mg-ATPase and Cl^- activated Mg-ATPase in fish olfactory mucosa are mutually dependent and these enzymes change the effect of GABA.

362. Symposium: Pheromone communication and interaction with hormones

M. Meredith, R.E. Johnston, R.I. Wood, L. Monti-Bloch, M.K. McClintock and C.J. Wysocki

Communication by chemical (pheromone) signals is important in many species, including mammals. Chemosensory and hormonal systems can interact in at least two ways: (i) chemosensory input, especially but not exclusively that through the vomeronasal organ, may elicit hormonal release, which, in turn, may facilitate behavioral or physiological responses; and (ii) hormones, especially steroids may be essential for some responses to chemosensory, including vomeronasal, input. Recent, still controversial reports, suggest that chemosensory communication may occur in humans via a residual vomeronasal organ, and that chemosensory/hormonal interactions also operate in humans.

In this symposium we will examine these matters critically. Johnston will explore the concept of pheromone communication and suggest that the notion of a single-chemical 'magic bullet' irresistably leading to a preprogrammed result is too simplistic; despite documented examples of special stimuli acting via the vomeronasal organ. Meredith will briefly review evidence for hormonal mediation of the effects of vomeronasal input; including a situation where vomeronasal and hormonal facilitation of a behavior appear to be interchangable—but where vomeronasal input appears important only in inexperienced animals. Wood will discuss evidence that the effectiveness of chemosensory input to particular brain nuclei depends critically on the simultaneous presence of steroid hormone within the same nucleus. Monti-Bloch will present his evidence that steroids may act as gender-specific chemical signals in humans, exciting an electrical response from the residual human vomeronasal organ and affecting human hormone levels. McClintock and Wysocki will begin the critical discussion of these presentations and of the 'state of the science' in these areas.

363. Vomeronasal,olfactory, hormonal convergence in the brain: cooperation or coincidence?

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Chemosensory cues from females act to facilitate mating in many species. In golden hamsters, hamster vaginal fluid (HVF) is the source of several substances, which attract males, elicit a rapid increase in plasma androgens (a result of release of luteinizing hormone: LH), and induce mounting of females. Some of the substances are detected via the vomeronasal organs, and removal of these organs results in impaired mating behavior in inexperienced males, and loss of the hormonal response to HVF. Mating behavior can be substantially restored by intracerebral injection of luteinizing-hormone releasing-hormone (LHRH). LHRH stimulates LH release but, here, may act within the brain, as LHRH analogs that do not release LH do facilitate mating behavior. LHRH also facilitates mating and courting behavior in various circumstances in other species and in both sexes. In male mice, chemosensory stimulation of LH release is also eliminated by vomeronasal lesions. Thus, LHRH neurons in the brain may be targets for vomeronasal system input and might be activated during chremosensory stimulation and mating. Using c-fos expression as a marker of neural activity, activation of LHRH neurons has been observed in mating female rats, but not in male rats or hamsters, possibly because levels of activation in males are low. Activation by chemosensory stimulation alone has not been reported. Anterior-preoptic LHRH neurons in male hamsters can be activated with electrical stimulation of the vomeronasal organ although few cells are activated at current-levels used in awake animals. LHRH injected intracerebrally causes an increase in fos expression in the medial preoptic area in inexperienced mating male hamsters, but not in non-mating males. During sexual experience, association of olfactory and vomeronasal inputs may link olfactory input to hormonal and behavioral outputs previously activated by the vomeronasal system—permitting

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mating and hormonal responses in experienced males even after vomeronasal lesions. Patterns of c-fos expression in HVF stimulated experienced animals also indicate an enhanced activation of main olfactory pathways.

Supported by NIDCD grant DC00906.

364. Spatial organization of odor-stimulated olfactory receptor neurons in the zebrafish olfactory epithelium

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Zebrafish odorant receptor (OR) genes are either expressed randomly or in overlapping concentric arrays. We used an activity-dependent marker to compare the spatial organization of odor-stimulated ORNs with previously reported OR gene expression patterns in adult zebrafish. AGB (agmatine, 1-amino-4-guanidobutane) enters cells through activated non-selective cation channels and, after fixation, can be visualized immunocytochemically with polyclonal anti-AGB primary and nanogold secondary antibodies followed by silver intensification. Since AGB is a highly-stimulatory odorant for fish (Michel *et al.*, 1996), we examined the spatial distribution of AGB-sensitive ORNs by exposing the olfactory epithelium to 3 mM AGB for 10 min (other odors were applied at 2 min intervals in the AGB background). The distribution of AGB and odor-stimulated ORNs was quantified in the 0.25 μm serial sections at 10 μm intervals. Positive immunostaining was darker than signals within the 95% confidence interval defining the background signal. Labeling was highest in the apical region of the organ near the opening. In this region, an average of 26% of the sensory epithelium was positively labeled during stimulation with AGB alone (n = 2 noses). AGB and glutamine or taurocholic acid labeled 32 and 30% of the apical sensory region, respectively. In the basal region, the positive label comprised <10% of the total sensory area. The proportion of ORNs labeled is higher than expected if an odorant activates only one OR. Possibly, the ligand binding sites of several zebrafish ORs recognize common structural features of the odorants tested.

We thank Dr Robert Marc for the generous gift of the anti-AGB antibody. Supported by NIH DC-01418.

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365. Activity dependent labeling of olfactory receptor neurons using agmatine reveals regional differences in sensitivity in the olfactory organ of the spiny lobster

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The olfactory organ, or antennule, of a mature spiny lobster *Panulirus argus* has ~1000 aesthetasc sensilla and over 300 000 olfactory receptor neurons. ORN cell bodies bodies are located in discrete clusters near the base of the sensillum. We are using an activity dependent labeling technique to quantify the number of odor-stimulated ORNs that innervate an aesthetasc sensillum. The activity marker is AGB (agmatine, or 1-amino–4-guanidobutane), a cationic guanidine compound that passes through non-selective cation channels (Marc, Soc. Neurosci. Abs. 21:781.6, 1995; Michel *et al.*, Chemical Senses 21:642, 1996). To label ORNs, an antennule was excised, cannulated and perfused with saline. Odors mixed in low-Na^+/Ca^{2+} artificial sea water with AGB were presented to the aesthetascs once a min for 60 min. AGB labeled cells were visualized using immunocytochemistry. We show that the intensity of labeling varies with odorant type and concentration. Labeling varies between regions of an antennule and between clusters in a region, suggesting that aesthetascs are not functionally equivalent coding units. These results suggest that ABG activity labeling is useful in studying the functional organization of the lobster's olfactory organ.

Supported by NIH grants DC000312 and DC01418.

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366. Characterization of bitter receptors that couple to gustducin/transducin

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Bitter and sweet tastes are believed to be transducted by receptor mediated mechanisms involving heterotrimeric G-proteins, second messenger-regulated effectors and G-protein-coupled receptors. Gustducin is a transducin-like G protein that we identified and cloned from rat taste cells. We have found that rod transducin is also present in vertebrate taste cells. We have isolated two different phosphodiesterases from bovine taste tissue: one is inhibited the other is stimulated by transducin. Behavioral and electrophysiological analyses of gustducin null mice indicate that gustducin is important to bitter and sweet taste transduction. Recent studies with transgenic mice expressing mutant forms of gustducin indicate that gustducin directly couples to both bitter and sweet taste receptors.

To understand the working mechanism of bitter signal transduction and physically characterize the gustducin/transducin-coupled receptors, we have used *in vitro* activation of gustducin/transducin to characterize bitter receptor activities from bovine taste membranes. The bitter receptors that couple to gustducin/transducin respond to a number of bitter compounds including denatonium, quinine, strychnine and nicotine. In addition, we have solubilized and maintained this bitter taste receptor activity. Further purification and characterization of this receptor is in progress. Immunohistochemical studies show that gustducin and transducin exist in the taste organ of several vertebrates including catfish, cow, mouse, rat, monkey and humans. Gustducin/transducin mediated bitter signal transduction appears to be the major taste signal transduction pathway in many vertebrate animals.

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367. NeuronDB (http://senselab.med.yale.edu/neurondb): a database tool for enhancing experimental analysis of neuronal properties

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Patch recordings in slice preparations are providing increasing data on the membrane properties of different types of neurons. Understanding the significance of a specific property at a specific site requires relating that property to the integrative context of that neuron, and comparing it with other neurons. We have begun development of database tools for this purpose, starting with our analysis of neurons in the olfactory pathway. NeuronDB is built using the Illustra object-relational database management system, made Web accessible through CGI programming. NeuronDB makes possible the storage of membrane property data in a comprehensive yet functional and searchable way by using canonical neuronal forms, consisting of the least number of compartments that allow for representation of their essential functional properties. In addition to the olfactory receptor cell and mitral cell, we have included the principal neurons in several central nervous regions to assure us of the generality of our compartmental scheme. We have created an inventory of membrane properties which can be assigned to compartments within any neuron. NeuronDB allows access to data by viewing the contents of a specific compartment of a given neuron, or by selecting a membrane property and viewing all of the neurons and compartments where that membrane property is located. Also, NeuronDB has a tool which searches for membrane properties within a generic compartment, allowing the user to view only those neurons which contain a specified membrane property in that compartment. These types of tools will allow the user to extract previously unrecognized patterns of molecular organization across different neurons in the nervous system. In addition to supporting experimental analysis, NeuronDB is being adapted to provide an interface to ModelDB, a database of compartmental neuronal models.

Supported by NIDCD, NASA and NIMH (Human Brain Project) and the National Library of Medicine's IAIMS Program. Illustra database software and support have been supplied by the Informix *Engines for Innovation* Research Grant Program.

368. The role of innervation in induction and differentiation of taste organs

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Recent studies are exploring the role of innervation in initial development of lingual taste organs: the gustatory papillae and resident taste buds. In the past, investigators in the taste field have often applied results from regeneration to developmental issues, and therefore a working assumption emerged that a taste nerve is essential in inducing epithelial cells to differentiate into taste bud cells. Less clearly stated has been an accepted role for nerves in induction and development of the papilla itself, the structure in which taste buds reside. It is now recognized that development and regeneration are very different processes in the gustatory system and data from numerous approaches have led some investigators to suggest that gustatory organs might begin to differentiate in the absence of innervation. In this symposium neurobiologists studying other sensory systems will join gustatory scientists in discussing nerve/target interactions in sense organ development, and specifically, the role of innervation in induction and differentiation of the gustatory papillae and taste buds. Charlotte Mistretta will introduce current issues and questions about initial development of taste organs. Louis Reichardt will provide an overview of sensory neuron development after targeted disruption of neurotrophin genes. Alun Davies will present data on neurotrophin expression during target/ganglion cell interactions in development of trigeminal sensory neurons. Bernd Fritzsch will compare nerve dependence of developing inner ear and taste buds. Christopher Nosrat will discuss neurotrophin expression in developing tongue and taste papillae, and effects of neurotrophin gene disruption. Bruce Oakley will consider development of circumvallate papilla and taste buds in neurotrophin gene knockout animals. Linda Barlow will discuss data from amphibian models on taste bud cell origin and differentiation. Robin Krimm will summarize patterns of innervation between developing taste ganglion cells and taste buds.

369. Olfactory bulb GABAergic neurons are more affected by mice staggerer mutation than dopaminergic neurons

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We have observed an important neuronal death in olfactory bulb of mice affected by staggerer mutation, a gene alteration which induce a large neurodegeneration in cerebellum and in olfactory system. Since olfactory system is very sensitive to a wide variety of brain dysfunctions in animals and in human diseases, we have tried to define, on this animal model of aging, the nature of the defects which leaded to neurodegeneration. According to our controls on 30, 90 and 120 days of age, degeneration affected first granular (GC) and periglomerular (PGC) cells and after, the mitral cells (MC). At 120 days, <30% of MC remained functionnal. Since cell death seemed mostly concerned with associative neurons which are of GABA- or dopaminergic types, we have investigated the functional status of olfactory bulb by electrophysiological, cytological analysis and pharmacological experiments. The influence of GABA and dopamine applications has been tested on the transglomerular potentials in normal and staggerer mice. Results in staggerer have shown that an important number of glomerules are not functional. The potentials induced by odorants (ammonia, amylacetate) cannot induce a complete potential, the response consisted thus in a slow and low voltage negative variation corresponding to PI, i.e. olfactory nerve depolarization. An important decrease in the sensitivity to GABA is also observed in mutants as compared with normal mice, since responses remained unaffected by iontophoretic applications of GABA or of bicuculline. On the opposite, dopamine ejection induced a significant decrease of the potential amplitude and lengthened latency. Considering the early appearance of GABA receptors in this structure, it can be conclude that the mutation affects the concerned neurones postnatally simply by their progressive disappearance. The impossibility of GC and PGC to renew from progenitor cells is probably the result of gene alteration. Two gene

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defects can be implied in staggerer neuro- degeneration: the RORalpha and/or an adhesion molecule (N-CAM) gene, the later alteration being perhaps an effect of the first. We are now investigating on these two possibilities.

370. Head-portion exposure to low-level X-rays reduces isolation-induced aggression of mouse, and involvement of the olfactory carnosine in modulation of the radiation effects

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We examined the effects of very low-dose X-irradiation on mouse's aggressive behavior using a resident-intruder paradigm.

Male ICR White Swiss mice became gradually calm, and showed remarkably quiet behavior 7–10 days after the whole body 5 or 15 cGy X-irradiation. Irradiation of the mouse's head-portion produced the same effects, suggesting the important role of the brain in this phenomenon. Furthermore, only the anterior part of the head (olfactory system) exposure also induced the remarkable suppression of the behavior. The calm behavior induced by low-dose X-irradiation might be related to the changes in the olfactory function.

We also obtained the data on brain biochemistry giving a further support for the above low-dose effects on the mouse behavior. The carnosine content decreased to <50% that of control. The content also decreased remarkably after the peripheral deafferentation with $ZnSO_4$. The results indicates that the olfactory exposure induced the similar effects as the $ZnSO_4$ treatment. Previous investigators have shown that $ZnSO_4$ treatment eliminated remarkably aggressive behavior. We think from these results that low-dose X-irradiation could induce subtle changes in olfactory physiology including the decline of carnosine level, resulting in depression of the behaviors.

371. Salty and sour transduction: multiple mechanisms and differences

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A possible pathway contributing to salty and sour transduction in mammalian taste cells is thought to be Na^+ and H^+ permeation through the amiloride-sensitive Na^+ channels. Patch-clamp recordings were made on non-dissociated mouse taste cells using a localized stimulating method to the apical membrane alone. Both $NaCl^-$ and citric acid-induced depolarizing responses were obtained from single taste cells regardless of those amiloride-sensitivity. The citric acid-induced responses were not suppressed by amiloride even in the amiloride-sensitive taste cells whose salt-induced responses were inhibited by amiloride. The reversal potential of citric acid induced current responses was ~60 mV on the average even when cells adapted to Na^+-free Tyrode solution. On the other hand, the reversal potential of NaCl-induced current responses varied from negative to positive potentials from cell to cell, indicating the contribution of multiple mechanisms to salt signal transduction. Furthermore, the ratio of the number of amiloride-sensitive taste cells to that of all the taste cells examined was different among strains. These results suggest that salt- and acid-induced responses are mediated by different transduction mechanisms in mouse taste cells and the difference in occurrence of amiloride-sensitive taste cells among strains comes from the genetic difference.

Supported in part by a grant from the Human Frontier Science Program Organization of France.

372. Unilateral olfactory functioning in patients with schizophrenia: relationship to clinical and MRI indices

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Deficits in olfactory processing have been well-documented in patients with schizophrenia. These deficits are seen early in the illness, and appear unrelated to neuroleptic use, smoking history, cognitive deficits and illness severity. Unlike other sensory systems, the primary olfactory projections are largely ipsilateral and may therefore be sensitive to lateralized abnormalities. However, no study has yet examined unilateral presentation of olfactory stimuli in patients with schizophrenia. Forty-six schizophrenia patients (24 men, 22 women) and 38 healthy controls (23 men, 15 women) were administered the University of Pennsylvania Smell Identification Test (UPSIT) and odor detection threshold for phenyl ethyl alcohol (PEA). Schizophrenia patients performed more poorly on the UPSIT for both nostrils relative to controls [$F(1,80) = 6.9$, $P = 0.01$]. Similarly, PEA thresholds were also significantly poorer for both right- and left nostrils relative to controls [$F(1,57) = 10.01$, $P = 0.002$]. No gender effects or gender'diagnosis interaction was observed for either task. No clear laterality of deficit was seen in nostril presentation for either identification or threshold performance. No relationships were observed between scores on tests of olfactory function and MRI measures for either group. These data suggest that patients with schizophrenia evidence bilateral deficits in olfactory identification and odor detection thresholds, and suggest a more generalized dysfunction in olfactory brain regions. Future studies examining the relationship between unilateral measures and functional imaging indices may help elucidate any possible lateralized relationships.

Supported by grants from NIMH MH43880 and NIDCD PO1-00161.

373. LHRH release in the brain after stimulation of the nervus terminalis in the Atlantic stingray, *Dasyatic sabina*

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The nervus terminalis (NT) of most vertebrates contains cells immunoreactive (ir) to LHRH, but no specific function of this system has yet been demonstrated. In the NT ganglia of Atlantic stingrays, LHRH-ir cells project fibers into the central nerve trunk, which enters the forebrain dorsally along the midline. In an attempt to elicit LHRH release from these cells *in vivo*, the chondrocranium was surgically removed from above the forebrain in anesthetized animals and stimulating platinum iridium hook electrodes were place under the peripheral trunk of the nerve distal

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to the main ganglion. Recording hook electrodes were placed 5–10 mm from the ganglia under the central trunk. The peripheral trunk was stimulated at a rate of 2 Hz with enough current to induce a saturated compound action potential in the central trunk. After stimulation for 45 min, cerebrospinal fluid (CSF) was withdrawn from the fourth ventricle. CSF samples were initially frozen with dry ice then stored in a –40°C freezer. Samples were later thawed and tested for LHRH content with a heterologous radioimmunoassay using mammalian (m) LHRH tracer and standards. The antiserum used in the assay (a gift from Dr Judy King) crossreacts with chicken LHRH-II, dogfish LHRH and salmon LHRH. Three out of the four samples from stimulated animals showed higher levels of displacement of radioactive tracer compared with unstimulated-sham controls, which indicate higher levels of LHRH. A dilution series was performed on one of the samples from a stimulated animal and tested with the assay. The resulting binding curve was parallel to that of the mLHRH standards, indicating we were measuring a LHRH-like substance in the CSF samples.

Supported by NIDCD 00471.

374. This bud's for you: transexual limb grafts produce functional chemosensory appendages in male fiddler crabs

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The fiddler crab, *Uca pugilator*, shows considerable sex-specificity in its chemosensory system. Chemosensory neurons in the feeding claw of a female are more sensitive than those in the male. Further, the female has two feeding claws, while the male has only one feeding claw, and another (major) claw that is used for mate signaling and intraspecific combat. In an effort to examine the both developmental plasticity and CNS versus peripheral aspects of sex-specificity, we have developed a technique involving transplantation of developing limb buds. Two week old developing buds, obtained by autotomizing feeding claws in females, were grafted onto male hosts in place of the major claw. Roughly 10% of the animals maintained this bud to yield a morphologically normal second minor claw on the 1st or 2nd subsequent molt. Physiological investigations confirmed that the grafted limb contains chemosensory axons. Initial evidence on sensitivity thresholds and dose-response functions show these neurons have properties that are more characteristic of chemosensors from female donors than male hosts. Behavioral studies indicate that these axons make functional connections in the CNS. Stimulation of the transplanted minor claw with chemical feeding stimulants evokes characteristic grasping and feeding motions, although only by the native feeding claw. The transplanted claw is at least equally sensitive, or perhaps more sensitive, than the male's original claw, again suggesting donor control over neuronal properties of the limb graft. Anatomical evidence shows that the transplanted claw has a normal axonal complement, while the structure of its CNS target is transformed from that characteristic of a major claw to that of a minor claw. The major claw is not known to be chemosensory, so the results of these experiments suggest considerable plasticity, such that the CNS can process chemosensory information when innervated by chemosensory fibers.

Supported by NIH-NIDCD 1R29-DC02731 to M.J.W.

375. Targeting olfaction

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We have developed a genetic approach to visualize axons from olfactory sensory neurons expressing a given odorant receptor, as they project to the olfactory bulb. This approach is based on gene targeting in mouse embryonic stem cells and exploits the histochemical axonal marker tau-lacZ.

Neurons expressing a specific olfactory receptor, P2, project to only two topographically fixed loci among the 1800 glomeruli in the mouse olfactory bulb. Axonal convergence is apparent in early embryonic stages and is clear at birth. Our data provide direct support for a model in which a topographic map of receptor activation encodes odor quality in the olfactory bulb.

A genetic receptor swap experiment suggests that the olfactory receptor plays an instructive role in the guidance process, but it cannot be the sole determinant in the establishment of this map.

376. The functionality of the human vomeronasal organ: influence on gonadotropin and testosterone plasma levels, and autonomic reflexes

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A number of steroidal substances that we call vomeropherins, have shown to stimulate the human vomeronasal organ (VNO) in picogram quantities. This is followed by changes in central nervous system and neuroendocrine function. Local application to the VNO of certain vomeropherins can modify the pulsatile release of luteinizing (LH) and follicle-stimulating hormone (FSH), change testosterone plasma levels, modulate autonomic reflexes, and induce behavioral responses. We propose that the central physiological and pharmacological effects of vomeropherins are mediated through chemoreceptors located in neuroepithelial cells of the human vomeronasal organ. This data is supported by experiments showing that vomeropherins like pregna-4,20-diene-3,6-dione (PDD), activate cationic conductances in isolated neuroepithelial cells from the human vomeronasal organ. The time course of the conductance change correlates with the electrogram (EVG) produced by local stimulation of the adult human VNO. The effect in the VNO is gender specific, and it is followed by statistically significant decrease of plasma LH, FSH, and testosterone levels in male subjects. LH and FSH levels did not change in female subjects. Putative vomeropherin receptors have been found to be highly specific. An example is the effect produced by EVG depolarization with vomeropherin androsta-4,16-dien-3β-ol in women. This effect can be measured immediately after administration to the VNO with picogram quantities of the active substance, and is accompanied by skeletal muscle relaxation, increased EEG alpha waves and increased parasympathetic tone. Conversely, its stereoisomer androsta-4,16-dien-3 alpha does not produce depolarization in the human VNO, and does not induce changes in autonomic reflexes. We have also studied 10 adult male patients with hypogonadotropic hypogonadism, in which we found a non functional vomeronasal-terminalis system, as tested with the methods

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mentioned above. Interestingly, four of the 10 patients had a normal functional olfactory system.

Our investigations provide evidence that in humans gonadotropin release can be modulated through the VNO and that there are functional connections between the adult human vomeronasal organ and a variety of hypothalamic areas.

377. Effect Of vomeropherin pregna-4,20-diene-3,6-dione on isolated cells from the human vomeronasal organ

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It was recently reported that pregna-4,20-diene-3,6-dione (PDD) produces depolarization of the human vomeronasal organ (VNO) epithelium, followed by a statistically significant decrease of the episodic release of LH and FSH in man (Berliner *et al.*, 1996).

Cells were obtained from the VNO of six young adult male volunteers and were either used fresh or after maintenance in monolayer short-term cell cultures. VNO neuroepithelial cells were identified under phase contrast optics by their distinctive bipolar asymmetric morphology, long neurite, and presence of microvilli. This cell type stains with neuronal markers, and its apical membrane binds antibodies against non-selective ion channels.

We studied the changes in conductance underlying the PDD-induced depolarization of VNO cells using the whole cell and patch clamp techniques. The cells were exposed to dosages of PDD from 10^{-4} to 10^{-7} M. The vomeropherin produced a dose-dependent transient inward current followed by a slow recovery to baseline. This prolonged slow recovery effect outlasted the pulse of the vomeropherin by 200–500 ms. In patch-clamp, stimulation with cAMP (10^{-5} M) activated clusters of channel openings. This activity, which also outlasted the drug pulse, was enhanced by PDD and blocked by substitution of Na^+ in the perfusate by either Cs^+ or $Choline^+$. Testosterone (10^{-7} M) and estradiol (10^{-7} M) did not produce an effect on VNO neuroepithelial cells. Also, other cell types present in the sample that did not stain with neuronal markers were not affected by PDD.

We conclude that PDD is acting on putative receptors in human VNO neuroepithelial cells, activating a cyclic nucleotide-dependent cation-permeable channel. Stimulation of the PDD receptor site is the earliest event in the chemotransduction process in the human vomeronasal organ.

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378. Olfactory specific receptor guanylyl cyclase as a modulator of cAMP level in the olfactory system

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Olfactory specific particulate guanyiyl cyclase (GC-D) has been recently cloned, however its role in olfaction has not been determined yet. In the current study, the role of the olfactory specific receptor guanylyl cyclase was investigated. Since the cGMP response is slower and delayed, compared with the rapid and transient responses of cAMP and IP_3, the cGMP response has been suggested to be involved not in odorant detection, but in desensitization or long-term activity-driven responses. Therefore, the effect of exogenous cGMP on cAMP levels was determined in olfactory cilia. The odorant-induced cAMP production was significantly increased by exogenous 8-bromo cGMP (8-br cGMP) in the isolated olfactory cilia. The addition of exogenous cGMP-dependent protein kinase (PKG) could mimic this effect. The addition of PKG inhibitor abolished this effect. In order to investigate the regulation of the olfactory ciliary receptor guanylyl cyclase, we purified the receptor guanylyl cyclases from the olfactory cilia. The purified receptor guanylyl cyclase was phosphorylated by cAMP-dependent protein kinase. The phosphorylation of the receptor guanylyl cyclases significantly reduced cGMP production. Thus, the potentiated cAMP signal can negatively regulate guanylyl cyclase to limit cGMP signal, and in turn, PKG-dependent cAMP production can be terminated.

Supported by NIDCD 00243 and NIDCD 01704.

379. Sublethal effects of pesticides on pheromonal mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr

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There is increasing evidence that pheromones are closely involved in the synchronization of spawning between male and female teleosts. In Atlantic salmon ovulated females release a priming pheromone(an F-type prostaglandin) within their urine, which when detected by the olfactory system of mature males, increases their levels of plasma sex hormones and expressible milt. This paper describes the impact of exposure to sublethal levels of three pesticides on the priming pheromonal system of Atlantic salmon. The studies examined two different aspects relating to the effects of exposure to an organophosphate (Diazinon), a carbamate (Carbofuran) and a triazine (Atrazine) pesticide on the responses of male salmon to the priming pheromone. First, an electrophysiological study was carried out to investigate the effects of the pesticides on the olfactory ability of mature male Atlantic salmon parr to detect prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Second, an endocrinological study was carried out to investigate the effects of exposure to the pesticides on the levels of plasma sex steroids and expressible milt in mature male parr after exposure to water-borne $PGF_{2\alpha}$. The results indicated that after short term exposure to environmental levels of the pesticides the ability of the male salmon to detect and respond to the priming pheromone was significantly reduced or abolished. The long term sublethal effects of the pesticides on reproduction and populations of Atlantic salmon are discussed.

380. Basic principles for chemoreception: lessons from the physics of chemical dispersion

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Chemical signals comprise four properties that can potentially contain important information for sensory systems. These

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properties are quality (chemical structure), quantity (concentration), spatial and temporal distribution. In most macroscopic situations, three of these properties; quantity, spatial and temporal distributions, are not due to any intrinsic properties of the molecule but are strictly due to the dispersion of signals through fluid flow. If we want to gain some insight into the dynamics of these three properties of chemical signals under natural conditions, it becomes imperative to examine the physical forces involved in chemical dispersion which is mainly fluid dynamics. The exact nature of the fluid flow, and thus the dynamics of chemical signals, will depend upon several features of the environments under study. These include sensory appendage morphology, spatial scales relevant to the behavior or neurobiology of the organism under study, and environmental parameters such as roughness elements. I will show several examples of chemical signals quantified under different physical conditions. These physical conditions, and the resulting signals, set constraints under which the morphology, physiology and behavior of the chemosensory system must evolve. By studying different habitats and the constraints within them, we hope to discover some broad principles that pertain to all chemosensory systems.

Supported by NSF grant # (IBN-9614492).

381. Representation and processing of odor molecule information in the olfactory bulb

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In the mammalian olfactory system, all the sensory neurons expressing a given odorant receptor gene project and converge their axons onto two or three glomeruli among ~2000 glomeruli in the main olfactory bulb. Thus, individual glomeruli seem to be a functional unit, representing one or a few type(s) of odorant receptors. In addition, olfactory sensory neurons in a given odorant receptor expression zone among four zones in the epithelium project their axons to a corresponding zone of the olfactory bulb. These axonal connection patterns underlie the response specificity of mitral/tufted cells in the bulb to odor molecules. Individual bulbar neurons associated with a glomerulus are activated by a range of odor molecules with a similar chemical structure, which can bind to an odorant receptor protein. The range of odor molecules to which a given mitral/tufted cell responds is called 'the molecular receptive range of the cell'. Here we report a local map of molecular receptive ranges of mitral/tufted cells in the rabbit main olfactory bulb. We report also that the molecular receptive range property differ systematically among mitral/tufted cells located in different zones of the olfactory bulb. Finally we provide evidence that lateral inhibition via dendrodendritic synaptic pathway in the olfactory bulb greatly enhances the tuning specificity of odor responses and thus contributes to discrimination of odor molecules.

382. A cyclic nucleotide-gated channel functions in *C. elegans*: olfaction, gustation, and thermosensation

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In the nematode *C. elegans*, behavioral genetics is a powerful tool to dissect neuronal functions at the molecular and cellular levels. We are interested in understanding the underlying mechanism of thermotaxis behavior that manifests the plasticity of the *C. elegans* nervous system. The *tax-4* mutants are unable to respond to temperature as well as to water-soluble and volatile chemical attractants. The molecular analysis revealed that the predicted *tax-4* gene product is highly homologous to vertebrate cyclic nucleotide-gated channels. Tax-4 protein expressed in HEK293 cells reconstituted a functional cyclic nucleotide-gated cation channel, indicating that Tax-4 is a *C. elegans* homolog of an alpha subunit of the channel. Further, the electrophysiological properties of Tax-4 channel suggest that Tax-4 functions only as a cGMP-gated channel *in vivo*. The green fluorescent protein (GFP)-tagged functional Tax-4 protein is expressed in olfactory, gustatory, and thermosensory neurons that mediate all the sensory behaviors affected by the *tax-4* mutations. The Tax-4::GFP fusion protein is partly localized at the sensory endings of these neurons. These results suggest that cGMP is an important intracellular messenger in the *C. elegans* sensory system. and that a cyclic nucleotide-gated channel is required not only in olfaction but also in gustation and thermosensation in *C. elegans*.

383. OCAM, a novel cell adhesion molecule related to zone-to-zone projection of primary olfactory axons

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Zone-to-zone projection of olfactory and vomeronasal sensory axons is a basic principle for organization of the mammalian olfactory system. Chemoreceptor expression zones of the olfactory and vomeronasal sensory epithelia are topographically and functionally mapped onto zonally arranged glomeruli in the main and accessory olfactory bulbs, respectively. Here we report the isolation of cDNA encoding OCAM (R4B12 antigen), an axonal surface glycoprotein expressed by subsets of both olfactory and vomeronasal axons in a zone-specific manner.

OCAM protein was purified from rabbit brains by immunoaffinity chromatography with monoclonal antibody R4B12-coupled Sepharose column, and amino acid sequences of several digested peptides were determined. OCAM cDNA clones were isolated by RT–PCR and plaque hybridization. OCAM is a novel cell adhesion molecule belonging to the immunoglobulin superfamily. As revealed by binding studies, OCAM has a homophilic binding activity. Detailed *in situ* hybridization and immunohistochemical analyses revealed that in both the main and accessory olfactory system, OCAM mRNA and proteins were expressed by sensory neurons in restricted chemoreceptor expression zones and that OCAM-expressing neurons projected

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their axons into the glomeruli in the corresponding zones of the main and accessory olfactory bulbs.

These results demonstrate a precisely coordinated zonal expression of chemoreceptors and OCAM, and suggest that OCAM may play important roles in selective fasciculation, and the formation and maintenance of zone-to-zone projection of the primary olfactory axons.

384. The effects of β-bungarotoxin on the morphogenesis of taste papillae and taste buds

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Although it as long been accepted that innervation by a taste nerve is essential for maintenance of taste buds, it is not clear what role, if any, innervation plays in the morphogenesis of taste papillae and taste bud development. The following study was undertaken to determine the effects of lack of sensory innervation on the development of taste papillae and the formation of taste buds. Timed-pregnant females (n = 3) at gestational day (gd)12 were anesthetized and the uterine horns exposed by laporatomy along the ventral midline. Embryos were impaled with micropipettes and a 1 μl solution (1 μg/μl) of β-bungarotoxin (β BTX), a neurotoxin that disrupts sensory and motor neuron development, was injected into the amniotic cavity using a Narishige microinjection system. Shams were injected with PBS. Fetuses were harvested at gd 18, one day before birth. β BTX-injected fetuses were flaccid, had a distinct cervical curvature and possessed palatal clefts. Four β BTX-injected embryos, two shams and two controls were fixed in buffered paraformaldehyde and processed for glycol methacrylate embedding. Two micron serial sections were examined for the presence and morphology of taste papillae and taste buds. No nerve profiles were observed in β BTX-injected tongues. Although circumvallate papillae were present on β BTX tongues, few fungiform papillae could be identified. These, found in the anterior tongue, were narrower than their control counterparts. Taste buds were present on a large percentage of fungiform papilla profiles (24%) and on circumvallate papillae in Sham and control fetuses; in contrast no taste buds were associated with taste papillae in β BTX fetuses. However, a few small groupings of 'taste bud-like' cells were observed in the palates of β BTX-injected individuals. These results suggest that innervation plays a significant role in taste papillae and taste bud morphogenesis. However, because affected fetuses were examined only at gd18, it is not known if this role is in the formation of these structures or in their differentiation and maintenance.

Supported by NIDCD 01657.

385. Urine-derived compounds decrease membrane conductance and cAMP levels in vomeronasal receptor neurons

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Current findings have suggested that signal detection, integration and transduction in the vomeronasal organ (VNO) may be different from mechanisms used in olfactory receptor neurons (ORN). To elucidate the cellular mechanism(s) involved, male urine-derived compounds, dehydro-*exo*-brevicomin (DHB) and *s*-butyl-dihydrothiazole (SBT), were tested in isolated VN receptor neurons (VNRN) and in VNO tissue of the female mouse. Two bioassays were utilized, whole-cell patch-clamp recordings and cAMP accumulation assays. Dissociated VNRN were voltage and current-clamped and their response to DHB and SBT were determined. Under voltage-clamp recording, ~26% of the VNRN responded to DHB and/or SBT with an outward current at negative holding potentials. The current reversed at ~+4 mV. Application of DHB and/or SBT to VNRN is voltage-clamp conditions decreased membrane conductance, and in current-clamp mode produced membrane hyperpolarization and/or a reduction in the firing of action potentials. No effect was observed with these two compounds in ORN. *In vitro* tissue and cell membrane preparations of the VNO of female mice were used to perform cAMP accumulation assays. The results show that while cAMP levels in VN cells increased after exposure to forskolin and GTP-γ-S, they decreased after exposure to DHB and SBT in a dose dependent manner. The decrease in cAMP levels occurred exclusively in the sensory and not in the non-sensory portion of the VNO. The general odorant, citronellal did not alter cAMP levels in VNRN. In contract, both the urine-derived compounds and citronellal increased cAMP levels in olfactory cilia. The combined data suggests that certain urine-derived compounds decrease membrane conductance and hyperpolarized VNRN and that the mechanism for the closure of the ion channel may involve a decrease in cAMP levels.

Supported by NIH grant DC 02120 from NiDCD.

386. Specific odor terminology: the case of Liwanzi (a Bantu language of Central Africa)

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The study of olfaction in occidental cultures suggests that languages use a rather poor and relatively non- specific set of terms to describe odors. In this paper we would like to suggest that this situation is not universal in the languages of the world. More precisely we would like to propose that there is a relation between the preciseness of the lexicon used by the speakers of a given language and the role played by odors in their culture.

The olfactory semantic field was investigated in liwanzi, a bantu language spoken by less than 10 000 speakers in the south-east corner of gabon (in a mixed savanna/equatorial forest ecological zone). Twenty three subjects were tested in their natural environment using artificial odorant stimuli (pure chemicals, mixtures or essential oils) presented in bottles. The results indicate that 13 different 'basic' terms exist in this language. By 'basic' we imply that these terms refer exclusively to the smell and are not linguistically derived from the name of the substance producing the odor.

Finally we will consider to what extend these 'basic' odor terms can help us understand the structure of the olfactory space in a way comparable to the work done by Berlin and Kay for the color space.

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Supported by the French Ministery of Research and Technology (Sciences de la cognition, N°92C0427, 3 novembre 1992).

387. Immunological and molecular characterization of the olfactory phospholipid transduction pathway

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Inositol phosphates have been implicated in some aspects of olfactory transduction in a variety of species (Restrepo *et al.*, 1996). This laboratory has previously shown that inositol 1,4,5-trisphosphate (IP_3) is involved in the excitatory odor response in the spiny lobster, *Panulirus argus*, and that plasma membrane-localized IP_3 receptors (IP_3Rs) and a G protein α-subunit of the Gq family have been implicated in this transduction pathway (Fadool and Ache, Neuron, 1992; Fadool *et al.*, 1995). We have also described the isolation of two cDNAs, isolated from the lobster olfactory organ, that encode an IP_3R and a Gq-type protein (Munger *et al.*, 1996). Here we describe immunological localization of these two proteins to the outer dendrites of the lobster olfactory receptor neurons (ORNs), the site of olfactory transduction. Olfactory sensilla (aesthetascs) were cut distally to permit access to the dendrites, then the tissue was incubated with one of two antisera: a polyclonal antibody (C-19, Santa Cruz Biotech) raised against a peptide that is absolutely conserved in the cloned spiny lobster Gq, or a polyclonal antibody raised against a unique peptide represented in the cloned lobster IP_3R (Ab 23341). Both antisera strongly labeled the ORN dendrites, exhibiting punctate staining; staining by both antisera was abolished with preabsorbtion by the antigenic peptides. The presence of both antigens in the outer dendrites implies roles in olfactory transduction for both proteins. Additionally, as there are no intracellular membranes present in the outer dendrites, localization of the IP_3R to this region of the ORNs demonstrates a plasma membrane localization of this integral membrane protein.

Supported by NIDCD grant DC01655.

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388. Learning and memory in the chemical senses

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There has been tremendous progress in research on human memory due to the availability of modern neuropsychology, neuroimaging and behavioral neuroscience. The rapid advances in knowledge of neuroanatomical substrates and memory systems have tremendous potential for application to the question of odor memory. There has been very little research on the effects of aging on odor memory; however, what does exist reveals significant influence of age on odor memory. Odor memory is particularly vulnerable to the degenerative processes involved in Alzheimer's disease (AD) and shows significant deterioration in patients who are classified as having mild AD. A specific aim of this symposium is to explore the opportunities for and to stimulate future research in the area of odor memory and specifically the effects of aging and dementia on odor memory. Two internationally renowned experts—one in human memory, Dr Larry Squire, and one in the area of hippocampal function, Dr Howard Eichenbaum—will provide a global view of research on memory systems. Dr Squire will emphasize the different types of memory that are supported by different brain systems, the use of animal modeling of human amnesia, and the application of modern technologies such as neuroimaging, cognitive neuroscience, and neuropsychological techniques to understanding the neuroanatomical basis of human memory. Dr Eichenbaum will focus on neuropsychological studies of memory in humans, animal studies of hippocampal function, and on theoretical and practical issues in assessing odor memory and its application in aging. A second specific aim of this symposium, to bring into focus the most recent findings in the area of odor memory relevant to aging, will be addressed by young scientists at the forefront of this area, who will present their most recent findings. Dr Squire will act as discussant after the shorter presentations, emphasizing the commonalities and departures from research on memory in other systems.

389. Apolipoprotein E and odor identification

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We have previously reported olfactory impairment in normal aging which is further affected in Alzheimer's disease. Patients with moderate dementia show losses in olfactory threshold, odor identification and odor memory. In these patients the sensitivity and specificity of olfactory testing is significant, with the greatest power of accurate diagnosis in the more cognitive olfactory tasks. In patients with very mild AD or in patients at risk for the disease because of their dementia status, losses are apparent for odor identification, odor recognition memory and odor threshold, with the best sensitivity in the identification task. Persons who are either heterozygous or homozygous for the e4 allele of apolipoprotein E have an increased risk of Alzheimer's disease, although they show no dementia in the preclinical period. Evidence of olfactory dysfunction in this population might be reflective of an incipient dementing process. We have recently examined olfactory function in a group of normal elderly persons who have undergone genetic testing for the APOE4 allele. These individuals consisted of all normal control subjects at the UCSD Alzheimer's Disease Research Center who had undergone both the genetic testing and testing for olfactory function. All had been diagnosed as normal by two different neurologists who applied the NINDS-ADRDA criteria for dementia. Persons with a history of alcoholism, drug abuse, learning disability or neurologic or psychiatric illness (including depression) are excluded from the normal control pool at the UCSD ADRC. In this population, persons with the APOE 4 allele showed significantly poorer odor identification than those without. Early appearance of olfactory

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deficits in the progression to AD in persons with the e4 allele suggests diagnostic utility in olfactory testing.

Supported by NIH grant # AG08203 to C.M. and AG12674 to M.B.

390. Age effects on central nervous system activity reflected in the ofactory event-related potential

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A series of studies of the olfactory event-related potential (OERP) examined the underlying central nervous system activity associated with age-related changes in olfactory functioning. Early (sensory) components of the OERP showed reduced amplitude and longer latency in elderly subjects, with larger effects on males. Amplitudes were already decreased at middle age. The late cognitive component, P3, showed a longer latency as well as a decreased amplitude in the elderly, with effect sizes for age significantly larger for the late component than for early components. The large effect on P3 latency suggested age-related slowing of olfactory information processing. Examining the relationship of the components of the OERP with neuro- psychological measures (CVLT, Trails A and B) as well as measures of odor learning and memory indicated that older subjects showed poorer performance on memory tasks as well as slower performance on the Trails tests. Performance correlated with the amplitude and the latency of the P3. Older persons with Down's syndrome (DS) develop neuropathological changes and eventually dementia. Because they also show impaired olfactory function, both early and late OERP components were assessed in this population, with direct comparison between adolescents and older adults. The older persons with DS showed lower amplitudes and longer latencies than normal individuals and significantly longer latencies than younger persons with DS. Taken together, these studies strongly suggest that the elderly brain shows smaller responses to odors, is less able to allocate attentional resources and slows in its olfactory cognitive processing. The OERP is a potent reflection of these changes.

Supported by NIH grant # DC02064 to C.M.

391. Effect of aldosterone on ion transport in the rat tongue epithelium *in vitro*

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The apical Na^+ channel in fungiform taste cells is regulated by hormonal and other factors, but this regulation is not well understood. Herness (1992) injected the mineralocorticoid aldosterone (ALDO) into rats 4–6 h before recording from the chorda tympani. ALDO increased the percent inhibition by topical application of amiloride of the neural response to hyperosmotic NaCl. This suggested that ALDO may increase the number of active amiloride-sensitive sodium channels in the apical membrane of taste cells, as it does in other epithelia. Based on these results, ALDO should increase the amiloride sensitivity of the short-circuit current (I_{sc}) response to luminal hyperosmotic NaCl solution in the dorsal anterior tongue epithelium, as measured *in vitro* in an Ussing chamber. We found that ALDO (0.1 or 1.0 μM), incubated with the epithelium *in vitro* for up to 18 h, had no effect on the amiloride sensitivity of I_{sc} in response to luminal 0.5M NaCl. Hormone injected subcutaneously into the animals 4–6 h before mounting the tissue in the chamber also had no effect. However when ALDO was injected three times over a 26 h period, the percent inhibition due to amiloride decreased in ALDO-treated tissues compared with controls (12 ± 2% compared with 25 ± 2%, mean ± SEM, $n = 6$). When spironolactone, an ALDO-receptor antagonist, was injected into both experimental and control animals (26 h injection), the difference in amiloride sensitivity between experimental and control tissues was no longer statistically significant. These data show an effect of ALDO on lingual epithelial ion transport. Further experiments will be required to reconcile these results with those obtained from neural recordings.

Supported in part by NSF Grant BNS-9196065; and the University of Delaware Charles Peter White Endowment Fund, Science and Engineering Scholars Program, and Honors Program.

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392. Differential distribution of LHRH isotype immunoreactivity in nervus terminalis ganglion of the bonnethead shark, *Sphyrna tiburo* and Atlantic stingray, *Dasyatis sabina*

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The elasmobranch nervus terminalis (NT) ganglia lie on nerve trunks connecting nasal epithelium and brain and are rich in LHRH immunoreactivity.

Immunocytochemical studies on bonnethead and stingray ganglia using antisera raised against dogfish (df)LHRH and salmon (s)LHRH (gift of Dr Nancy Sherwood) show distinctly different patterns of immunoreactivity.

Antiserum GF-5, raised against sLHRH, has been shown by others to react with a broad spectrum of LHRH isotypes. GF-5 antiserum reacts with approximately half of the ganglion cells in bonnethead showing a pattern similar to that shown in previous studies. In addition, the many varicosities of neuronal fibers in the peripheral and central trunks as well as throughout the ganglion show intense immunoreactivity.

Antiserum 7CR-10, raised against dfLHRH, has been shown by others to be actually quite specific for chicken (c)LHRH-II. We report a strikingly different labeling pattern compared with GF-5 or any previous antisera. Intense immunoreactivity is shown in the varicosities of fibers in the peripheral and central trunks and coursing throughout the ganglia of both elasmobranchs. Small (9–13 mm) immunoreactive club-shaped structures are found scattered throughout the bonnethead ganglia but are absent in the stingray ganglia. Using confocal microscopy, these club-shaped structures appear to be formed by the convergence of multiple fibers and terminate on blood vessels within the ganglion.

These data suggest that the multiple isotypes of LHRH

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expressed in elasmobranch NT ganglia may have distinct physiological roles.

Supported by NIDCD 00471.

393. Comparison of the zonal expression of pheromone receptors and OCAM in the vomeronasal sensory epithelium

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OCAM (R4B12 antigen) is a novel cell adhesion molecule which may be involved in the development of zone-to-zone projection of primary olfactory axons in both main and accessory olfactory system.

In this study, *in situ* hybridization analysis with OCAM cRNA probe was performed in the vomeronasal organ of the mouse accessory olfactory system. The results showed that OCAM expressing vomeronasal sensory neurons were located in the apical zone in the sensory epithelium. In addition, we compared the expression pattern of OCAM with that of pheromone receptors in the vomeronasal sensory epithelium. We obtained fragments of the mouse pheromone receptor cDNA by RT-PCR technique using degenerate primers based on the sequences conserved among the rat pheromone receptor cDNAs. Nucleic acid sequences of the PCR fragments showed a striking homology with previously reported pheromone receptors. *In situ* hybridization analysis was performed on sections through the vomeronasal organ using OCAM and the cloned pheromone receptor cRNA probes. We discuss the implications of the coordinated distribution of OCAM and pheromone receptors for the development of zone-to-zone projection of vomeronasal sensory axons to the accessory olfactory bulb.

394. Sensory evaluation of binary mixtures of sweeteners and an orange aroma

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The application of mixtures of sweeteners can improve the sensory quality of beverages with a low sucrose content. The study of sweetness flavour interactions in these soft drinks requires a fundamental understanding of the behaviour of the sweeteners. Mixtures of sucrose and maltitol, aspartame or Na-cyclamate were studied in the presence of an orange aroma. A quantitative descriptive analysis was used to indicate differences between the flavour profiles of equisweet mixture solutions and to investigate the effect of an addition of orange aroma. Comments on the attribute aftertaste further specify the mixture solutions.

Beidler's mixture equation was found suitable to compose equisweet mixtures of sucrose and maltitol as well as equisweet mixtures of sucrose and aspartame. A different approach was used to eliminate possible effects of synergy in mixtures of sucrose and Na-cyclamate. The percentage of sucrose was fixed at a certain level, and then the amount of Na-cyclamate was determined to obtain a mixture solution at 10% Sucrose Equivalent Value. The mean scores for sweetness in the descriptive analysis are an indication of the accuracy of the methods chosen.

Supported by EC grant PL94-102.

395. Effect of gelatin (a working model for PRP) on the sensory astringency of 5-caffeoyl-o-quinic acid and tannic acid

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The feature that accounts for astringency in food phenolics (tannins) is the ability of these compounds to bind and precipitate salivary proteins (mainly the proline-rich-proteins or PRP) producing a sensation of dryness and puckeriness in the mouth and a prolonged aftertaste.

Although not strictly homologous, gelatin PRP and the PRP in saliva share compositional and structural features. Studies on interactions between gelatin and phenolic compounds provide a practical model to gain insight into sensory astringency. We concentrated on a small phenolic structure, 5-caffeoyl-*O*-quinic acid (5-CQA), and reported the effect of gelatin on 5-CQA by equilibrium dialysis and by UV difference spectroscopy. The spectral characteristics of early interactions (10–20 s), depended on 5-CQA concentration and on the ratio gelatin/5-CQA. Similar results were observed when salivary PRP/5-CQA were used. A way of relating the spectroscopic results to sensory astringency was to modify the PRP/5-CQA ratio by artificial means and study the perceived differences in astringency.

The present work investigates differences (triangular tests) between the astringency of 5-CQA and that of 5-CQA + gelatin. As an overall reference similar tests are reported using tannic acid (which has been shown to elicit a strong response). Finally, a time-intensity study on aqueous solutions of tannic acid (TA), TA + gelatin and gelatin, reveals relevant temporal factors.

Although, under the experimental conditions, the majority of comparisons revealed statistically significant differences, in the time-intensity study no significant differences were found between TA + gelatin and gelatin, neither for the intensity nor the total duration of the response. Moreover, for the fading of the response there was no difference between TA + gelatin and TA.

The main temporal contribution to the difference is in the onset of the response and can be related to the spectral differences observed in the earlier studies. It will be shown that these results represent an important step in our understanding of the mechanisms of astringency.

396. Direct measurement of concentration of chloride in the newt olfactory cell

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It has been reported that the Ca^{2+}-activated Cl^- efflux at the cilia amplifies the depolarizing odor response in the olfactory cell. For this efflux, high intracellular concentration of Cl^- $[Cl^-]_i$ is required. In fact, the high $[Cl^-]_i$ was suggested by the reversal

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potential of the later phase of the odor-induced current response measured by the perforated patch technique. Aiming at confirmation of this unique function of Cl^- among the neuro-systems, we challenged to measure $[Cl^-]_i$ by a Cl^--sensitive fluorescent dye, *N*-(6-methoxyquinolyl)-acetoethyl ester bromide (MQAE).

By incubation with 5 mM MQAE for 10–30 min, MQAE was loaded into the solitary olfactory cells prepared byenzymatic dissociation. Fluorescence (500–580 nm) images of the cells perfused under a microscope were captured under the excitation light at 380 nm with a high sensitive silicone intensified target camera. Their fluorescence intensity (FL) was read out with an image analyzer (ARGUS-100, Hamamatsu Photonics). Fls were measured first in the normal perfusing solution, then in the solutions containing various $[Cl^-]_i$ and ionophores, tributyltin and nigericin, that caused the change of $[Cl^-]_i$. By the calibration curve for the $[Cl^-]_i$ and FL obtained as above, the original $[Cl^-]_i$ was estimated to be 10–20 mM. Thus, contrary to our expectation, the $[Cl^-]_i$ was relatively low. Unfortunately,the cilia were too thin to capture their fluorescenceimage with our system. Possible local accumulation of Cl^- in the cilia is to be examined.

397. Evaluation of aromachemicals and perfumes by contingent negative variation

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Perfume has been found to effect the psychophysiology of humans. The Contingent Negative Variation (CNV) has been a useful electrophysiological method to study this effect. Previous studies showed significant stimulating and relaxing effect on CNV of both caffeine and diazepin, respectively. When some aromachemicals, essential oils and fragrances have been presented to humans via the olfactory system, significant differences between fragrant and non-fragrant trials have been found. Even though the effect of perfumes and aromachemicals have been obvious, the relationship between CNV and other methods to understand human behavior have not been well studied. We will discuss the effects of several common perfumes on CNV and its use in determining consumer preference.

398. Identification of aromas using polymer-film-coated quartz resonator chemical sensor in conjunction with neural network

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The objective of this work is to demonstrate a novel chemical sensor system using polymer-film-coated quartz resonator in conjunction with neural network pattern recognition for identification of aromas from several kinds of alcohol or vinegar. The aroma sensing system proposed here consists of two polymer-film-coated quartz resonators and a personal computer for principal component and neural network pattern recognition analysis. The proposed aroma sensing method has the following features: (i) the frequency change as a function of time is detected using two-kinds of quartz resonator coated with alkyl-resin-film or urethane-resin-film which is formed by dipping method when the sensor is exposed to aroma from each alcohol or vinegar; and (ii) the parameters which characterize the shape of transient response curves (frequency change versus time) are used for the pattern recognition. The result of principal component analysis showed that the responses for individual arom tend to cluster in discrete sections of space with well defined boundaries. In back-propagation neural network analysis, the recognition probability was 100% for four kinds of alcohol or four kinds of vinegar.

Supported in part by Foundation from the Ministry of Education, Science and Culture of Japan.

399. Salivary gland expression of Leu 7 and neuron specific enolase: a comparison study of normal tissue with Sjogren's disease and radiation sialadenitis

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Background: neuropeptides play an important role in salivary gland development as well as regulation of a variety of biological processes. Leu 7 is a natural killer cell antigen which recognizes myelin-associated glycoprotein. Neuron Specific Enolase (NSE) is an isoenzyme specific to neurons and neuroendocrine cells. The present study demonstrates changes in expression of the neuropeptides and Leu 7 (CD57) at various ages of the normal gland and in the pathologic processes of Sjogren's and radiation.

Materials and methods: normal cases (young, 2; adult, 2; elderly, 6) by age and cases of Sjogren's (7) and radiation change (3) were selected from the files. Expression of the neuropeptides was studied using immunohistochemical methods with antibodies directed against Leu 7 (CD57, produced at the University of Iowa) and NSE (Biogenics), and visualized with avidin–biotin complex.

Results: in the salivary gland, nerve fiber labeling with Leu 7 is minimal at younger ages, and moderate in adults and elderly. NSE labels nerve fibers uniformly throughout all ages. The ductal epithelium is minimally labeled in the elderly only with both Leu 7 and NSE. In Sjogren's, Leu 7 is seen focally in acinar cells and occasionally in both nerve fibers and the epithelial cells of larger ducts. NSE labels nerve fibers in some (30%) of Sjogren's cases. Irradiated salivary glands show increased labeling for both Leu 7 and NSE in the epithelium of small ducts, nerve fibers, and also in atrophic glandular elements.

Conclusion: these observations suggest that changes in Leu 7 expression are associated with the pathogenesis of Sjogren's syndrome. Radiation produces an increased production of both neuropeptides in the salivary gland.

400. Oral astringency caused by stannous ions: sensory and biochemical correlations

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Stannous fluoride causes a sensation of astringency or drying for many people. The response to stannous was found to be highly variable between subjects, and was also variable for the same

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subject depending on test conditions such as time of day and previous food intake. Under controlled test conditions, the astringency had a dose-response correlation with stannous fluoride concentration. Saliva composition and sensitivity to the astringency of stannous was studied to determine whether any correlations existed. Total saliva protein concentration, saliva flow rate, and separated saliva proteins did not show any strong correlation with response to the astringency of stannous fluoride. As with many astringent compounds, stannous fluoride causes precipitation of many saliva proteins. *In vitro* precipitation of saliva proteins with stannous fluoride revealed an inverse correlation between the concentration of remaining soluble saliva proteins after precipitation with stannous fluoride and sensitivity to stannous astringency. In other words, subjects with a high concentration of soluble saliva proteins remaining after precipitation with stannous fluoride sensed low astringency from stannous fluoride.

Stannous fluoride causes the precipitation and/or denaturing of saliva proteins that lubricate the oral cavity (stathrin, mucin, PRPs). Loss of functional lubricating proteins causes the sensation of astringency or drying. Other astringent compounds (acids, tannins or plant polyphenols) act via the same biochemical mechanism.

401. Molecular approaches to the vertebrate olfactory system

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How does the olfactory apparatus of vertebrates detect and discriminate thousands of odors? Our approach to elucidating the mechanisms of olfactory discrimination involves the characterization of odorant receptors and the neural pathways that they activate. We are also interested in the developmental mechanisms which are responsible for specifying odorant receptor expression in olfactory neurons and the pathfinding of these cells' axons to their appropriate targets.

Our laboratory uses primarily the zebrafish and the mouse to study signal transuction and development of the vertebrate olfactory system. To examine the roles of different intracellular messengers in olfactory signal transduction, we have created a 'knockout' mouse in which the gene encoding the olfactory cyclic nucleotide-gated cation channel has been disrupted by homologous recombination. In addition to providing a means to assess the roles of different second messengers in olfactory signaling, these mice provide an excellent model system in which to study the role of neuronal activity in the development of the peripheral olfactory system.

In another series of experiments, we are using the zebrafish to examine the regulation of the odorant receptor gene family as well as the development of synaptic connections in the olfactory bulb. The zebrafish is particularly amenable for the study of these issues, owing to the anatomic and numerical simplicity of its olfactory system and the ease with which we can observe the developing nervous system in living embryos.

402. Beyond maps: dynamic and distributed processing of tactile information in the brain

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The rat trigeminal somatosensory system was used a model to investigate how populations of neurons, located at multiple processing stages of this sensory pathway, represent tactile information following passive and active stimulation of facial whiskers. Contrary to the traditional view, our results revealed the existence of highly dynamic and distributed representations of tactile information, not only in the somatosensory cortex, but also in the thalamus and even in the trigeminal brainstem complex. In these structures, we identified broadly tuned neurons with multiwhisker receptive fields (RFs). In the thalamus, a large percentage of neurons exhibited shifts in the spatial domain of their RFs as a function of post-stimulus time. During these shifts, the center of the neuron's RF moved across the whisker pad from caudal to rostral whiskers, but not in the opposite direction, suggesting that these spatiotemporal RFs may encode directional information. Simultaneous recordings of the activity of cortical and thalamic neurons revealed that thalamocortical ensemble responses increase in a nonlinear fashion according to the extent and spatial orientation of the multiple-whisker stimuli. Supralinear responses were seen more frequently with vertically- than horizontally oriented stimuli suggesting that thalamocortical interactions generate complex spatial transformations and that these are used by rats for tactile discrimination. During active tactile exploration, we observed that cortical, thalamic, and brainstem neurons can exhibit widespread 7–12 Hz synchronous oscillations, which began during attentive immobility and reliably predicted the imminent onset of rhythmic whisker twitching. Initially, each oscillatory cycle began as a traveling wave of neural activity in cortex and then spread to the thalamus. Just prior to onset of rhythmic whisking, the oscillations spread to the spinal trigeminal brainstem complex. Thereafter, the oscillations at all levels were synchronous with whisker protraction. We interpreted these results as evidence that distributed synchronous activity in the trigeminal system may encode not only sensory information but also the onset and temporal domain of tactile exploratory movements.

403. The cloning and characterization of soluble guanylyl cyclases in the olfactory system of the moth *Manduca sexta*

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Pheromonal stimuli have been shown to elicit a slow sustained increase in cGMP in insect antennae (Ziegelberger *et al.*, 1990). In addition, NADPH diaphorase staining suggests that nitric oxide, and by extension cGMP, plays an important role in the antennal lobes (Müller and Hildebrandt, 1995). To understand better the role of cGMP in the olfactory system of *Manduca sexta* we have used cGMP immunocytochemistry to determine which cell types show high levels of cGMP and we have begun to clone and

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characterize the soluble guanylyl cyclases (sGCs) expressed in the olfactory system of *Manduca sexta*.

In mammals, active sGC is a heterodimer composed of an alpha and a beta subunit. These subunits are encoded by independent genes. We have obtained complete cDNA clones from *Manduca sexta* encoding three different SGC subunits, one alpha and two beta. The sequences of these cDNA clones, their relationship to previously described SGCs, and their localization to particular cells within the olfactory system of *Manduca sexta* using in-situ hybridization will be described. To complement the SGC studies, we have performed cGMP immunocytochemical staining in the antennae and antennal lobes using an antibody generated against fixed cGMP (De Vente *et al.*). We have shown that this antibody stains specific subsets of cells, including several in the lateral cell-body group of neurons in the antennal lobes and sensory neurons along with their respective support cells in the antennae.

Supported by DFG grant STE/5-1 to M.S., NIH training grant NS07363 to A.N., NIH NS29740 to D.B.M. and NIH NS28495 to J.G.H.

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404. Age-associated differences in olfactory functioning in persons with Down's syndrome

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Recent research suggests that individuals with DS invariably develop Alzheimer's disease type neurofibrillary tangles and neuritic plagues by age 30. These neuropathogical abnormalities are salient in regions used for processing olfactory information. In fact, a number of studies have shown deficits in odor detection, identification and recognition memory in DS individuals who live to their third and fourth decade of life. However, to date, the performance of young DS individuals, who presumably have not yet developed these plaques and tangles, has not been compared with older individuals with DS. Thus, the present study examined the performance of adolescent (ages 11–18 years) to middle-aged (ages 26–56 years) persons with DS on a number of olfactory measures. All subjects performed a forced-choice, two-alternative, ascending odor threshold task. In addition, all subjects' odor identification ability was assessed using a picture-based identification test and the University of Pennsylvania Smell Identification test. Results revealed that young DS subjects had greater odor sensitivity than older DS subjects $P < 0.05$. The results of this cross-sectional study suggest that olfactory deficits seen in older DS individuals, particulary measures with few cognitive demands, maybe a result of disease progression.

Supported by NIH grant # AG08203.

405. Expression of cDNAs encoding sweetness-inducing proteins, miraculin and curculin in *Escherichia coli*

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Miraculin, which exists in the pulp of *Richadella dulcifica*, has the unusual property of modifying a sour taste into a sweet taste. The DNA encoding matured miraculin was cloned into an expression vector, pET-32a. Thioredoxin-miraculin fusion protein expression plasmid was transformed into *Escherichia coli* strain AD494. The presence of expressed fusion protein in the supernatant of the cell extract was confirmed by SDS–PAGE and immunoblotting using anti-miraculin serum. The fusion protein purified by Chelatiing Sepharose affinity chromatography gave a single band having a molrcular mass of 76 kDa on SDS–PAGE. In the presence of β-mercaptoethanol, the fusion protein gave a single band having a molrcular mass of 38 kDa on SDS–PAGE. Theae results suggest that the non-reduced fusion protein is a dimer of the reduced fusion protein. Assay of the activity of expressed miraculin is in progress.

Curculin, which exists in the pulp of *Curculigo latifolia*, has a sweet taste and the taste-modifying activity. After curculin, water elicits a sweet taste and sour substances induce a stronger sense of sweetness. Expression of curculin was performed by the same procedure as that of miraculin. The presence of expressed fusion protein in the supernatant of the cell extract was confirmed by SDS–PAGE and immunoblotting using anti-curculin serum. Assay of the activity of expressed curculin is in progress.

406. Expression of *c-fos* in central vomeronasal pathways after activation of accessory olfactory bulb by metabotropic glutamate agonist

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Granule cells of the accessory olfactory bulb (AOB) are inhibitory interneurons that express metabotropic glutamate receptors of the mGLUR2 type. Activation of these receptors disinhibits AOB mitral cells by preventing release of GABA from granule dendrites in response to activation through their reciprocal synapses with the mitral cells. In female mice, infusion of the mGluR2 agonist, DCG-IV, into the olfactory bulbs has been shown to modify behavior thought to depend on vomeronasal sensory input. In our experiments, immunocytochemistry for *c-fos* expression was used to measure neuronal activity in male golden hamsters resulting from AOB activation by the group II mGluR agonist L-CCG-I. Freely moving animals implanted with bilateral cannulae were given two (same side) unilateral injections of L-CCG-I (25 nmol in 0.5 µl), into the caudal olfactory bulbs and AOBs. Injections were separated by an inter-injection interval of 30 min. After a further 60 min, animals were overdosed with sodium pentobarbital and perfused with 4% paraformaldehyde. Vibratome sections (50 µm) were stained for *c-fos* expression using a human anti-Fos antibody (Genosys), the ABC reaction and DAB. Fos positive nuclei were counted, using computer-enhanced image analysis software, in tissue sections representative of central targets of the AOB

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efferents. Our findings show a significant increase in numbers of Fos-stained neurons in the anterior medial amygdala on the drug-infused side as compared with the control (non-injected) side, but no comparable increase in other AOB targets such as the posterior medial amygdala or in more central regions such as the caudal medial preoptic area. Changes in main olfactory pathways are currently under investigation.

Supported by NIDCD 00906.

407. Odor memory in normal aging and in Alzheimer's disease

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Whereas episodic (e.g. recognition) and semantic (e.g. identification) memory for odors have been studied separately in normal aging, considerably less is understood regarding recall of odors (verbal recall of presented odor items) which requires accurate memory of both episodic and semantic nature. To acquire information on age-associated differences in odor recall and on the processes and strategies involved, a design was employed which compared performance on the California Verbal Learning Test (CVLT) and an analogous odor test. The CVLT quantifies such variables as learning strategies, learning rate across trials, consistency of item recall across trials, learning errors in recall, and recognition memory. Thus, these aspects were obtained for both olfaction and audition with the addition of odor identification. Results suggest that both immediate and delayed recall of odors, both free and cued, is affected by normal aging, perhaps more so for olfaction than for audition, and that the odor-recall decline cannot simply be referred to poor identification. Instead, this decline may be referred to restricted use of semantic-clustering strategies and common use of intrusions among elderly. Learning and consistency in odor recall across trials as well as recognition memory and identification for odors were also poor in the elderly. Olfactory decline in memory in normal aging is, however, far from as affected as in Alzheimer's disease (AD). The early neuro-pathology in AD in the cerebral regions that process olfactory information evokes attention directed towards the issue of olfactory tests for early diagnosis of AD. Two studies of persons with questionable AD (impairment in two or more areas of cognition but still absence of functional changes as reported by significant others) demonstrated very early and significant deficits in both recognition memory and identification of odors. Although further research is required, the findings from these 'preclinical' cases imply that performance on olfactory-mediated tasks may contribute to early diagnosis of AD.

Supported by NIH grants AG08203 and AG04085 (C.M.).

408. Neurotrophins in the tongue; expression patterns in rodents and humans, relation to innervation, and studies of neurotrophin knockout animals

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During the past several years, more light has been shed on many aspects of the gustatory system including taste transduction mechanisms, origin of taste buds and involvement of neurotrophins (specifically BDNF) in the innervation of the gustatory system.

In embryonic rats, the timing of arrival of gustatory (e.g. chorda tympani) and somatosensory (e.g. trigeminal) nerves is matched and preceded by BDNF and NT3 mRNA expression respectively. Earlier in-oculo transplantation studies have shown the competence of lingual, but not other epithelium in giving rise to taste buds. Precedence of innervation by neurotrophin mRNA expression is another example of prespecialization of lingual epithelium based on the type of neurotrophin expressed. Neurotrophin mRNA expression persists into adulthood, BDNF mRNA in taste buds and NT3 mRNA in somatosensory-related areas (two new such structures have recently been reported in rodents). The same pattern of neurotrophin expression is also seen in humans.

Conventional gene-knockouts allow studies of consequences of development in the absence of a given protein. Indeed, BDNF knockouts show clear anatomical, histological and physiological deficits in their gustatory system. In NT3 knockouts, on the other hand, the gustatory apparatus appears intact while there is severe loss of somatosensory innervation. Compensatory mechanisms must often be taken into account when interpreting the net effects of a given null-mutation. However, lingual gustatory and somatosensory systems, to a large extent, show a non-overlapping dependence on BDNF and NT3 respectively.

Supported by the Swedish Medical Research Council, USPH grants (AG, BG, NIDA) and the Swedish Dental Association

409. PTC and PROP behave differently in discrimination tests

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Better discrimination was possible between PTC solutions and the pure solvent when the solvent was a tasteless low concentration NaCl solution to which the subject had adapted, than when the solvent was purified water. The reverse was true for PROP. The aftertaste of PROP served as the cue for its identification in the discrimination testing and it was more intense in the water solvent than in the tasteless NaCl solvent, thus eliciting better discrimination. The aftertaste of PTC tended to persist while the distilled water solvent was being tasted; it did not persist while the tasteless NaCl solvent was being tasted. This persistence caused a tendency to confuse the solvent with the PTC solution, eliciting worse discrimination for PTC in the distilled water solvent. Thus, the differences in discrimination for PROP and PTC in the different solvents were caused by differences in the intensity and persistence of aftertastes rather than a greater sensitivity to the tastes of PROP or PTC per se. This has consequences for the traditional approaches to measuring taste sensitivity which generally assess how well a subject can discriminate a solution from its solvent. It also indicates that PTC and PROP are not necessarily equivalent indicators of 'taster' versus 'non-taster' status.

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410. Neurotrophin gene knockouts and the development of mouse peripheral gustatory apparatus

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We evaluated null mutant mice corresponding to one of the three neurotrophin ligands for TrkB: BDNF, NT3 or NT4. The vallate gustatory epithelium of *nt3–/–* mice and of *nt4–/–* mice appeared to be normal. However, *nt3–/–* mice lost about half of the putative somatosensory axons in the dorsal surface of the vallate proper, and lost all axons in the vallate fringing epithelium (dorsal surface of the outside trench walls). In contrast, the developmental absence of brain-derived neurotrophic factor in *bdnf–/–* mice led to sparse innervation of the vallate trench walls. We concluded that normally developing somatosensory neurons require NT3 while taste neurons require BDNF. In addition to sparse innervation, *bdnf–/–* mice had two further vallate defects: fewer taste buds and a reduction in the area of the gustatory epithelium. These were permanent defects, already present at birth, persisting to postnatal day 7, and worsening by postnatal day 12. Such developmental defects were widespread, affecting other taste papillae and taste buds in the oral cavity of *bdnf–/–* mice. To assess whether any taste buds were functional, we presented water, sucrose, and quinine. Suckling to sucrose was vigorous and equally prompt in *bdnf–/–* and *bdnf+/+* mice. We propose a gustatory developmental model that accounts for these three prenatally originating defects as well as for comparable defects observed in earlier postnatal experiments. In developing mice, BDNF sustains taste fibers that in turn support vallate papilla maturation and subsequent taste bud formation.

Supported in part by grants from NSF and NIH-NIDCD.

411. Receptor neuron responses to aggregation pheromone compounds in the desert locust, *Schistocerca gregaria*

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The antenna of *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) possesses three olfactory sensillum types as previously shown in scanning and transmission electron microscopic studies. In the current study, recordings from single receptor neurons present in sensilla basiconica, s. coeloconica and s. trichodea in immature adults revealed that: (i) mature adult-produced aggregation pheromones stimulated receptor neurons in basiconic sensilla with increasing concentrations, whereas at higher doses they inhibited the neural activity of receptor neurons in coeloconic and trichoid sensilla; (ii) nymphal-produced aggregation pheromone compounds (consisting of short chain aliphatic acids and the corresponding aldehydes) stimulated receptor neurons in coeloconic sensilla at low concentrations, but at higher doses the receptor neurons in basiconic sensilla were also stimulated; (iii) receptor neurons in sensilla trichodea were stimulated by a compound found in a preferred host plant of *Schistocerca*. This compound has also been found to be produced by mature solitarious females. Nymphal-produced aggregation pheromones and general plant odours stimulated receptor neurons in trichoid sensilla at higher concentrations. Our results show that immature *S. gregaria*, though not producing aggregation pheromone compounds, is able to detect aggregation pheromones produced by the nymphal stages and by mature adults through different antennal receptor neuron types.

Supported in part by the Swedish Agency for Research Cooperation with Developing Countries (SAREC).

412. Lack of synergistic umami responses in gustatory cortex in rats

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A mixture of two representative umami-substances, Na-glutamate (MSG) and 5′-guanyl-monophosphate (5′-GMP), yields synergistic responses in sucrose-best neurons in the chorda tympani and brain-stem nuclei of rats. Reports have been made on cortical responses to single umami substances only, but not to the mixture. In our study, we examined whether the mixture yielded synergistic responses in cortical taste neurons in rats, and whether treatment of the oral cavity with gurmarin, an antisweet agent, affected them. We recorded single taste neurons from the gustatory cortex of urethane-anesthetized, adult SD rats, by applying taste stimuli to the oral cavity. Neurons were classified according to the best stimulus which produced the largest responses among the four basic tastes (0.1 M NaCl, 0.5 M sucrose, 0.01 N HCl and 0.02 M quinine–HCl). In some neurons, MSG (0.3%) was more effective than any of the 4 basic tastes. Synergistic responses were evaluated by average ratio of responses to 0.27% MSG + 0.03% 5′-GMP to those to 0.3% MSG. No synergistic responses was seen in the sucrose-best neurons. Gurmarin did not affect responses to sucrose and MSG + GMP in one and the same neuron. Our results indicate that synergistic umami responses was not transmitted from the brainstem to the cortex in rats used. This may be attributed to the nutritional state of the animal, as suggested by behavioral studies.

Gurmarin was given by Dr T. Imoto, Tottori University of Supported by Society for Research on Umami Taste.

413. Intracellular Ca^{2+} responses to dextromethorphan in isolated taste cells of the mudpuppy

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A variety of substances with different chemical structures elicit a bitter taste. Recent studies have shown that several different transduction mechanisms are used for bitter taste (for review see Kinnamon and Margolskee, 1996). We have found that the bitter substance denatonium (DN) increases intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) via the phospholipase C (PLC)–inositol 1,4,5-triphosphate (IP_3) pathway in taste cells of the mudpuppy, *Necturus maculosus* (Ogura *et al.*, 1997). In the present study, we tested whether dextromethorphan (DEX), a bitter antitussive, uses the same transduction mechanism as DN in taste receptor cells of the mudpuppy. $[Ca^{2+}]_i$ was measured in isolated single taste cells using the Ca^{2+} sensitive-dye, fura-2. DEX (2.5 or 5 mM) increased $[Ca^{2+}]_i$ by 53% over resting levels in most DN-sensitive taste cells.

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The onset of the $[Ca^{2+}]_i$ response to DEX was slower and more gradual than the response to DN. Immediately after washing out DEX, a transient increase of $[Ca^{2+}]_i$ (off-response) was observed in ~50% of the cells tested. The $[Ca^{2+}]_i$ response to DEX was inhibited when intracellular Ca^{2+} stores were depleted with thapsigargin (1 μM), suggesting that DEX releases Ca^{2+} from the intracellular Ca^{2+} stores. These results are similar to those obtained with DN. In contrast, the PLC inhibitor U73122 (5 μM) failed to inhibit DEX $[Ca^{2+}]_i$ responses, suggesting that the PLC–IP_3 pathway is not involved in the response to DEX. Since U73122 completely inhibits the response to DN, DEX and DN appear to use different pathways for transduction in mudpuppy taste cells.

Supported by Procter & Gamble Company, NIH grants DC00244 and DC00766 to S.C.K.

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414. Possible glutamate perception involved in umami transduction in mouse taste cells

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Transduction mechanisms for umami are believed to involve membrane bound receptors on taste cells. However, details of the transduction for umami are still unknown. In mammals, umami taste is probably transduced by means of several receptor types that may be related to glutamate receptors of the vertebrate central nervous system. Recently, metabotropic glutamate receptor 4 (mGluR 4) has been shown to be expressed only to mouse taste buds. The potent agonist of metabotropic glutamate receptor 4 (mGluR 4), L-AP4 (2-amino-4-phosphonobutyrate) and umami taste substance monosodium glutamate (MSG) elicited similiar tastes in rats. To elucidate the taste receptor mechanisms for umami, we examined the responses of isolated mouse taste cells to MSG or L-AP4, by using whole-cell patch-clamp technique. Data were obtained from taste cells isolated from female mice (89 weeks old) of the C3H/HeJ and C57BL/6J strains, which are highly sensitive to MSG. Under voltage clamp (holding potential –80 mV) with K^+ containing pipette, bath application of MSG (10 mM) elicited both inward currents (17/38) with an increase in membrane conductance and outward currents (9/38) with a decrease in membrane conductance. L-AP4 (1 mM) stimulation elicited only outward currents (11/35) with a decrease in membrane conductance at a holding potential –80 mV. Further, with the Cs^+ solution in the patch pipette, MSG stimulation elicited only inward currents (6/17) with a increase in membrane conductance, while we have been unable to observe any responses in L-AP4. These results suggest that MSG perception mediates via metabotropic glutamate receptor 4 (mGluR 4) that decreases a membrane conductance, and/or another receptor type that increases a membrane conductance.

415. Rapid disappearance of actin filaments in epithelial cells around taste pores of rat fungiform papillae after selective resection of the chorda tympani nerve

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To clarify the trophic action of the gustatory nerve on the maintenance of taste pores in rat fungiform papillae, effects of gustatory denervation on the actin filament organization in epithelial cells around taste pores were examined by observing actin filaments stained with rhodamine–phalloidin under a confocal laser microscope. Ring-shaped fluorescence was observed in the epithelial cells around taste pores. The fluorescence around taste pores began to disappear one day after chorda tympani nerve resection and then almost disappeared at day 3. In contrast, after lingual proper nerve transection these rapid changes were not detected.

It is concluded that the chorda tympani nerve plays a predominant role in actin filaments encircled the taste pores.

416. Cyclic GMP induces anion current in frog taste cells

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Although cyclic AMP is considered to be an intracellular second messenger in taste transduction, apical localization of guanylate cyclase in mammalian taste bud cells suggests that cyclic GMP rather than cyclic AMP may be involved in the transduction. In the present experiments, the effect of intracellular application of 8-Br-cGMP from a patch pipette was analyzed in isolated bullfog taste cells using the whole-cell patch clamp. Intracellular dialysis with 1 mM 8-Br-cGMP in KCl-internal solution induced an inward current of –140 ± 20 pA (mean ± SE, $n = 21$) accompanied with a conductance increase at a membrane potential of –50 mV in rod cells. The cGMP-induced inward current was observed in 47% of rod cells, but not in forked cells. Extracellular application of 2 mM dibutylyl cGMP also elicited a similar response in rod cells, but extracellular 2 mM 8-Br-cGMP inhibited voltage-gated outward current nonspecifically. The reversal potential of the cGMP-induced inward current was –12 ± 2 mV ($n = 21$). Exchanging of intracellular Ca^{2+} buffer from 1 mM EGTA to 10 mM BAPTA did not influence the current, indicating that change of intracellular Ca^{2+} concentration may not be involved in the response. The cGMP-induced current was inhibited by external 2 mM Cd^{2+}, but not by eliminating external Na^+. The inward current disappeared when the intracellular Cl^- was decreased from 104 to 4 mM. The reversal potential of the current shifted positively when the extracellular Cl^- was decreased from 121 to 6 mM. The results suggest that cyclic GMP can activate the Cd^{2+}-sensitive anion channel in the rod-type frog taste cell.

This study was supported in part by a grant-in-aid (No. 08672131).

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417. Differential immunoreactivity of homologous presynaptic protein, Complexin I (921-S) and Complexin II (921-L) between the main and accessory olfactory bulbs

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Although it has been reported that some presynaptic proteins are involved in the modulation of the neurotransmitter secretion, the action of Complexin I (921-S) and Complexin II (921-L) has not yet been clarified. Therefore, in order to examine their function on development of olfactory sense, we carried out Complexin I (921-S) and Complexin II (921-L) immunohistochemistry on the main and accessory olfactory bulbs of mice and rats at several ages. It has been clarified that DNA sequence of these proteins are completely homologous. For the immunohistochemical method, monoclonal antibody was used as the primary antibody and biotinylated anti-mouse Ig G antibody as the secondary antibody. The signals were visualized with DAB–nickel staining following ABC reaction. Although few signals was detected in both bulbs at the preweaning stage, there are some signals only in the main olfactory bulb of adult animals. Strong Complexin I (921-S)-immunoreactivity is detected at the external and internal plexiform layers, whereas Complexin II (921-L)-immunoreactivity is detected only in a very deep area of the granule cell layer. These results suggest the possibility that the presynaptic protein Complexin I (921-S) is involved in dendrodendritic reciprocal synapses between mitral and granule cells, whereas Complexin II (921-L) acts on granule cells.

418. Glomerular development in slice and organotypic cultures from moth olfactory lobe

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With the goal of understanding the mechanisms underlying the sensory axon-induced formation of olfactory glomeruli, we are preparing both slice and organotypic cultures of the developing olfactory (antennal) lobe of the moth *Manduca sexta*. These cultures will allow us to manipulate specific cellular interactions in the antennal lobe without the confounding effects of disrupting the three-dimensional architecture that is likely to be an important feature of the cellular interactions that lead to glomerulus formation.

Slice cultures, maintained in Millicell wells for 3–7 days, show well-maintained neuropilar structures, BrdU labelling in the glial-cell population of the lobe that is comparable in pattern to that seen *in vivo* at the age of dissection, approximately normalmorphology of antennal-lobe neurons, and large regions of ultrastructurally intact synaptic neuropil. The regions normally occupied by olfactory sensory axons show obvious disruption, commensurate with axotomy.

Organotypic cultures consist of the entire antenna, antennal nerve, and antennal lobe, thereby maintaining structural continuity between the olfactory sensory epithelium and the brain. Using DiO to label the receptor axons, we have found that after 2 weeks *in vitro* many of the axons are tipped with growth cones and as a group they are organized into glomerulus-like structures.

Thus, we have laid the groundwork that demonstrates that the slice and organotypic cultures maintain many of the important features of glomerulus development and that now permits experiments to perturb neuron-glia and neuron-neuron interactions in a controlled *in vitro* environment.

Funded by NIH grant # 28495.

419. Odor interaction: an integrated view on quality and quantity

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Several models of how odors mix to form new percepts have been presented during the last decades. Almost all of these models have concerned the perceived intensity of mixtures rather than the perceived quality of mixtures. In 1994 Olsson suggested an integrated model for how perceived intensity and quality of the mixture can be predicted from the perceived intensities of the single substances. The model can be viewed as an extension of vector summation. The current study reviews support for this model. In doing that, assumptions of the model are defined and tested against data. It is shown how odor interaction, when the process is seen from the view of the observer, reduces to a set of simple rules that are consistent across levels of intensity and combinations of odorants.

420. Molecular cloning of calpain from taste epithelium of the channel catfish, *Ictalurus punctatus*

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Transduction in taste cells likely involves calcium signalling. In taste cells from many species, including the channel catfish, *Ictalurus punctatus*, taste stimuli often induce inward calcium currents. The calpains are calcium-activated neutral proteases. In other calcium signalling systems, a transient rise in intracellular calcium often induces the co-translocation of calpains and PKC from the cytoplasm to the plasma membrane, where PKC, receptors and cytoskeletal proteins are degraded by calpains. We hypothesize that calpains contribute to the adaptation and/or synaptic modulation mechanisms in taste cells. Polyclonal antibodies were raised in guinea pig using as antigen an electroeluted ~80 kDa protein band from a SDS-PAGE of a partial membrane preparation of catfish barbel epithelium. These antibodies were used to screen a λ-ZAP cDNA library constructed from catfish barbel epithelium. Of the several positive phage plaques, we characterized a 2.4 kb calpain large subunit cDNA from the library. The clone has an open reading frame of 2061 bp, encoding a polypeptide of 687 amino acids with a predicted molecular weight of 78 kDa. The amino acid sequence shares ~50% homology with chicken, rat and human novel type calpains. The encoded amino acid sequence possesses calcium-binding domains (four EF-hand structures) and the conserved Cys, His and Asn residues at the active site. These sites are known to be conserved among the calpains from mammals to fruit fly. Anti

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bodies raised againsth the N-terminal 3–16 amino acid sequence recognized the are surrounding the taste pores and the upper regions of the bud body as well as some taste cells within the bud.

Supported in part by NIDCD 00356, NIDCD 01838 and a grant from the Department of Veterans Affairs.

421. Oral irritants can modify responses of rat gustatory nerve fibers to NaCl

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Single- and multi-fiber preparations of the rat chorda tympani (CT) nerve were used to study the effects of both capsaicin, the pungent component of red peppers, and ibuprofen, nonsteroidal anti-inflammatory drug (NSAID), to salt-taste transduction. (i) Capsaicin selectively suppressed the responses to NaCl of the chorda tympani nerve fibers (N-type) that are sodium-specific and poorly sensitive to potassium. Among the more broadly responsive, cation sensitive fibers (E-type), there are two sub-types both of which responded to capsaicin but in different ways ('enhanced' type and 'suppressed' type). The suppressive effect continued for at least 20 s after the simultaneous application of 100 ppm capsaicin–100 mM NaCl. These observations indicate that capsaicin caused both suppression and enhancement of responses of rat CT nerve fibers to NaCl. (ii) 10–100 mM ibuprofen was suspended in Borate–Na_2CO_3 buffer (pH 7.5) or dissolved in the same buffer at pH 10.0 (ibuprofen–sodium salt). The former suspension shows tingling sensation on the tongue and strong stinging sensation on the throat with some time lag after its application, but no irritation was shown by the latter solution. The response of all multi-fiber preparations of chorda tympani nerve to 100 mM NaCl were suppressed by ibuprofen suspension (pH 7.5). The suppressive effect appeared 10–20 s after the simultaneous application of 10–100 mM ibuprofen–100 mM NaCl. On the other hand, ibuprofen–sodium salt solution (pH 10.0) had little or no effect on CT nerve response to NaCl. From these results, it is suggested that there is an interrelationship between the oral-irritation and the taste suppressing effect evoked by ibuprofen.

422. Reaction time to NaCl, KCl and NaCl/KCl mixtures

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Homogeneous taste mixtures have usually shown hypoaddition, i.e. mixture intensity is lower than the sum of intensities of each salt taken in isolation. The present study focuses on the possible neurophysiological basis of hypoaddition using another psychophysical approach: taste reaction time (TRT). TRT may likely correlate with receptor binding and subsequent subcellular transduction kinetics which also takes place in the ms interval. Reaction time to salty taste (RT) was measured using a 'go–no go' procedure and a computerized record. Fifteen stimuli, five for each pure salt and five for salts mixture (ratio 1:1), ranged from 5×10^{-3} to 1 M.

RT showed a linear negative correlation with concentration increase in agreement with Pieron's law. Slopes for NaCl, KCl and NaCl/KCl were respectively 0.282 ± 0.018, 0.295 ± 0.025, 0.318 ± 0.029 (ms M^{-1} ± SE). Maximum RTs were respectively 1929 ± 130, 2241 ± 196, 2400 ± 205 (ms ± SE). Differences in the kinetic behavior of the salts and its mixture could only be detected comparing concentrations within each experimental situation. Differences in RT ($\neq$, LSD test, $P < 0.05$) between molar concentrations (M) were found: for NaCl $0.010 \neq 0.032$, for KCl $0.010 \neq 0.032 \neq 0.100 \neq 0.320$ and for NaCl/KCl $0.005 \neq 0.010 \neq 0.032 \neq 0.100 \neq 0.320$. Differences between experimental situations were observed only for NaCl 0.010 M $\neq$ NaCl/KCl 0.010 M.

No changes were observed between mixture RT and RT for each pure salt. This effect might be related to intensity hypoaddition. A sustained tendency to higher RT values for KCl and NaCl/KCl was evident. The different RTs for NaCl and NaCl/KCl observed at low values (0.010 M) might result from possibly different modulatory effects of the two salts which occur only at low concentrations.

423. Respiratory monitoring and olfactory stimulus delivery apparatus for use with brain electrical activity recording

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Steady state probe topography (SSPT) has revealed useful temporal aspects of brain electrical activity in a variety of testing paradigms. Accurate timing of 'task' delivery is needed for synchronization and subsequent analysis of the 64 channel electroencephalographic (EEG) signals. We have developed a system which can deliver odours during normal respiration and provide the accurate timing needed for concurrent SSPT, based on steady state visual evoked potentials (SSVEP). A purpose-built microprocessor-based system records respiration *via* an oxygen therapy mask and a pneumotachograph and sends digital samples to a serial port. Software running on a lap-top computer monitors the sampled respiratory flows and determines: peak flow rate, respiratory rate, and the start and end of each breath. At the required point in each breath the software sends a signal to the microprocessor to drive one of two stepper motor driven syringes. These gas-tight syringes contain either medical air or the odourant and with the attached small bore tubing, the gas can be delivered to the face mask where the ends of the tubing are placed close to the external nares. The SSVEP recording system is triggered so that the continuous EEG signals can be divided into stimulus-based frames. An optional cognitive 'task' is provided on a monitor allowing the subject to press a button for 'smell' or 'no smell'. The system is currently in use to obtain high quality SSVEP signals while the subjects (six male and six female) are exposed to either an air or butanol stimulus with each breath. The results of extensive testing of the system to determine the actual relationship between the derived topographic brain electrical activity patterns and the presence of butanol are reported.

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424. Using sensory and instrumental data to interpret the effect of storage at elevated temperatures on aroma of Chardonnay wines

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The effect of elevated temperatures during storage on wine aroma was determined for commercial Chardonnay wines. To characterize the changes in aroma, wines stored at 40°C for 0, 15, 30 and 45 days were profiled by descriptive analysis by a trained panel. Volatiles recovered by solvent extraction were separated by gas chromatography (GC) and identified by GC–mass spectrometry (MS). Of the 67 compounds quantified by GC, six were selected which best fit the sensory space of the products by principal component analysis of instrumental variables (PCAIV). The outcome of this analysis is a mapping of the wines based on the six compounds only, which is as close as possible to the sensory map of the product obtained by a principal component analysis of the sensory data. This sensory map revealed that heated storage decreased intensity of fruity and floral notes, while increasing in attributes such honey, butter/vanilla, oak and rubber. The PCAIV map which provided a chemical interpretation of the sensory data revealed that control and 15 day stored wines were higher in *trans*-2-hexenoic acid and two unknown compounds, while the 30 and 45 day times wines were higher with pentolactone, diethyl butanedioate and an unknown peak.

425. Antagonistic bindings of polysaccharides to the sugar receptor proteins and their inhibitory effects on the feeding behavior of the blowfly, *Phormia regina*: two types of putative sugar receptor proteins in the leg of the fly

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The blowfly, *Phormia regina*, has the taste sensilla in the tarsi of the forelegs, and searches foods with the taste sensor. When the tarsal sensilla were stimulated with sugar solutions at higher concentrations than a feeding threshold, the fly shows proboscis extension reflex. This feeding behavior was depressed by antagonistic polysaccharides added to a stimulant sugar solution. Two kinds of polysaccharides, starch and levan, which would bind two different types of sugar receptor molecules, depressed the feeding behavior to different groups of sugars, respectively. Based on the quantitative analysis of the behavioral experiment, we determined the dissociation constants of the receptor-antagonist complexes. In either case for starch or levan, the dissociation constant was identically obtained, regardless of the starvation level or the feeding threshold of the fly. With starch- and levan-affinity electrophoreses, we also detected the putative sugar receptor proteins in the tarsal extract of the fly. Starch and levan competitively bound these proteins with different groups of sugars, respectively. Thus, the sugar selectivities of these proteins were consistent with the sugar-selective depression of the proboscis extension reflex by starch and levan. The dissociation constants determined by the quantitative analysis on the electrophoreses of the proteins were identical with those obtained by the behavioral experiments, respectively.

426. Changes of smell sensitivies and olfactory bulb structures of sturgeons under the impact of crude oil

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Investigations of the single effect of crude oil extracted from deposit 'Neftyanye Kamni' (concentration in water 50 ml/l) on behavior and olfactory balb structure were accomplished on plant-fed Russian sturgeon juveniles at age of 6 months. Fishes kept in pure water served as controls.

Fishes were taken for experimentation after staying for 1, 3 or 5 days in polluted water. Experiments to determine smell sensitivity were conducted in a U-figured aquarium, and shifts in cytoarchitectonics of olfactory balb were studied by the Nissl method. After staying in oil-polluted water for 1 day, considerable changes in animal behavior were observed. The most prominent changes were noticed on fish kept in polluted water for 5 days. The sturgeons fed poorly, did not react to external signals and were motionless. They reacted indifferently to substances (repellents and attractants) to which they were exposed in theaquarium, whereas controls revealed high sensitivity to these substances. 1–2 days after displacement from tanks with toxicants into the tanks with pure water, partial rehabiliation of sensitivity to smell stimuli was noticed.

427. GPI-anchored receptors in *Paramecium*

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Glycophosphatidylinositol lipids can anchor proteins into membranes. Surface antigens of parasitic protozoa and mammalian folate receptors are perhaps the best known of GPI-anchored proteins. Although it seems unlikely, these proteins can function in signal transduction, as in the ret receptor complex. We show evidence that the large surface glycoproteins of *Paramecium tetraurelia* are GPI-anchored, as are other smaller surface proteins. GPI-anchored proteins can be harvested with PLC treatment of *Paramecium* surface membranes or more conveniently with salt/ethanol washes of whole cells. Inhibitor studies show that this latter treatment appears to activate a surface lipase or less likely a protease. Chemoreceptors appear to be among the smaller GPI-anchored proteins because antibodies against the proteins of the salt/ethanol wash (produced by J. Forney) inhibit chemoresponse to a subset of chemoattractants, including folate. Chemoresponse to NH_4Cl, which does not require a receptor, is not inhibited by these antisera. (Mutant cells missing the large surface antigens but displaying other surface proteins were provided by J. Forney for these assays.) A 61 kDa protein from the salt/ethanol washes specifically elutes from a folate affinity column and is a candidate for the folate chemoreceptor. We are currently harvesting this protein for N-terminal sequencing.

Supported by NIH and the Vermont Cancer Center.

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428. Citrate enhances olfactory receptor activity to amino acids and triggers oscillatory receptor responses in the channel catfish

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Citrate (trisodium), an acidulant and preservative in food processing, is used commercially as a flavor modifier and was recently shown to enhance taste responses in fish (Ogawa and Caprio, 1995; Davis and Caprio, 1996) and mammals (Gilbertson *et al.*, 1995). We now report that the moderatley stimulatory citrate, a normal constituent of cellular metabolism, is also a potent enhancer of asynchronous olfactory receptor responses (EOG and multiunit recordings) in the channel catfish, *Ictalurus punctatus*. In addition, synchronized oscillations of olfactory receptor neural activity known as 'peripheral waves' (PW) (range 16–31 Hz) can also occur in response to moderate concentrations (0.1 mM–1.0 mM) of binary mixtures of amino acids with trisodium citrate, whereas neither stimulus alone at the concentration tested results in wave phenomena. The result that PW activity is detectable with microelectrodes placed only in the sensory region of an olfactory lamella indicates that olfactory receptor neurons are capable of generating synchronized responses. With increasing stimulus duration, the stimulus concentrations required to trigger PW activity decreases while PW duration increases. PW activity across different specimens is classified into five different patterns and each pattern is generally reproducible with repeated stimulus applications. Neither the specific pattern nor the frequency of the PWs appear related to stimulus quality. Current studies are aimed at determining whether PW activity affects neural processing within the olfactory bulb. The frequency (13–17 Hz) and amplitude of olfactory bulbar field potentials (FP) are not affected by PW activity. PWs do not drive olfactory bulbar FPs as vigorous bulbar oscillations occur in response to odorants in the absence of PW activity. Ongoing studies will determine if PWs affect the processing of odorant information within the olfactory bulb.

Supported by NSF IBN 9221891.

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429. Scanning electron microscopy of denervated taste buds in hamster: morphology of fungiform taste pores

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Taste pores of fungiform papillae are critical for taste function. Taste nerve injury affects the pore, rendering it refractory to staining with vital dyes. Whether pores of denervated fungiform papillae disappear or undergo more modest structural changes to account for diminished staining was the subject of the present study. The chorda tympani was severed unilaterly and the anterior tongues of the animals ($n = 4$) were prepared for scanning electron microscopy after 31 days of survival. Taste pores were found on 90% of control fungiform papilllae ($n = 74$). They were round openings, 1.8 ± 0.8 μm (mean diameter), formed by the free margins of 1–4 keratinocytes, and centered in hillock-shaped elevations (7.5 ± 2.6 μm) of the papillary surface. Hillocks were encircled by a trench-shaped depression which, in turn, was surrounded by a circular epithelial ridge. The pores were morphologically distinct, each one led to a channel that penetrated more or less deeply into the papilla. The experimental papillae had markedly fewer pores. Definitive pores were present on only 50% of denervated papillae ($n = 66$). Half of the poreless papillae, retained a small (4.06 ± 1.8 μm) hillock and a subtle indentation in place of the pore. The remaining papillae had flat apical surfaces. The pores that remained were smaller (1.1 ± 0.6 μm), and led to shallower channels than control pores. These persistant pores were associated with hillocks, trenches and ridges that were more variable and less distinct than those of control papillae. In summary, fungiform pores are associated with specialized surface features of the papillary epithelium. Denervation results in changes that range from pore disappearance to the shrinkage and atrophy of epithelial structures surrounding the pore.

Supported by NIH grant DC01091.

430. SSVEP changes in response to olfactory stimulation

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The location, nature and characteristics of brain activity during detection and identification of odours are of importance if brain function techniques are to be of value to sensory systems. Steady state probe topography (SSPT) has been used in our laboratory to record steady state visual evoked potentials (SSVEP) which have demonstrated cognitive task related changes in a variety of testing paradigms. The enhanced spatial (when using 64 channels) and temporal resolution (380 ms) of the system enable the brain electrical activity changes occurring before, during and after delivery of an odour to be examined. We have developed a system which can deliver odours during normal respiration and the accurate timing needed for SSVEP recordings. The system is based on the premise that a subject breathing butanol compared with filtered medical air will demonstrate SSVEP topographic changes associated with detection and identification of butanol. During our experiments the subject has either an air sample or an equal volume of butanol injected into the inspiratory airflow. These are randomized and every breath has the same stimulus system with no known clues as to the differences apart from detection. The results from a panel of 10 female subjects—who all identified the butanol correctly—showed that butanol delivery resulted in sequences of changes in SSVEP topography (amplitude and latencies) which involved parietal, frontal and temporal regions. While consistent with other studies (parietal changes), our results revealed more dynamic temporal changes involving prefrontal and parietal regions at different periods around odour delivery.

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431. Artificial olfactory systems employing distributed processing

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Recent advances in chemical sensor technology combined with a resurgence of interest in classifier systems, has led to a rapid expansion in the development of artificial odour sensing or so called 'electronic nose' systems. To date, this research effort has generated practical odour sensing instruments that are capable of some discrimination between a variety of simple and complex odours. However, this advance has been achieved with little more than cursory consideration of the biological olfactory pathway and its architecture.

In view of the recent advances in understanding of the key sensory information processing principles exploited by the olfactory bulb and cortex, we are now presented with an opportunity to evaluate and reassess the architecture for an electronic nose that is both biologically more credible as well as promising a performance improvement in the practical system.

In this paper, we examine the parallels that exist between the current understanding of the biological olfactory pathway and its analogue, the electronic nose. These are shown to be manifold, ranging from the method of odour delivery, nonspecific sensor/receptor response, sensor/receptor preprocessing, to *Content Addressable Memory (CAM) function.*

Both systems need to overcome similar operating problems, such as sensor/receptor drift, degeneration and poisoning, limited sensor/receptor sensitivity, discrimination of odour quality invariant of intensity, and also identification of particular odour components within a mixture of background odours. For the first time, we will consider the application of existing computational neuroscience (PDP-based) models of the olfactory pathway to arrays of artificial and nonspecific odour sensors. It is demonstrated that a multisensor array combined with such models of information processing are capable of odour discrimination. Finally, the opportunities for the use of such models in artificial olfaction for overcoming some of the aforementioned limitations in future odour sensing technology are discussed.

432. The role of olfaction in acquired food aversions in the elderly

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Elderly people report fewer acquired food aversions (AFAs; aversions that result from association of ingestion of a food with discomfort or illness) than do young adults. In 1990 Bartoshuk and Wolf argued that odors play an important role in AFAs and that the elderly have a lower incidence of acquired food aversions because of their decreased olfactory sensitivity. When subjects completed an interview about food aversions and underwent olfactory threshold determinations, we found only a modest difference between subjects with good and poor olfaction in number of aversions reported. However, it is possible that an interview format is less than optimal for the detection of olfactory-loss effects because when foods are reported upon from memory subjects may be able to fill in from memory for the missing sensory information. So, our next effort was a prospective examination of acquired food aversions in cancer patients who were receiving chemotherapy. Subjects (28–71 years of age) underwent olfactory testing just prior to their first chemotherapy treatment. They were also given a novel flavor of hard candy to eat during that session (mango or kiwi) which they rated for liking. The other flavor of novel candy served as the control. During a follow up session, subjects again rated their liking for the candies. Among subjects who experienced nausea, greater olfactory sensitivity was found to be related to greater decreases in liking for the candy eaten prior to chemotherapy. This result is consistent with an important role of olfaction in AFAs and with the idea that olfactory loss may be one of the mechanisms for the decreased incidence of acquired food aversions in the elderly.

Supported by AG09892.

433. Odorant-binding proteins, structural aspects

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Odorant-binding proteins (OBPs) are small soluble proteins, discovered at the beginning of the eighties at about the same time in the olfactory system of vertebrates and insects. They are highly exposed in the nasal mucus of vertebrates and in the sensillar lymph of insects, where they may account for the great majority of soluble proteins. Their low molecular weight and exceptional stability, as well as their unusually high concentration, have made purification and structural characterization particularly easy. At present, complete amino acid sequences have been established for nine vertebrate OBPs and for more than twenty insect OBPs, while partial sequence information is available for a much larger number of these proteins.

Vertebrate OBPs belong to the large family of carrier proteins called lipocalins, several members bind and transport hydrophobic ligands in aqueous biological fluids. Particularly interesting is the similarity between OBPs and some lipocalins present in organs and fluids known to be specific pheromones releasers. These include the urinary proteins of mouse (MUPs) and rat(alpha2-u), the salivary proteins of mouse and pig, and the hamster vaginal protein aphrodisin.

The three dimensional structure of bovine OBP, recently resolved, is similar to that of urinary proteins and share with all lipocalins the typical β-barrel motif. A particular feature of bovine OBP is the rather unusual phenomenon of 'domain swapping', that contributes to the stability of its dimeric structure.

Insect OBPs do not bear any sequence similarity with vertebrate OBPs, nor with other classes of proteins. Their three-dimensional structure, not yet experimentally resolved, is predicted to be mostly composed of a-helix segments and is stabilized by three intramolecular disulphide bonds. Both invertebrates and in insects three to five types of OBPs have been described in each species. Their structural differences indicate the presence of distinct classes of these proteins and may suggest a role of OBPs in odour discrimination.

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434. ErbB-3 and ErbB-4 expression in the mouse olfactory system

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The interplay between neuregulins and the ErbB receptor family has a pivotal role in the development of several tissues, including the nervous system, and is maintained in the adult olfactory system where extensive plasticity and neurogenesis are retained. In the present work we show the immunohistochemical localization of ErbB-3 and ErbB-4 in the olfactory system of adult normal and lesioned mice. The expression of ErbB-3 is demonstrated to be restricted to the ensheating cells of the olfactory nerve and to few substentacular cells of the olfactory epithelium (OE). ErbB-3 staining circumvents the glomeruli but is never observed inside them or elsewhere in the adult olfactory bulb. Conversely, ErbB-4 immuno-reactivity is found in all the periglomerular and mitral/tufted cells of the olfactory bulb (OB) and to a minor extent in the olfactory neurons and basal cells of the OE. Interestingly enough, cells coming out from the rostral migratory stream of the subependymal layer (SEL), as well as isolated cells in the granule cell layer, possibly migrating cells, strongly stained for ErbB-4 expression. Lesions of the olfactory epithelium have been performed by unilateral intranasal irrigation with $ZnSO_4$ and 3 weeks after the irrigation, the olfactory bulbs were analysed for olfactory marker protein (OMP), tyrosine hydroxylase (TH), ErbB-3 and ErbB-4 expression. In the deafferented OB, the drastic loss of immunoreactivity for OMP was accompained by a strong reduction of ErbB-3 expression. Most of the deafferented dopaminergic interneurons switched off TH expression. In the deafferented periglomerular and mitral/tufted cells ErbB-4 expression was turned off and down, respectively. No differences were noted at the granular cell layer level in the deafferented OB with respect to control. Taken together our results suggest that, in normal conditions, neuregulins are involved in the survival of the ensheating cells of the olfactory nerve through ErbB-3 activation and in the functional activity of postsynaptic neurons through ErbB-4 activation.

Supported by MURST and CNR.

435. The role of the odor intensity in the interaction between olfactory and gustatory cues in conditioned flavor aversion (CFA) in Wistar rats

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Using a behavioral coding system, we previously showed that odor (almond), taste (saccharin) and an odor/taste compound produced strong flavor aversions. The conditioned stimuli elicited a pattern of agitated behavior characterized by increased general activity, rearing, and the specific mimetic response 'gaping'. In the compound-experimental group an overshadowing effect for the odor component by the new taste was found. However, the role of olfactory stimuli in compound with taste is still controversial.

In the current study we were interested to elucidate whether the odor intensity played a crucial role producing the effects of potentiation or overshadowing in CFA. To prove this hypothesis, we conducted a series of experiments with various odor intensities (almond) conditioned alone or in compound with taste. Animals were tested for their aversions both in a one-bottle and a two-bottle choice procedure.

Our results indicate that odor intensities, which were only able to establish a slight aversion when conditioned alone, were potentiated when conditioned in compound with taste. Whereas intensities, which are easily conditioned tend to be overshadowed. This effect was obvious under the one-bottle test procedure. Under the two-bottle choice procedure no differences between the odor alone condition and the compound condition could be observed.

436. Na,K-ATPase subunit isoforms in the rat olfactory mucosa: demonstration by *in situ* PCR

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Na,K-ATPase is a transmembrane enzyme vital in the maintenance of the electrochemical gradients of all mammalian cells. The enzyme exists as a heterodimer of equimolar amounts of two polypeptide subunits: α and β. At present, three isoforms have been identified for each subunit in mammals. Current evidence suggests that each α isoform can associate with each β isoform to generate a functioning holoenzyme. The physiologic significance of this is not yet fully understood but different isoforms appear to demonstrate different biochemical properties. Moreover, in many systems a cell and tissue specific pattern of expression has been described which suggests different roles for the various combinations. In the olfactory mucosa, Na,K-ATPase has been demonstrated in multiple cell types and a complex pattern of isoform expression might be expected. In the current study, we have utilized the technique of *in situ* PCR to demonstrate the presence of the α1 and β1 isoforms in the olfactory mucosa of the rat. Specific RNA probes were generated from linearized plasmids containing short cloned sequences of the rat Na,K-ATPase subunit isoform genes. Results confirm a complex pattern of isoform expression in the various olfactory cell types. This study suggests that specific isoforms of Na,K-ATPase may play specialized roles within the olfactory mucosa and further establishes the importance of this enzyme to the peripheral mechanisms of olfaction.

437. Responses of trigeminal nerve fibers to thermal and chemical stimulation in rats

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Prior research in our laboratory showed evidence of two distinct groups of thermally sensitive fibers within the lingual branch of the trigeminal nerve, which may also respond to chemical stimuli. This study sought to confirm the method of classifying lingual neurons and investigated the chemical sensitivity of these neurons when adapted to 35 and 25°C. The criterion of a neural response to a stimulus was defined as activity exceeding 1.64 standard deviations of the prior 20 s baseline activity. Forty-seven lingual neurons increased their firing rate in response to a gradual temperature decrease from 35 to 10°C at a rate of 1°C/s. Based on the response characteristics of threshold, time-to-peak, and range

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of sensitivity, we identified the neurons as either Group 1 or Group 2. Group 1 neurons (n = 19) responded to a smaller change in temperature, exhibited faster time-to-peak activity, and responded to a smaller range of temperatures compared with Group 2 neurons (n = 28). After thermal classification we examined the responses of the neurons to 10s chemical stimulation by NaCl (150 mM), glucose (150 mM), citric acid (0.3 mM), and quinine–HCl (3 mM), at 35 and 25°C temperature adaptation conditions. Tactile sensitivity was also assessed. Of the Group 1 neurons, 91% were chemically sensitive, and 96% of the Group 2 neurons were chemically sensitive. A greater percentage of Group 1 neurons responded to NaCl at 35°C (70%) than at 25°C (30%) and a greater percentage responded to glucose at 25°C (50%) than at 35°C (20%). Equal percentages of Group 1 neurons responded to citric acid and quinine for both temperature conditions. In Group 2 neurons, a greater percentage responded to NaCl at 25°C (58%) than at 35°C (42%) and a greater percentage responded to citric acid at 25°C (58%) than at 35°C (38%). The response patterns of the chemical stimuli were similar indicating that thermally-sensitive lingual neurons provide information regarding the presence of chemical stimulus rather than its quality.

This research was supported by Grant DC-02641.

438. Effects of insulin-like growth factor-I on olfactory neurogenesis *in vivo* and *in vitro*

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The rate of neurogenesis in the peripheral olfactory neuroepithelium is regulated by unknown mechanisms. To study neurogenic regulation, we infused growth factors known to alter CNS neuroblast division into the noses of newborn Sprague–Dawley rats, i.e. when the epithelium is still developing. Growth factors or carrier solution (0.9% NaCl with 0.25% bovine serum albumin to prevent non-specific binding) were applied (10 µl) to the left nostril once per day on P1, P2 and P3 (P1 = day of birth), and the animals were sacrificed on P4 by decapitation. After paraformaldehyde immersion fixation, cryostat sections of the olfactory area of the nose were immunostained for the proliferating cell nuclear antigen (PCNA). Sections were position-matched by turbinate structure and then, on a midline septum section, epithelial height and area of PCNA staining at the base of the epithelium (which represents division of primarily neuronal precursors) were measured by image analysis. Both were significantly increased by rat insulin-like growth factor-1 (IGF-1) (20 ng/ml, 2.6 nM), but not insulin (20 ng/ml, 2.6 nM) or an IGF-1 derivative, LongR31GF-1 (200 ng/ml, 22 nM), that does not bind to the IGF-1 binding proteins (IGFBPs). Thus IGF-1 selectively increases the rate of neurogenesis and its actions may be modified by interaction with one or more of the six IGFBPs. Other growth factors are being examined. To examine *in vitro* effects, cultures of newborn rat olfactory mucosal cells were plated on astrocytes as previously described (Pixley, 1992). BrdU (10 µM) was added for the first 3 days, rinsed out and the cultures were fixed after 5 days. Addition of rat IGF-1 (20 ng/ml) at plating and feeding (3 DIV) increased the number of BrdU-positive cells and cellular aggregation over that seen with human IGF-1 (20 ng/ml), and measures for both factors were greater than in control cultures. The different effects of rat versus human IGF-1 suggest species differences in either receptor or IGFBP binding. Thus rat IGF-I alters olfactory neurogenesis *in vivo*, and cell division and aggregation *in vitro*.

Supported in part by NIDCD 002106.

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439. Inhibitors of sensillar esterase block reversibly the responses of moth pheromone receptor cells

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The pheromone of *Antheraea polyphemus* is slowly (within min) degraded by a sensillum lymph specific esterase. The effects of two volatile alkyl-thio-trifluoro-methyl-ketones with alkyl chain length of six (C6S) and ten carbons (C10S), which selectively inhibit the activity of the sensillar esterase, were tested.

Single sensillum recordings from sensilla trichodea were performed on isolated antennae either held in a continuous stream of clean air, or exposed to an airstream containing one of the esterase inhibitors. Afterwards the antennae were stimulated with the pheromone components (*Z*,*E*)-6,11-hexadecadienyl acetate and (*Z*,*E*)-6,11-hexadecadienal. Both esterase inhibitors caused a reduction of electrophysiological responses to each of the two pheromone compounds. The C10S was slightly more effective then C6S. An almost complete block of the receptor potential amplitude and nerve impulse response was achieved within seconds of exposure to the inhibitors. Responses of both pheromone receptor cells were reduced to the same extent. The half-times of rise and decline of the receptor potential remained unaffected in most cells. The responses to pheromone partially recovered to ~50–70% within several minutes in clean air.

An inhibition of the sensillar esterase cannot explain the observed effects. The inhibitors could either react directly with the receptor molecules, thereby inhibiting the action of the pheromone, or bind to the pheromone binding protein (PBP) and prevent the formation of the pheromone–PBP complex stimulating the receptor.

440. Ultrastructure of taste receptor cells in active and hibernating ground squirrels

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Differences in taste bud ultrastructure between active and hibernating animals were investigated in the Siberian ground squirrel, *Citellus undulatus*. Compared to active summer animals, taste bud volume and the development of the taste pore showed little change while an animal was hibernating. However, impressive differences were observed in nuclei and cytoplasmic organelles of all elongated cells within taste buds. During deep hibernation, the nucleoli of elongated non-dark cells had few, but large fibrillar centers (a sign of inactivity). In the cytoplasm

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the number of free polyribosomes (polysomes) was sharply decreased; ribosomes were seen mainly as (non-synthesizing) monosomes and more seldom as membrane-associated ribosomes. The endoplasmic reticulum (ER) volume was reduced, to the extent that only separate and rare cisternae of smooth and rough ER remained. The large vacuoles, which are typically found in many taste cells of active animals, were absent during hibernation, and the Golgi apparatus was disassembled into numerous vesicles. Of these, many had small diameters near 60 nm, while a few had larger diameters near 300 nm. Secretory organelles (dense granules in dark cells and dense-cored vesicles in type III cells) were absent during hibernation, the lateral plasma membrane was smooth and signs of exocytosis were not found. Provoked arousal, during which the brain temperature increased from 2–4 to 36°C within 2–3 h, was characterized by a re-organization of the nucleoli, suggesting renewed synthesis of pre-ribosomal particles in the second hour, and formation of free and ER-associated polysomes in the protoplasm. Vacuolar enlargements of the ER and secretory granules reappeared at this time. Our findings suggest reduced protein synthesis and reduced sensory function during hibernation and a striking remodeling of ultrastructure towards the active state primarily in the second hour of arousal.

441. Right-unilateral UPSIT scores negatively correlate with 'typical' depression scores in patients with seasonal affective disorder: a preliminary report

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Since olfaction modulates seasonal rhythms in some animals, we investigated whether phenyl ethyl alcohol (PEA) detection thresholds and University of Pennsylvania Smell Identification (UPSIT) scores differ between patients with seasonal affective disorder (SAD) and normal controls, and in depressed versus light-treated SAD patients. Preliminary results of an ongoing study are presented for 11 SAD patients and 10 controls. PEA tests were administered bilaterally and the UPSIT unilaterally, with two of four booklets administered to each side of the nose in random order. PEA thresholds and UPSIT scores did not differ significantly between SAD and control subjects, or between depressed and remitted SAD patients. However, in depressed SAD patients, a highly significant negative correlation ($r = 0.78$; $n = 11$; $P < 0.004$) was found between right unilateral UPSIT scores (regardless of handedness) and the 'typical' depression scores on the Self-Assessment Mood Scale for SAD (SAM-SAD). If confirmed in additional subjects, the side-dependent relationship between olfaction and depression could prove interesting in light of other research suggesting CNS laterality in emotional processing, mood regulation and dysregulation.

Supported by NIMH and by NINCD grant PO1 00161, NIH.

442. Comparison of brain activation following stimulation with odors and vomeropherins

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Recent evidence shows that human adults possess a functional vomeronasal organ (VNO). An electrophysiological response has been obtained from the epithelium of the VNO and specific changes in hormonal levels have been observed following application of synthetic pheromones (vomeropherins). These findings suggest a functional vomeronasal-pituitary axis in humans, which may be mediated via hypothalamic and other brain regions.

Functional magnetic resonance imaging (fMRI) may enable localization and identification of the neural substrates underlying pheromone processing in the human brain. To this end we applied methods of air dilution olfactometry that we have successfully employed in localizing brain regions involved in olfactory processing.

Two young male volunteers were scanned during stimulation with six alternating 60 s blocks of the odorless vomeropherin estra-1,3,5(10),16-tetraen-3yl acetate alternated with a diluent. Functional data were acquired from eight 4 mm slices, 2 mm skip, taken at an oblique plane from frontal to temporal pole (31″ to AC–PC) at 1.5T using a T2*-sensitive gradient echo spiral sequence (TR = 720, TE = 40, flip angle = 65). Data were analyzed using the cross-correlation method. Significant activation ($P < 0.025$) was seen in the amygdala, hypothalamus, and thalamus. In similar experiments, stimulation with the olfactory stimuli vanillin and decanoic acid and the trigeminal stimuli valeric and propionic acid induced significant activation in 13 subjects in the piriform, entorhinal, orbitofrontal, insular, ventromedial frontal, and superior temporal cortices, as well as in the amygdala and thalamus. Patterns of activation induced by vomeropherins versus patterns of activation induced by olfactory and trigeminal stimuli were markedly different.

However, due to adaptation in the VNO, we were forced to use a relatively long duration and low frequency of alternating blocks (0.0083 Hz). Sham runs revealed a high level of random noise in the functional image at this low frequency. Thus, additional subjects are being scanned in order to address the validity of these preliminary findings regarding activation following stimulation with vomeropherins.

443. Olfactory and irritant responses to a mixes and single VOCs in man

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In most indoor air situations people are exposed to more than one air pollutant and complaints about air quality often focus on odor and irritation. When chemicals are the origin of complaints, the sources can be diverse and therefore the exposure is to a mix of chemicals and each situation is likely to have a different mix of chemical. It is of interest to determine if, within a mix of 21 VOCs,

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a particular chemical or group of chemicals is driving the responses. To do an exhaustive exposure to all combinations of the chemicals and concentrations is impossible. The mix used in this experiment consisted of 65% xylene and butyl acetate and the remainder was a mix of various concentrations of 19 other VOCs. It was decided that an exposure to an equimolar concentration of a logical subset of the chemicals would be appropriate. Thus, the exposure regimen at 6.4 mg/m^3 toluene equivalent was the 21 chemicals, xylene alone, butyl acetate alone, xylene plus butyl acetate, 19 chemicals without xylene and butyl acetate, and a clean air control. Equimolar concentrations were used because presenting the VOCs in the ratios found in the mix would present a greater concentration of some VOCs than others. Each of the 100 female subjects participated in only one 6 h. exposure. Odor and irritant as well as symptoms questions were responded to at intervals during the exposure. Odor and irritant questions were presented in three formats; magnitude estimation, analogue scaling, and categorical format. Analysis of this groups design was by a repeated measures ANOVA. There were significant linear trends indicating that adaptation occurred to the odorous stimuli. Reported nasal mucosal irritation showed a similar trend of adaptation to the continuous exposure. Interestingly, there was no significant difference between exposures, implying that there was no difference in response to chemicals when presented as singles, pairs, mixes at the same molar concentration. There was no evidence for synergism or additive effects. This is an abstract of a proposed presentation and does not reflect EPA policy.

444. The effects of capsaicin on flavor intensity in foods: a time intensity study

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Recent evidence suggests that oral irritation does not suppress the odor component of flavors, even when the sweetness of the flavor is suppressed. However, these data come from studies of model solutions, rather than from studies of foods in which such interactions typically take place. In the present study, we wished to determine the effects of oral irritation produced by capsaicin on the flavor intensity of foods, in this case tomato and apple purees. In addition, since it is possible that irritation at low intensities may actually enhance flavor intensity, we examined a range of capsaicin concentration starting at very low intensities. A group of 10 subjects who had received extensive training in rating flavor and irritation intensities rated the flavor intensity of the purees to which had been added capsaicin in the following concentrations: 0 (vehicle only), 0.3, 0.6, 1.2 and 2.4 ppm. The lowest concentration had previously been determined to be above threshold in these foods for all subjects. Using a computer mouse, the subjects moved a cursor on a graphic rating scale displayed on the screen of a computer. The computer sampled the position of the cursor three times/s. Subjects made continuous ratings until the flavor intensity was rated at zero (up to a maximum of 300 s). Measures of time to maximum flavor intensity, maximum flavor intensity, rate of decay of flavor intensity, and time to zero flavor intensity were derived. The results are discussed in terms of the role of pungency in food flavors and flavor-taste-pungency interactions.

445. Human pheromones: releasers or primers—fact or myth?

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Both the pheromone concept and the first isolated compounds with pheromonal activity were defined and/or isolated from insects. Chemical signals in insects have a high degree of specificity with specific responses, perhaps involving some degree of genetic programming. These well-defined responses, which include overt displays of attraction and copulation mediated solely by chemical signals are described as releaser effects on behavior. More subtle neuroendocrine effects have been demonstrated in rodent and domestic animals resulting in changes in reproductive cycle length and timing as well as puberty onset and pregnancy block. These types of endocrine-mediated changes are described as primer effects. Humans have both sources of putative chemical signals and an olfactory system to receive them; however, our overt responses to odors are confounded by past experiences, other sensory inputs and the context in which an odor is perceived. Therefore, one would not expect to see observable 'releaser' effects in humans.

Despite no hard evidence supporting the possibility of rapid, odor mediated changes in behavior, numerous fragrances, or additives for fragrances, which perpetuate the myth that an odor can make one attractive or irresistible to members of the opposite sex have been or are being sold. In some instances, the working definition(s) of pheromones have been altered to fit the marketing of certain products. Most products being sold as pheromones appear to mainly contain chemicals from two categories of human odors: C_2–C_5 aliphatic acids and C_{19}-steroids. In this presentation we review the available evidence supporting and refuting the existence of human pheromones, the pathways by which they are detected and their putative activity.

Supported in part by NIH grant DC-01072 (to G.P.).

446. Long acting modified OB protein decreases spontaneous feeding in rhesus monkeys

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OB protein (also known as leptin), the protein product of the ob gene, plays a role in the regulation of feeding, energy balance, and metabolism. Systemic administration of OB protein reduces food intake in rodents. The half-life of recombinant OB protein is <20 min. We have examined the effects of subQ administration of a novel modified recombinant human OB protein preparation (MOD-OB) with a prolonged half life on spontaneous feeding in 4 adult rhesus monkeys (*Macaca mulatta*). Monkey biscuits and water were available ad libitum throughout the studies and food intake was measured daily. The experiment was divided into five 8 day phases: (1) baseline food intake without injections; (2) vehicle

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injections; (3) MOD-OB injections; (4) vehicle injections; and (5) baseline food intake without injections. Each monkey received a subQ injection of vehicle or MOD-OB each morning of phases 2, 3 and 4. In Experiment 1, the daily dose of MOD-OB was 1 mg/kg. At the end of the MOD-OB treatment phase, mean intake was suppressed 5% (± 5.7 SEM) from baseline, but returned to the baseline level during phase 4. In Experiment 2, the daily dose of MOD-OB was 4 mg/kg. At the end of the MOD-OB treatment phase, mean intake was suppressed 13% (± 4.8) from baseline. By the end of phase 4, mean consumption was 26% (± 4.9) below baseline; recovery of intake took place during phase 5. Therefore, the peripheral administration of MOD-OB for 8 days caused a long lasting, dose dependent suppression of the spontaneous daily food intake of rhesus monkeys. The time course of the suppression of feeding was consistent with the pharmacokinetics of MOD-OB. These results suggest that OB protein is one of the multiple factors involved in the regulation of spontaneous feeding, food selection, and energy balance in rhesus monkeys. If the metabolic and neuroendocrine effects of OB protein in rodents also occur in monkeys, these would provide additional inputs that may influence the chemical senses and their contributions to food selection.

447. Olfactory neuroepithelium explants induce tyrosine hydroxylase expression in cultured rat olfactory bulb neurons

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There is a close correlation between expression of tyrosine hydroxylase (TH) by juxtaglomerular (JG) neurons of the olfactory bulb and innervation of the bulb by primary sensory olfactory axons. Lesion of the olfactory nerve results in selective downregulation of TH in JG neurons. In reversible lesions, TH expression is upregulated as newly formed primary sensory olfactory axons reinnervate the olfactory bulb. However, little is known about the mechanisms regulating this apparent dependence of TH expression on innervation by primary sensory olfactory axons. It has been hypothesized that the expression of TH is regulated by trophic factor release and/or synaptic activity from primary sensory olfactory axon terminals. To begin to test these hypothesis we examined TH expression in co-cultures of dispersed olfactory bulb cells and olfactory neuroepithelium slice explants. TH positive neurons in control dispersed olfactory bulb cell cultures alone represented only a small fraction of the total population of cells present in the culture. However, when olfactory neuroepithelial slice explants were co-cultured with the dispersed olfactory bulb cells there was a mean 2.7-fold increase in the number of TH positive neurons. The increased number of TH positive neurons were dispersed randomly throughout the culture, and often were not in contact with primary sensory olfactory axons. These data suggest that the TH/dopamine phenotype in cultured olfactory bulb neurons can be induced by the presence of primary sensory olfactory axons, but is not dependent on direct axonal contact. Therefore, it is possible that an, as yet, unidentified diffusible molecule is released by primary sensory olfactory neurons and is capable of inducing TH expression. This molecule could act directly upon olfactory bulb neurons to induce TH or could exert its effects through secondary actions on other cell types present in the cultures. The present *in vitro* co-culture system provides a useful experimental system for testing these possibilities.

Supported by NIH grant NIDCD-00347.

448. Functional heterogeneity of periglomerular cells in rat olfactory bulb slices

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Periglomerular (PG) cells have been studied with the patch-clamp technique in slices of the rat olfactory bulb at 30°C. The analysis of current-clamp data reveals that these cells have different responses to the injection of sustained depolarizing currents (SDC), suggestive of functional heterogeneity. The ionic bases of these differences have been elucidated by voltage-clamp analysis. PG cells can be classified into three groups, based on the composition of ionic conductances. Na^+-conductance, g_{Na}, is present in all the PG cells studied. However, the first group displays two K^+-conductances (delayed rectifier, g_{KV}, and fast transient, g_A) of equivalent amplitude, and under current-clamp conditions shows the usual outward rectifying behaviour in response to SDC. The second group has a large g_A and a small or absent g_{KV}. It follows that after sustained depolarizations, leading to inactivation of g_{Na} and of g_A, these neurones behave as ohmic elements, as normally observed only at hyperpolarized potentials, and do not show outward rectification under current-clamp conditions. A third group, has mixed properties. Zinc (10–300 mM) affects g_A but not g_{KV}. The inactivation process is strongly altered by Zn^{2+}, the activation process less so; open-channel conductance is not affected. The effect of Zn^{2+} is unlikely to be due to surface charge screening, or to a mechanism involving channel block. In view of the substantial presence of zinc ions in the olfactory nerve terminals, the actions of Zn^{2+} on the A-current might be of some relevance for physiological function. The three main voltage-dependent currents present in PG cells (I_{Na}, I_A and I_{KV}) have been kinetically characterized at 27°C, and an HH numerical model of the neurone has been developed.

Supported in part by MURST (40%).

449. GABAergic inhibition of periglomerular cells in rat olfactory bulb *in vitro*

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Periglomerular (PG) cells of rat olfactory bulb have been studied with the patch-clamp technique in the whole-cell configuration. Most of the cells presented a rich spontaneous synaptic activity, which included bicuculline-sensitive and kynurenate-sensitive events. GABAergic spontaneous events (sIPSP) could be isolated with 1 mM kynurenate or by holding the membrane at 0 mV with E_{Cl} = –70 mV. sIPSP occurred at the frequency of ~1 Hz, their amplitude distribution could be fitted by several Gaussians having the same interpeak distance, and the decaying phase of the synaptic currents could be fitted by a single exponential with a

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time constant of 11 ± 1.5 ms at −70 mV. sIPSP were blocked by bicuculline 10 mM and always reversed at the chloride equilibrium potential for different $[Cl_o]/[Cl_i]$ ratios. GABAergic synaptic potentials could also be observed in PG cells following direct stimulation: electrical stimuli applied to the glomerular layer could evoke large bicuculline-sensitive synaptic responses in PG cells. These responses could be easily differentiated from those following the olfactory nerve (ON) stimulation on the basis of the pharmacology, kinetics and absence of some typical feature of the ON response (e.g. paired-pulse depression). It is concluded that GABArgic synaptic contacts exist between PG cells. The existence of these connections requires a revision of the currently accepted OB wiring scheme, where the PG cells are supposed to send their inhibitory axons only to projection neurones. Together with the growing histochemical and functional evidence that PG cells actually are an heterogeneous population, these data suggest that the role of PG cells could be more complex than previously supposed.

Supported in part by a grant to Prof. V. Perri (MURST 40%).

450. The role of semantic memory in olfaction

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Semantic memory refers to overlearned information, knowledge of objects and concepts, including their associations. Tests of category verbal fluency (VFT), in which a subject is presented with a category such as 'animals' and asked to name as many exemplars that come to mind, are thought to be a good measure of semantic memory. Little is known about the role of semantic memory for odors. Our goal was to explore this relationship through an olfactory analogue of the VFT to better understand the storage and recall process for odors in healthy adults. In the present study, participants were presented with two odorants, one at a time, and asked to name as many odors as the stimulus brought to mind. Participants were then asked to identify the odor. It was hypothesized that labeling the odor, whether the identification was correct or incorrect, would facilitate access to the semantic network for odors and thus, lead to an increased number of responses. An ANOVA revealed that subjects who correctly labeled an easily identifiable odor did in fact name more exemplars on the fluency task than those who failed to identify the stimulus (P = 0.001). Participants had more difficulty when the odor was not easily identifiable. The results revealed that any label given an odor significantly increased the number of exemplars (P = 0.009). These findings suggest that any labeling of the odor facilitates greater responses on the fluency task. That is, it appears that once a label has been given to an odor, other exemplars within that semantic network are more readily available. These results may have further implications for clinical populations who suffer from both olfactory and semantic memory deficits, such as patients with Alzheimer's disease.

Supported by NIH grant # AG08203 to C.M.

451. Spatial–temporal masking in olfaction

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In olfaction, it is not possible to determine which nostril is being stimulated, i.e. lateralize, when a pure olfactory substance, e.g. phenylethyl alcohol or vanillin, is administered into one nostril, and, simultaneously, an odorless, solvent blank into the contralateral nostril. We subjected volunteers to extensive training, with feedback on each trial, in an attempt to determine whether it was possible, in these well-trained subjects, to overcome this apparent impossibility. We failed to obtain any evidence to support the notion that a pure olfactory stimulus could be lateralized when the odorant and blank were presented simultaneously. The task, however, became simple when the odorant and the blank entered each nostril sequentially. We investigated, using a two-channel olfactometer, temporal parameters that enabled such discrimination. We controlled the duration of odorant and blank air puffs, as well as their mutual timing, to determine the threshold stimulus onset-disparity, i.e. the interval between stimulus onset and blank onset, that resulted in an inability to lateralize. Latencies shorter than the threshold interval would be perceived as simultaneous stimulation. We determined that the onset interval was between 200 and 400 ms, depending on the duration of the stimuli (a shorter interval was noted for stimuli of 150 ms duration relative to 300 and 450 ms stimuli). This was also true when two odorants were applied, rather than an odorant and a blank, and the subject was instructed to focus on the sequence of odorant delivery and side of stimulation. The temporal onset-threshold was the same for lateralization and for order of stimulation. Whether the olfactory system per se mediates this discrimination or whether inputs from olfaction and chemesthesis, via trigeminal free nerve endings stimulated by air-stream onset, combine to allow this discrimination has yet to be determined.

Supported by NIDCD P50 DC00214 and the Eugene Garfield Federation.

452. Floral scent and its role(s) in hawkmoth attraction

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Hawkmoths (Lepidoptera: Sphingidae) are important pollinators of pale, tubular, night-blooming flowers, many of which produce copious nectar and a sweet, powerful scent. We used chemical analyses, video recordings of wild and lab-reared moths, and manipulative experiments to characterize floral fragrances from a guild of Sonoran Desert plants, and to investigate their function in hawkmoth attraction. We observed considerable variation in chemical composition and complexity of floral scents, with no universally present compounds or compound classes. However, rates of scent emission per gram of flower were relatively constant across plant species, suggesting that hawkmoths may use a variety of floral scent blends as distance attractants. Both male and female naive, lab-reared *Manduca sexta* moths showed olfactory-guided flight to flowers in wind tunnel assays. In contrast, wild, presumably experienced hawkmoths (*Hyles lineata*, *M. sexta* and *M. quinquemaculata*) entered floral populations by flying into the

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wind, then flew directly from flower to flower, suggesting olfactory guidance from a distance and visual guidance from close range. Finally, we covered flowers of *Hymenocallis sonorensis* (Amaryllidaceae) with plastic and cloth bags to decouple olfactory and visual cues. A significant majority of approaches and landings by wild hawkmoths occurred on open control flowers, indicating that both olfactory and visual cues are required to elicit floral visits. Thus, floral scent attracts hawkmoths from a distance, works in concert with visual cues to elicit landings, and may promote associative learning and floral constancy in experienced hawkmoths.

Supported by NSF # IBN-9511742 and NIH # T32AI 07475.

453. The influence of product color on the perception of fragrance by trained and untrained subjects

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Anecdotal consumer reports as well as experimental work indicate that color affects the perception of fragrance quality. The aim of this study is to investigate whether the impact of color is as strong for trained assessors as it is for untrained consumers. A group of naive consumers and a Givaudan–Roure trained sensory fragrance panel participated in this study.

The panelists evaluated three types of fragrances: herbal-green, fruity-sweet and citrus. The fragrances were presented in a shampoo base and each fragrance was presented in four different color bases: pastel-green, yellow-orange, creamy-white and rosy-red. Each fragrance was selected to be congruent with one of the colors. The color–fragrance congruency was independently determined on the bases of a Givaudan–Roure color–fragrance synesthesia study. Herbal-green fragrance was strongly associated with the color green, fruity-sweet with orange and red, and citrus with orange and yellow.

Both the naive subjects and the trained panelists were given a list of sensory attributes to use during evaluation. This was done to ensure that subjects in both groups had the same sensory vocabulary at their disposal. The subjects' task was to indicate which attributes best described each fragrance sample. They were free to choose as many or as few attributes as they saw fit.

The main results of the study show that (i) naive and trained subjects did not use the same set of attributes to describe the fragrances: trained panelists used attributes that were consistent with the dominant notes in the fragrance more frequently than naive subjects; and (i) base color had a significant effect on the choice of attributes used to describe the fragrance, but Naive subjects were significantly more influenced by it.

454. BDNF and NGF differ in promoting neurite outgrowth from cultured embryonic rat geniculate and trigeminal ganglia

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Neurotrophins are produced in peripheral and central targets of developing sensory ganglion cells and have selective roles in ganglion cell survival and differentiation. However, participation of neurotrophins in neuron support in the cultured geniculate ganglion has not been explored, even though a role for brain derived neurotrophic factor (BDNF) in gustatory neuron maintenance has been suggested from various studies. We compared effects of exogenous BDNF and nerve growth factor (NGF) on neurite extension in cultured embryonic geniculate and trigeminal ganglia. Ganglia were dissected from rat embryos at 16 days of gestation (E16; vaginal plug = E0) and placed on coverslips previously coated with bovine corneal endothelial cell basement membrane. Cultures were maintained in DMEM/F12 plus 1% fetal bovine serum and 2% B-27 supplement (Gibco), with and without exogenous BDNF (10 ng/ml) or NGF (10 ng/ml) (Alomone Labs). After 30 h, cultures were fixed and processed for immunocytochemistry with antibodies to neurofilaments or PGP 9.5. Geniculate ganglia exposed to BDNF had massive neurite outgrowth in small and large fascicles, in a halo around the entire explant. In contrast, geniculate ganglia exposed to NGF or no exogenous neurotrophin had very few neurites extending from the explant. In trigeminal ganglia exposed to exogenous BDNF there was moderate neurite extension. However, NGF promoted substantially greater neurite outgrowth than BDNF; without any added neurotrophin very few neurites extended. We conclude that at E16, BDNF supports neurite growth in geniculate ganglion cells, whereas NGF is not effective. These neurotrophins have differing effects on cultured trigeminal ganglia. Coupled with data from our lab that trkB receptors for BDNF are present in geniculate ganglia *in vivo* at E16, the *in vitro* results make a strong case for a role for BDNF in support of gustatory ganglion cells.

Supported by Grant DC00456, National Institute on Deafness and Other Communication Disorders, NIH.

455. Do Asian elephants, especially males in musth, chemically signal via volatile compounds in breath?

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During musth in male Asian elephants alterations in behavior occur and dominance rankings may shift. Concurrently, serum testosterone concentrations fluctuate, physiological perturbations occur, and the composition of chemicals in various body emissions changes dramatically. Previous studies have demonstrated that a spectrum of volatiles in the secretions from temporal gland of males elicit retreat behavior by female elephants. (This gland is unique to elephants and in male Asian elephants is only overtly active during musth.) Captive elephants, especially males, and particularly males in musth, forcefully exhale at each other. Younger, subdominant males may retreat or mock-challenge more dominant, older males during musth, subsequent to exposure to breath-emitted volatile chemicals. The current study reports the spectrum of volatile compounds, C3 through C14, present in the breath of nonmusth male Asian elephants and compares these chemicals with volatiles emitted during musth. Not only are specific volatiles in breath distinctive during musth as compared with non-musth, but also during the progression of the musth episode both the composition and abundance of certain compounds changes. Breath volatiles that are characteristic of musth, are remarkably similar the bioactive volatiles emanating from the temporal gland secretions. which contain a distinctive combination of ten light volatiles (eight ketones, an alcohol and frontalin). Interestingly, during musth eight of these ten distinctive

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compounds are a significant component of male elephant breath; only 2-methyl-3-pentanol and frontalin are not detected.

456. Response characteristics of olfactory receptor neurons(ORNs) from elderly humans differ from those of younger subjects

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A gradual loss of olfactory capability with age is a common but not universal phenomenon, and mechanisms underlying this loss are not understood. To investigate whether changes in ORN function may contribute to age-associated olfactory impairment, calcium imaging techniques were used to study response characteristics of ORNs isolated from 12 biopsies from healthy elderly subjects (66–84 years, $n = 8$). Forty-two ORNs were studied using odorant mixtures comprised to distinguish between distinct second messenger pathways of calcium influx ('mix A', adenylate cyclase-mediated; 'mix B', phospholipase C-mediated). In spite of reports of an age-related increase in patchiness of olfactory epithelium, nine of 12 biopsies produced >1 responsive ORN. An unusually high percentage of cells from this population responded to *both* odorant mixtures (7/27 responsive cells), compared with ORNs from younger subjects (0/43). In addition, the majority of cells responding to one mixture responded to mix B (15 mix B-responsive/41 tested versus 5 mix A-responsive/41 tested). In contrast, ORNs from younger subjects responded to mix A or mix B with similar frequency. Interestingly, elderly subjects also exhibited significantly higher detection thresholds than younger subjects (a subset matched to the elderly for gender and test date) for phenylethylalchohol, a component of mix A, but not to lyral, a component of mix B. These studies suggest that individual ORNs from older subjects do not show generally reduced odorant responsivity, but that age-related changes in ORN selectivity may contribute to changes in olfactory performance.

Supported by NIH grants DC-00566, DC-00214 and DC-00014, and by a grant from the Veterans Affairs Department.

457. Odor similarity judgments analyzed by multidimensional scaling analysis classify Alzheimer's patients and normal controls with high accuracy

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Patients with Alzheimer's disease (AD) demonstrate deficits in olfactory and cognitive functioning. Given that a definitive diagnosis of AD is reached only upon autopsy, there is currently a great need for tests that can accurately diagnose patients early in the disease process. Thus, the current study assessed the sensitivity and specificity of tests of cognitive and olfactory functioning in a group of AD patients and normal elderly controls. Subjects' odor detection threshold and ability to name odors, objects, and colors were measured. In addition, studies have shown that AD patients perform particularly poorly on tasks of semantic memory, which represents our knowledge for objects, concepts, and sensory experiences, including their meanings and associations. Thus, given that the sensitivity and specificity of the association networks for odors have not been studied in patients with AD, similarity judgments previously obtained for odors, using a triadic comparison task, and analyzed with multidimensional scaling analyses (MDS) were examined. Using vision as a comparison modality, previously obtained similarity judgments for colors analyzed with MDS techniques were also examined. A series of logistic regression analyses were performed in order to assess each test's ability to correctly classify patients and normal controls. The analyses revealed that the odor naming and the odor association tasks had exceptionally high overall classification rates, 95 and 87.5%, respectively. The color naming and color association tasks, however, were both quite poor at classifying AD patients (25 and 58%, respectively), presumably due to the fact that the striate cortex is relatively spared from early changes in this group. These results suggest that olfactory tests which are both sensory- and cognitively based (i.e. odor naming and odor association tasks) are quite sensitive and specific for classifying AD patients and normal elderly subjects and may prove to be very helpful tools in the early diagnosis of these patients.

Supported by NIH grant # AG08203 to C.M.

458. Stimulus fluctuation in the measurement of odor detection thresholds by static olfactometry

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Increased interest in the field of human olfaction has led to a rapid growth in the number of studies of human odor perception. As previously noted, studies of odor perception entail issues of stimulus control that can contribute to much of the variation in the sensory response. Static olfactometry (i.e. squeeze-type bottles with a range of concentrations of an odorant in solution) offers the most practical, inexpensive and uncomplicated method for presentation of olfactory stimuli, yet introduces serious limitations in stimulus control. A systematic analysis of the stimulus concentration is needed to assess (i) the actual vapor concentrations that are available to be delivered; (ii) concentration changes that occur with usage and over time; and (iii) the potential contamination of the stimuli. Such factors may be particularly important when assessing thresholds for odor detection, where even small changes in stimulus concentrations might compromise results and interpretations. To explore the extent to which time-dependent changes in concentration for several commonly used, olfactory stimuli could affect sensory measurements, we obtained odor detection thresholds repeatedly from the same set of bottles at regular intervals for three weeks. We then compared those sensory thresholds with the headspace concentrations of the bottles, as measured by gas chromatography immediately before and after sensory testing. The vapor concentration in the bottles decreased both with time and with usage. The human psychophysical data revealed variation in thresholds that were attributable to the physical changes in the stimuli, thus reaffirming the importance of monitoring the actual stimulus delivered in olfactory experiments.

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459. Viral replication in olfactory receptor neurons and entry into the olfactory bulb and brain

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I will describe our ongoing studies of the interaction of the mouse host and vesicular stomatitis virus. When VSV is applied to the nasal neuroepithelium, it initially replicates in the olfactory receptor neurons and is transmitted along the olfactory nerve to the CNS within 12 h. In the olfactory bulb, the virus replicates invasively through the layers of the olfactory bulb, reaching the olfactory ventricle by day 4–5 and hindbrain regions by day 8 post-infection. In mice, infection may result in a 50% mortality rate. I will describe the crucial host innate and specific immune responses responsible for restricting viral propagation and caudal spread of the virus. I will describe a therapy which results in enhances clearance of virus and survival of the host which may be applied to human encephalitis. The effect of the sex of the host in restricting viral disease will also be discussed. The role of specific mucosal humoral immunity and systemic cellular immunity in prevention of infection will be described.

460. Mammalian cells expression of mice olfactory receptors from MHC class I region for ligand identification

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Olfactory receptors (OR) are seven transmembrane proteins representing several families of the G-protein coupled receptor (GCPR) superfamily. Although a large number of putative OR have been cloned in different species, their ligand specificity remains most of the time unknown. Several polymorphic genes encoding putative OR have been mapped in the MHC class I region in mice. These receptors may play a role in mating preferences for genetically different partners.

In order to identify specific activating ligands, we have cloned receptors from individuals of a mice kindred in an eucaryotic expression vector. A COS cell line has been established that contain a permanent reporter gene sensitive to receptor activation, allowing *in vitro* ligand screening.

461. Properties of inositol 1,4,5-trisphosphate-gated conductances in patches excised from the soma of rat olfactory neurons

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It is generally accepted that inositol 1,4,5-trisphosphate ($InsP_3$) plays a role in olfactory transduction. However, the precise mode of action of $InsP_3$ remains controversial. We have characterized the properties of conductances activated by addition of 10 μM $InsP_3$ to excised patches from soma plasma membrane of rat olfactory neurons. $InsP_3$ induced current fluctuations in 22 of 78 inside-out patches. These conductances could be classified into two groups according to the polarity of the current at a holding ptotential of +40 to + 60 mV (with Ringer in the pipette and pseudointracellular solution in the bath). Conductances mediating outward currents could be further divided into large (64 ± 4 pS, n = 4) and small (17.5 ± 1.6 pS, n = 14) conductance channels. Both small and large conductance channels were nonspecific cation channels. The large conductance channel displayed bursting behavior at +40 mV (in ms: t_{burst} = 130, $t_{flicker1}$ = 15, $t_{flicker2}$ = 2), and flickering increased at negative holding potentials to the point where single channel currents were no longer discernible. The small channel did not display flickering behavior. The conductance mediating inward currents at +40 to +60 mV reversed at +73±4 mV. The current traces displayed considerable fluctuations, and single channel currents could not be discerned. Current fluctuations returned to baseline after removal of $InsP_3$. The power density spectrum for the excess noise generated by $InsP_3$ followed a $1/f$ dependence indicative of conductance fluctuations in the channel mediating this current. These experiments demonstrate the presence of plasma membrane $InsP_3$-gated channels of different ionic specificity in olfactory receptor cells.

Supported by NIH DC00566 and DC00214.

462. Ultrastructure of the taste buds of the cave-dwelling amphibian *Proteus anguinus* (Caudata)

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Proteus anguinus is a neotenic cave-dwelling amphibian which lives endemically in the subterranean waters of a few caves in the calcareous mountains which border on the Adriatic Sea. the eyes of *Proteus* are reduced during early development. in this study, we investigated the taste buds (TBs) of this highly protected animal which is well adapted to an extreme biosphere, and compared them to data from epigean relatives. One animal originating from Planina cave (Slovenia) served for this investigation. After MS-222 anesthesia, the animal was fixed in a 2% glutaraldehyde–2% paraformaldehyde mixture and was processed for electron microscopy. Samples of distinct head and mouth regions were investigated using scanning- and transmission-electron microscopes. TBs occur in the lips and in the mouth cavity. They are located in hillock-like papillae or do not top the surface of the epidermis. In longitudinal sections, the TBs are ovoid or pear-shaped and are ~120 μm high and 80–100 μm wide. The sensory epithelium comprises ~80–100 elongated cells of different electron densities (light and dark cells). Apically, light cells terminate either in one or several large receptor villi, in one thick and short receptor villus or in several undivided short and relatively thin microvilli. They are rich in smooth endoplasmic reticulum, Golgi-systems, mitochondria and microtubules and possess, especially in their basal lobar processes, numerous light- (30 nm) and dense-cored (60 nm) vesicles. Dark cells reach the receptor area with relatively small and often branching microvilli. They are also rich in organelles and contain, especially in their supranuclear portions, numerous granulated mucous vesicles. Basal cells rest on the basal lamina of the TB. They have long processes which intermingle with the basal processes of light and dark cells and the nerve fiber plexus. Afferent synapses connect

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light- and dark-basal cells (presynaptic sites) and nerve fibers (postsynaptic sites). Rarely, there are also efferent synapses between nerve fibers (presynaptic sites) and light cells. Characteristically, their postsynaptic site is marked by a subsynaptic cisterna. TBs seem to be organized like the TBs in other caudata, but in those no efferent synapses were reported so far.

463. Salty taste enhancement with amino acids

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Enhancement of salty taste has important implications for the reduction of sodium in foods as well as for understanding the mechanisms of salty taste transduction. Certain amino acids have previously been reported to enhance the saltiness of NaCl. To further investigate this, subjects rated saltiness and other taste attributes of solutions containing various concentrations of NaCl and L-arginine HCl (Arg) or L-asparagine (Asn) and all possible binary combinations using the method of magnitude estimation. Results from these studies showed that 50 and 75 mM NaCl was rated as saltier with the addition of 50, 75 or 100 mM Arg, while Arg alone was not found to be salty. This trend was not seen for Asn which had no effect on saltiness. The enhancing effects of Arg are concentration dependent: low to moderate concentrations increased the saltiness of NaCl by ~100%, whereas at higher concentration mixtures of NaCl and Arg, only slight enhancement was seen. The second part of this study examined the salty taste enhancing effects of 25 and 50 mM L-arginine HCl, L-asparagine, and L-lysine HCl (Lys) in 50 or 75 mM NaCl solutions. These amino acids were chosen because all three contain at least one nitrogen in the side chain. Both Arg and Lys have basic (charged) side chains and Asn's side chain is neutral (amide). Pairs of samples were presented in a forced-choice pairwise comparison protocol in which subjects indicated which of the two samples was saltier. Under almost all concentrations of amino acid and NaCl the Arg/NaCl solutions were significantly more salty than all other samples. The saltiness of the solutions were ranked as: Arg/NaCl > Lys/NaCl > Asn/NaCl = NaCl. If Na^+ (and Li^+) salts elicit a salty taste by passing directly through an epithelial ion channel, then the amino acids Arg and Lys may modify this channel to increase inward conductance, thereby enhancing the salty taste of NaCl.

Paul Breslin is a Morley R. Kare Fellow. This work was also funded by NIH grants R29 DC02995 (P.A.S.B.) and DC00882.

464. Na,K-ATPase isoform expression in the olfactory mucosa: analysis by quantitative RT-PCR

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The enzyme Na,K-ATPase, known as the sodium pump, is a plasma membrane enzyme that uses the energy from the hydrolysis of ATP to maintain the electrochemical gradients for sodium and potassium in all mammalian cells. The enzyme itself is a heterodimer composed of equimolar amounts of two non-covalently linked polypeptide subunits termed α and β. The transmembrane α subunit is associated with the catalytic properties of the enzyme while the β subunit is critical for membrane insertion of the holoenzyme. Both subunits are encoded by multigene families and, at present, three isoforms for each subunit have been identified in mammals (α1–3 and β1–3). Several lines of evidence suggest that each α subunit can associate with each of the β subunits to form a functioning holoenzyme. The physiologic significance of this diversity is not yet well understood but a cell and tissue specific pattern of expression exists which suggests different roles for the various isoforms. In epithelial cells and exocrine glands the α1b1 combination predominates while neurons preferentially express the α3b1 isoforms. Glial cells, which are specialized to deal with high extracellular potassium levels tend to express α2b2. The olfactory mucosa is a neuroepithelial tissue specialized for secretion, chemoreception and neurotransmission. Multiple lines of evidence suggest that Na,K-ATPase maintains the ion gradients necessary for the processes to take place. In this study we have utilized the technique of quantitative RT–PCR to determine the differential expression of the various Na,K-ATPase isubunit isoforms in order to further characterize this enzyme in the olfactory mucosa. We note that all subunit isoforms are expressed in this complex tissue. This finding supports the concept of an integral role for Na, K-ATPase in the peripheral mechanisms of olfaction.

465. Development of the olfactory sense organs on the antenna of the fruitfly *Drosophila melanogaster*

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There are three morphologically distinct types of sense organs located on the third segment of the antenna of the fruitfly *Drosophila melanogaster.* The structure of each sense organ is made up of three non-neuronal support cells and is innervated by between one and four neurons. The neurons project in the antennal nerve to the glomeruli of the antennal lobe. Our previous experiments had shown that the development of the olfactory sense organs is somewhat unique and follows neither the principles described for the development of the mechanosensory organs nor that for the compound eye. In an effort to further elucidate this mechanism, we are carrying our genetic screens using behavioral and morphological criteria to identify genes involved in the development process. We will discuss our results with some of these genes which appear to act early in the development of the sense organs and which appear to be involved in the specification of the type of sense organ and its position on the surface of the epidermis.

466. Olfactory neuron specific expression of SNMP-1, a novel membrane protein of olfactory cilia of the silk moth *Antheraea polyphemus* with homology to the CD36 family of membrane proteins

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A 67 kDa protein was purified from the ciliary membranes of sex-pheromone-specific olfactory neurons of the wild silk moth *Antheraea polyphemus.* Expression analysis of the corresponding cDNA clone, referred to as SNMP-1, by Northern blot and

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ribonuclease protection assays (RPAs) show that SNMP-1 is present in at least two forms, and that SNMP-1 expression is restricted to the antenna. Further analysis of SNMP-1 expression by *in situ* hybridization and immunocytochemical analysis show that SNMP-1 mRNA is expressed in olfactory neurons, and SNMP-1 protein is localized to the cilia, dendrites, and somata, but not the axons. Antennal RPAs using tissue from different stages of adult development show that a low level of SNMP-1 expression initiates following the birth of olfactory neurons, but dramatically increases following the completion of morphogenesis. This correlates with the expression profiles of several antennal specific molecules that are directly involved in adult odor processing.

Homology searches using the NCBI Blast server indicate that SNMP-1 is homologous with the CD36 family of proteins, an as yet small family of receptor-like membrane proteins which include members in vertebrates, arthropods, and nematodes. Human CD-36 is a receptor for a variety of ligands, is expressed in a multitude of tissue types, and has been shown to interact with molecules implicated in signal transduction. SNMP-1 represents the first member of the CD-36 family identified in neuronal tissue. Homology between SNMP-1 and CD-36, the localization of SNMP-1 protein to the antennal dendrites, and the expression of SNMP-1 mRNA late in adult development argue that SNMP-1 has an important role in silk moth olfaction.

Supported by NIH NIDCD DC-00588 and USDA CSRS 94-37302-0615.

467. The representation of umami taste in the primate primary and secondary taste cortex

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There is a population of neurons in the primary taste cortex in the frontal operculum and insula and in the secondary taste cortex in the orbitofrontal cortex of the macaque with responses to sodium glutamate, and this population has responses which are different to the responses to the prototypical tastants glucose, sodium chloride, hydrochloric acid, and quinine (Baylis and Rolls, 1991). Extending this analysis, it was shown first that single neurons that had their best responses to sodium glutamate also had good responses to glutamic acid (e.g. 0.05 M); second that the responses of these neurons to the nucleotide umami tastant inosine 5′-monophosphate (e.g. 0.0001 M) were more correlated with their responses to monosodium glutamate than to any prototypical tastant (Rolls *et al.*, 1996); third that there is a partly sensory-specific decrease in the responses of these neurons produced by feeding satiety; and fourth that some of these neurons in the orbitofrontal cortex respond to umami taste and also to olfactory stimuli associated with the taste of glutamate. These latter experiments, and psychophysical studies in humans, suggest that part of the basis for umami flavour may be a combination of taste inputs from the glutamate system and associated olfactory stimuli.

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468. Taste and olfactory processing in the primate orbitofrontal cortex

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The primate orbitofrontal cortex contains not only the secondary taste cortex but also the secondary and tertiary olfactory cortices. In these regions, the representation of the flavour of food is formed, by combining onto single neurons olfactory and taste inputs. We have now shown that this representation of odour is built for at least 35% of neurons by learned association with taste, and that the responses of these neurons decrease to zero to the food with which the monkey is satiated, but not to other foods (see Rolls, 1996, 1997). Olfactory as well as taste sensory-specific satiety is thus represented in the primate orbitofrontal cortex.

There are also oral somatosensory inputs to the orbitofrontal cortex, and information about the texture of food converges onto some taste and olfactory neurons in this region. Such neurons are able to represent the presence of fat in the mouth.

In investigations of whether there are similar areas in humans, we present fMRI results showing a gustatory representation in the medial orbitofrontal cortex distinct from the olfactory representation in the right orbitofrontal cortex, and a separate representation of affectively positive tactile stimuli in the left human orbitofrontal cortex.

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469. Defective olfactory neuronal development in Rett syndrome

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Rett syndrome is a severe neurodevelopmental disorder that affects primarily females and results in motor dysfunction, mental retardation and often seizures. After apparent normal infancy, the behavioral and functional regression becomes obvious. There is a concomitant deceleration of head growth and decreased volume of grey more than white matter. Analyses of post-mortem brains have suggested abnormalities in neuronal size and dendritic arborization, the etiology of which is unknown. The olfactory receptor neuron is an acceptable model for the assessment of neuronal development, and is accessible for biopsy in the living. Biopsies of four girls with Rett syndrome have been obtained under local anesthesia, and show a lack of mature olfactory neurons. There is no immunoreactivity for olfactory marker protein (OMP). Sustentacular and probable basal cells have been identified, and await further clarification. These studies demonstrate a clear abnormality of the olfactory receptor neurons in girls with Rett syndrome. Further studies of these abnormalities hold promise in defining the pathophysiology of Rett syndrome specifically, and in neuronal maturation in general. It may also allow a marker for Rett syndrome to be identified.

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470. Apolipoprotein E and Alzheimer's disease: the tip of the susceptibility iceberg

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Apolipoprotein E (APOE) is a true susceptibility polymorphism of the common form of Alzheimer's disease (AD). There are three APOE alleles (s2, s3, s4) that are universally distributed in the population with some variation in allele frequency due to racial and ethnic differences, and are associated with different risks and age of onset distributions. In multiple studies, the positive predictive value for symptomatic possible or probable AD patients who carry at least one a4 allele was consistently >95%. Thus, really in the clinical course of dementia, when diagnoses are only 60%–70% accurate, the presence of an a4 allele raises the diagnostic accuracy of AD to >95%. With the anticipation of a second major late-onset AD susceptibility locus on chromosome 12, a matrix of relative susceptibility risks in the population raises many ethical and social questions associated with pre-clinical prediction.

The metabolism of apoE (protein) in the brain is a new and exciting area of neurobiology research made relevant by the association with AD. We have constructed transgenic animals using large human genomic fragments containing human APOE on an APOE-deficit mouse background as well as homologous recombination experiments replacing mouse APOE with human APOE promoter elements. NOT the human APOE gene coding sequence, is associated with the human pattern of intraneuronal apoE immunoreactivity.

471. Receptor neuron responses to naturally produced host plant odours in the moth species *Heliothis virescens*: gas chromatography linked to electrophysiological recordings from single receptor neurons

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Gas chromatography linked to electrophysiological recordings from single receptor neurons were employed to identify volatile compounds released by plants that are detected by receptor neurons in the tobacco budworm moth *Heliothis virescens*. Volatiles of various host (sunflower, tobacco and tomato) and non-host (e.g. spruce and juniper) materials were collected by a «head-space» procedure and separated in the gas chromatograph (GC). Two different GC-columns were installed, each with a split at the end, leading half of the effluent to the GC-detector and the other half out of the GC-oven, over the insect antenna during recordings from single receptor neurons. Thus, each neuron was tested for all compounds of a volatile mixture, separated in both a polar and a non-polar column. The chemical structures of the compounds were identified by coupled gas chromatography–mass spectrometry (GC-MS). Most receptor neurons RNs responded selectively to one or two compounds, in the latter case clearly strongest to one of them. For instance, one neuron type responded to two monoterpenoids, identified by GC-MS as β-myrcene and trans-β-ocimene and confirmed by retesting with synthetics. Another type of RNs was recorded in 75% of all experiments. These RN responded exclusively to two compounds, and clearly strongest to one of them. Preliminary GC-MS analyses showed that both were sesquiterpenes. Alltogether, 70 RNs recorded were classified in 12 neuron types, each type including neurons with identical response spectra, i.e. responding to the same compounds. No overlapping of response spectra were found between neurons of different types. These results suggest that plant odour information in *H. virescens* females is received by a small number of specialist RN types and mediated to the brain in a labelled-line mechansim.

472. Dissociation of odor intensity and pleasantness judgments: effect of temporal lobe lesions

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Odor pleasantness and odor intensity judgments are often confounded. To dissociate these two aspects of odor perception, we designed a test in which the odor quality and concentrations were selected to minimize correlation between intensity and pleasantness. In a pairwise comparison task, 25 healthy subjects compared all pairs in a set of 10 odors according to intensity, and separately according to pleasantness. The results showed a highly significant difference between intensity and pleasantness judgments for the same odors ($F = 35.7$, $P < 0.0001$), showing that the two aspects of the stimuli can be dissociated in healthy subjects, and that the test might be used to investigate affective judgments in individuals with temporal-lobe damage.

For Experiment 2 we retained six of the odors for a similar, shorter test for use with patients: 22 epileptic patients with temporal lobe lesions (12 left, 10 right) and 19 healthy controls were tested. Because amygdala is damaged to varying extents in patients with temporal-lobe lesions, we predicted that they would be impaired in the pleasantness, but not intensity judgments.

The preliminary results on this small sample indicate that the mean ratings of the 6 odorants does not differ among the groups, neither for intensity nor for pleasantness judgments. However, consistency of ranking in the right temporal-lesion group differs very significantly from the left and the controls ($F = 5.45$, $P < 0.008$). Contrary to our hypothesis, this difference was observed for intensity as well as pleasantness judgments. The finding that only the right-damage group differed from normal is in keeping with previous results showing a relative predominance of the right hemisphere in olfaction.

These results will be discussed according to the extent of damage in medial temporal-lobe structures.

473. Triangle and same–different methods of discrimination for mustard: differential effects of memory and irritation

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The triangle triadic method and the same–different paired method

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are both discrimination methods that can be applied to foods. They have the advantage that the nature of the difference does not have to be specified as with 2-AFC or 3-AFC methods, making them convenient for testing naive consumers. Both methods were used for discriminating between two confusable Dijon style mustards; the mean d' value was significantly higher for the same–different test (2.1 versus 1.05). This could be because the same–different test demands less memory load using only two stimuli. It could also be because the gradually increasing irritation elicited by the stimuli caused masking, the irritation increasing further with more stimuli in triadic presentations. Thus, the same subjects had also, in counterbalanced order, performed same–different methods with a 'dummy' stimulus tasted beforehand which was not assessed. This arranged that the order and number of stimuli encountered in the triangle and the same–different methods were the same. The mean triangle and same–different d' (1.4) were not significantly different, once the masking effects of irritation had been equated. This suggests that such masking effects played a more important role than memory in eliciting higher d's for the same–different test for mustard. This was supported by subjective reports.

474. Olfactory deficits in schizophrenia and major depression

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Olfactory functioning in psychiatric disorders like schizophrenia and major depression is of increasing interest because of the close neuroanatomical links between olfactory pathways and known structural abnormalities. The most often reported deficit in schizophrenia is a reduced ability to identify odours without acuity deficit (olfactory agnosia). Various studies indicate that this impairment is unrelated to neuroleptic use, overt psychopathology, and smoking, that it is not only secondary to attentional deficits and more common among males than females. Less is known about olfactory functioning in major depression.

The goal of the present study was to assess various olfactory subfunctions beside olfactory identification in schizophrenia and major depression. Olfactory evaluation was performed with 38 schizophrenic patients, 24 patients with major depression and 55 normal controls.

Our data show reduced ability to identify odours in schizophrenia and major depression. Furthermore, schizophrenics were impaired in nonverbal olfactory discrimination. No impairment was found in patients with major depression concerning the ability to discriminate odours and both groups had normal olfactory acuity. While patients with schizophrenia did not differ significantly from patients with major depression in olfactory identification, there was a difference in the ability of olfactory discrimination.

The obtained results suggest that also major depression is accompanied by an olfactory agnosia and that olfactory discrimination deficits are more prominent in schizophrenia, which most likely reflect dysfunctions of orbitofrontal cortex.

475. Quantitative olfactometery for threshold or evoked response testing

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A digitally controlled quantitative olfactometer has been developed for testing solid, liquid or gas source materials in half or quarter log steps. Three configurations have been evaluated: (i) human evoked potential testing, (ii) human threshold testing and (iii) canine threshold testing. The emitted odorant concentrations have been calibrated by three methods: gravitametrically, total hydrocarbon analysis and absorbent trapping mass spectroscopy. Seven odorant compounds, four liquids and three solids, have been evaluated: (i) acetic acid, (ii) isoamyl acetate, (iii) *n*-butyl alcohol, (iv) pyridine, (v) methylisothiocyonate (MITC), (vi) cocaine and (vii) TNT. The olfactometer has been found to be reliable and stable over a very large range of concentrations. Human odor thresholds have been evaluated and found to be at lower levels and with significantly less variance than previously reported. The olfactometer has several technical innovations that make it more reliable than traditional devices: (i) it does not produce aerosols; (ii) it avoids stainless steel; (iii) the odorant concentrations are maintained without condensation; and (iv) oxidation of the source odorant does not occur. For the generation of evoked potentials the device has been configured to trigger either: (i) randomly, (ii) on inhalation or (iii) on exhalation. It produces odorant pulses 20–500 ms long with an external trigger for averaging.

476. Distribution and subcellular localization of AChE and ChAT in the glomerular layer of the carp olfactory bulb

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The cholinergic neuronal structures and their synaptic connections in the glomerular layer of the carp (*Cyprinus capio*) olfactory bulb were analysed using AchE and ChAT cytochemistry. Enzymes are found in some receptor axon's terminals, asymmetrical axo-dendritic synapses and in mitral cell's dendrites. Some asymmetrical synapses between mitral cell's dendrites and dendrites of intrinsic neurons contain end products of both enzymes too.

Ultrastructural localization of AChE is different from that of ChAT. AChE is found on the outer surface of axon and dendrite membranes, while the ChAT reaction end product occurs inside of ChaAT-positive structures.

The data suggest that population of asymmetrical axo-dendritic synapses in glomerular neuropil is heterogeneous in terms of their chemical mediators and includes subpopulation of cholinergic synapses. The same is true for dendro-dendritic synapses which connect mitral cells and intrinsic neurons. Cholinergic subpopulation exists also among synaptic contacts of these types. Our results show that peripheral input of carp olfactory bulb includes cholinergic synaptic connections. Such organization of cholinergic synapses in the carp olfactory bulb differs from that in mammals.

Supported in part by RFFI project N 96-04-50752.

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477. Cellular and molecular neuropathology of the olfactory epithelium and central olfactory pathways in neuropsychiatric disorders in late life

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The ability to detect environmental odors decreases with normal aging. However, specific deficits in odor detection, identification, and memory have been recognized in a variety of disorders such as Alzheimer's disease (AD), various neurodegenerative movement disorders, and more recently, the neuropsychiatric illness, schizophrenia. The presence of dystrophic neurites observed upon histological examination of the olfactory epithelium (OE) in these disorders has been thought to contribute to the olfactory dysfunction. Molecular characterization of abnormal sensory epithelial neurons and their processes in these illnesses find immunoreactivity to abnormally phosphorylated tau proteins, other microtubule-associated proteins, neurofilament proteins, neural cell adhesion molecules, and various synapse related proteins. Furthermore, amyloid precursor protein and β-amyloid is present in most cases. However, these morphologic and molecular features are also seen in the OE of most normal elderly control cases. In contrast, neuropathological studies have found selective vulnerability of central olfactory regions for the accumulation of neurofibrillary pathology in AD and for cytoarchitectural and cytoskeletal protein abnormalities in schizophrenia. Thus, it is likely that the olfactory impairments associated with these diseases is due to damage within central olfactory pathways in combination with the less specific impairments associated with age-related sensory neuroepithelial abnormalities.

478. A role for tyrosine phosphorylation in olfactory glomerulus development in *Manduca sexta*

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Glia-neuron interactions are critical for the proper development of olfactory glomeruli in the moth, *Manduca sexta*. To better understand these cell–cell interactions it is important to identify the molecular events that mediate them. To identify molecular mechanisms mediating intercellular interactions in a developing system, one could look for molecules that receive or transmit signals at the extracellular surface or for changes in patterns of intracellular signaling molecules. In an initial screen for molecular events that correspond to developmental changes in olfactory glomeruli, I have chosen to monitor the post-translational modification, tyrosine phosphorylation. Tyrosine phosphorylation is known to mediate intracellular changes from the cytoskeleton to the nucleus in response to a myriad of extracellular cues. Modulations of tyrosine phosphorylation that correlate with cellular changes during glomerulus formation potentially represent a number of converging extracellular or diverging intracellular molecular cascades that mediate essential developmental events. A monoclonal antibody to phosphotyrosine and confocal microscopy were used to monitor phosphotyrosine levels in the developing olfactory lobe of *Manduca*. Immunoreactivity was strongest in the regions of the lobe known to contain the growing tips of antennal-lobe neurons and the developing synaptic neuropil. Tyrosine phosphorylation has been shown to play an important role in both neuronal process outgrowth and in synapse formation. In dissociated cultures, phosphotyrosine immuno reactivity was found in the growth cones of some antennal-lobe neurons and double-label immunofluorescence studies of intact lobes show that phosphotyrosine immunoreactivity colocalizes with the growing tips of at least one type of antennal-lobe neuron, the local interneurons. To examine more closely the relationship between phosphotyrosine levels and synapse forming regions, I have cloned Manduca synaptotagmin from a pupal cDNA library. The amino terminus of this clone has been subcloned into a bacterial expression system. The purified peptide will be used as an antigen for polyclonal antibody production. The antibody will be used in double label confocal microscopy studies to look at the association between tyrosine phosphorylation levels and synapses in the developing antennal lobe. Funded by NIH grant # NS20040.

479. Application of the atomic force microscope to the study of the vomeronasal neuroepithelium

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The atomic force microscope (AFM) is a type of the scanning probe microscopes (SPMs) which give three-dimensional images by measuring interactions between the scanning probe and sample surface. AFM has been expected to be a promising tool for biological material research because it can be used for both conductive and insulating materials in liquid as well as in the air. The resolution of AFM largely depends on the condition of sample surface as much as the sharpness of AFM tip. Although AFM has resolved atomic details on the sample surface under appropriate conditions, a high resolution for biological samples was rarely obtained so far.

We developed a new method of sample preparation for tissue sections which gave us AFM images comparable to the images obtained by the transmission electron microscope (TEM). Most subcellular structures of vomeronasal epithelium observed with TEM, including the inner membrane of mitochondria, junctional complex between neuron and supporting cells, and individual microvilli were observed with AFM. We obtained AFM and TEM images of the same area of the same sample.

We also propose another possibility of AFM application to the vomeronasal study. As the AFM tip contacts with sample surface directly, physical properties of the sample surface as well as its topography can be obtained. So far the binding forces between avidin-biotin and antigen-antibody have been measured by using AFM. Such reports encourage us to study the interaction between pheromones and their receptors with AFM. We are planning to functionalize AFM tips with ligands and map the distribution of their receptors on vomeronasal epithelium.

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480. Temporal process from receptors to higher brain in taste detection studied by gustatory-evoked magnetic fields and reaction times

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Using MEG and a taste stimulator with rapid-rise time, we previously located the primary gustatory area in the human cerebral cortex and also investigated the relation between the onset latency of the gustatory-evoked magnetic fields (GEMs) and reaction times (RTs) in different taste qualities. In the present study, we investigated the temporal process from receptors to the higher brain in taste detection based on the results of the GEMs and RTs of different tastes. We used 100 mM, 300 mM and 1 M NaCl and 3 mM saccharine. The duration of each stimulus was 400 ms. The inter-stimulus interval was ~30 s. The temperature of both taste solution and deionized water was maintained at the same as that of the tongue. Four subjects participated in this experiment. The 64-channel whole-head SQUID system (CTF Systems Inc., Canada) was used to measure GEMs. The sampling rate was 250 Hz and the low-pass filter was 40 Hz. In each subject, GEMs and RTs to a given taste were measured separately by applying 40 trials of stimulation. After each trial of both measurements, subjects showed a perceived intensity by using their fingers. In the GEM study, the trials contaminated with eye movements were rejected and the remaining trials were averaged. Averaged GEMs were superimposed on the same sheet with all 64 channels to measure the onset latecy of GEMs from the stimulus onset. RTs and onset latencies of GEMs were longer for saccharine than NaCl, and the value of RTs minus the onset latency of GEMs from RTs, presumably indicating the time for higher brain process plus motor process, did not differ between 3 mM saccharine and 1 M NaCl. With increased concentrations of NaCl, RTs became shorter, but onset latencies of GEMs remained constant. Sweet taste took a longer time than salty taste at receptor process including the time for diffusion to receptors.

481. Taste-associated odor learning in the rat

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Most foods have their own odors and tastes. We can recognize foods by their flavors, mixtures of odors and tastes. It is supposed that odors and tastes of foods are easily associated after exposure to them. No systematic study, however, has been documented on this topic except for the 'taste-potentiated odor aversion'.

We designed a new method to study an association between odor and taste in the rat. Wistar male rats were housed in home cages individually. They were deprived of water in the home cages and were allowed to drink in a circular open-field apparatus (0.76 m in diameter) from small glass dishes (3 cm in diameter). They were then exposed in their home cages freely to 0.005 M Na–saccharin and 0.0001 M quinine hydrochloride which contained either iso-amylacetate (5 ml/100 ml) or almond essence (5 ml/100 ml) for >2 days. They were again water deprived and tested for odor preference in the open-field apparatus equipped with eight dishes (1 ml of test solution in each): four contained iso-amylacetate + water and the rest almond + water as test stimuli. Naive rats showed equal preference to the two test solutions, but trained rats preferred to drink water with the odor previously associated with saccharin.

To elucidate the brain sites responsible for this odor-taste association learning, experiments have been performed in brain-lesioned rats with the same procedure as described above. Data so far obtained suggest that rats with lesions in the orbitofrontal or cingulate cortices showed weak or no preference to the saccharin-associated odor, and rats with lesions in the amygdala or gustatory cortex showed rapid extinction of preference to the saccharin-associated odor than control rats did.

These results suggest that this method is convenient and useful for studying taste-associated odor learning and memory.

482. Taste effectiveness of alcohols in the rat

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Alcohols, especially ethanol, are entertained as luxury drinks by humans. However, the effects of alcohols on the peripheral gustatory system including receptors and taste nerves have not been studied much. Two experiments were designed to study the gustatory effects of eight alcohols such as methanol, ethanol, ethylene glycol, 1-propanol, 2-propanol, propylene glycol, 1,3-propandiol and glycerin in male Wistar rats.

In Experiment 1, neural responses to each alcohol at 1 M concentration were recorded electrophysiologically from the chorda tympani and glossopharyngeal nerves. The responses to the four alcohols such as ethylene glycol, propylene glycol, 1,3-propandiol and glycerin which have two or three hydroxyl groups were larger than those to the other alcohols in both nerves. The responses to these alcohols were suppressed to ~20% of the pretreatment control responses by treatment of the tongue with 2% pronase E, a proteolytic enzyme. The response patterns of 93 single chorda tympani fibers in response to these four alcohols were similar to those of 0.5 M sucrose and 20 mM quinine–HCl.

In Experiment 2, the rats which acquired conditioned taste aversions to alcohols with two or three alcoholic hydroxyl groups also avoided 0.5 M sucrose and 0.1 mM quinineHCl. However, the aversion did not generalize to NaCl and HCl.

The present results suggest that alcohols exert their taste effects by binding to a protein receptor with binding sites for more than two alcoholic hydroxyl groups, and that alcohols have a taste similar to the taste of both sucrose and quinine.

Supported by grants from the Japanese Ministry of Education (No. 0745748 and 07838016).

483. Individual odour signatures of related and non-related male koalas (*Phascolarctos cinereus*)

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The koala (*Phascolarctos cinereus*) is probably one of the best recognized and most admired native Australian marsupials and is

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distributed in the eastern part of the continent from southern Victoria to northern Queensland. Adult animals weigh between 8 and 14 kg whereas the males are up to 50% larger than females. The koala is herbivorous and its diet contains only *Eucalyptus* leaves which are processed with the help of a bacterial biota in the large caecum. Only the male Koala shows a large sternal gland (up to 85 mm × 50 mm) which undergoes discernible seasonal changes in activity and is used for scent-marking on trees.

Our study investigated the chemical composition of the sternal gland secretion of the koala by using gas chromatography–mass spectroscopy (GC-MS) and looked at the variation of scent composition in related and non-related males. The chemical constituents of the sternal gland of the koala showed that a range of fatty acid derivatives, monoterpenes and sesquiterpenes as well as long-chain hydrocarbons were present in the secretion and some of those compounds were possibly connected to the diet.

High resolution GC-MS analysis revealed two oximes present in the secretion that previously were only found in the urine of guinea pigs. Furthermore, a previously unknown sulphur compound and three nitriles could be identified in considerable amounts, one of which had previously been identified in the chest gland secretion of the thick-tailed galago (*Galago crassicaudatus*).

In a second step the variation in scent composition was compared between three individuals using 31 identified compounds, equalling between 20 and 25% of the total peak area. Their relative abundance in the secretion was determined by integrating their peak area and their relation was analysed using principal component analysis (PCA). The consequences of those individual variations will be discussed in detail.

484. Extracellular field potentials and inhibition in the olfactory lobe of crayfish

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Electrical stimulation of crayfish olfactory afferents results in complex extracellular field potentials (EFPs) in the olfactory lobe (OL) of a perfused crayfish brain. Four components of the EFP can be repeatedly identified: two small positive going components followed by a large, short duration, negative component and a long duration repolarization. The first two positive going components most likely represent afferent activity because they are unaffected by perfusion of the brain with low calcium saline. Components 3 and 4 are abolished by low calcium saline perfusion indicating that these represent post synaptic activity. Double pulse afferent stimulation (orthodromic) or preceding an afferent stimulus with stimulation of the output neurons (antidromic), significantly reduces the amplitude of components 3 and 4 of the EFP, suggesting inhibition. These putative inhibitory effects are reduced by perfusing the brain with 0.0003 M picrotoxin. Preparations exhibit spontaneous, sometimes oscillatory, bursts of activity during and after picrotoxin treatment. The results suggest the presence of GABA modulated inhibition acting at both the input and output of the crayfish olfactory lobe, consistent with immunohistochemical GABA labelling in the cap and column regions of the OL glomeruli.

Supported by Australian Research Council.

485. Neuronal plasticity and induced cell death in the olfactory system of the crayfish

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Freshwater crayfish increase in size throughout their relatively long lives (~8 years). This growth is associated with an increase in olfactory receptor neuron (ORN) number, olfactory lobe output neuron number, and olfactory lobe volume. Turnover of the ORNs occurs at each molt. Damaged ORNs are regenerated. Crayfish olfactory neurons therefore exhibit plasticity in which ORNs and higher order neurons may retain an interactive flexibility throughout life. To test this possibility, ORNs were removed by cutting one antennule off sexually immature juvenile, or mature adult crayfish. This leads to a decrease of up to 60% in the volume of the olfactory lobe on the operated side, compared with the unoperated side. The output neurons of the olfactory lobe on the operated side in these animals are also reduced in number by 15–20%. This reduction in the output neuron number after ORN axotomy could represent a transsynaptically induced effect. We therefore searched for cell death in the output neurons of juveniles after unilateral antennular ablation using the TUNEL method. We find ~three times as many labelled output neuron somata on the operated side of the brain compared with the unoperated side. Labelled cells on the unoperated side of these animals, raises the possibility of neuron turnover in the olfactory lobe output neurons.

Supported by Australian Research Council.

486. Pax6 protein is expressed throughout development in the olfactory system, eye and brain of *Xenopus laevis*

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The transcription factor Pax6 has been shown to play an important role in the development of the vertebrate olfactory system. The *Pax6* gene encodes a protein that contains two conserved DNA binding domains called the *paired* domain and the *paired*-type homeodomain. In addition, it has a PST transcriptional activation domain located at the carboxy terminus. Mice and humans with homozygous *Pax6* mutations in either the *paired* box or the transcriptional activation domain have severe developmental defects in the eye, nose, and brain. Two different *Pax6* transcripts have been isolated from a *Xenopus* cDNA library which differ in their predicted carboxy-terminal amino acid sequence. One of these transcripts is nearly identical to the highly conserved sequence found in humans, mice and *Drosophila*. The other transcript lacks a 151 bp sequence, resulting in a novel predicted amino acid sequence at the 3′ end of the protein. PCR analysis has shown that both of these transcripts are present throughout *Xenopus* development. We have obtained an antibody, graciously provided by Dr. R. Reed, that binds specifically to the 3′ transcriptional activation domain of the mouse Pax6 protein. Therefore, this antibody only binds to the *Xenopus* form of Pax6 that contains the 3′ amino acid sequence homologous to that found in the mouse. Expression patterns not specific to either transcript have been determined through *in situ* hybridization. We have found that the Pax6 protein containing the 3′ sequence homologous to the mouse is expressed in the *Xenopus* eyes and

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brain from late embryonic stages through metamorphosis. Later stages revealed presence of the protein within the olfactory bulb in the glomerular, mitral, and granule layers, in cells lining the lateral ventricles and in the accessory olfactory bulbs. Staining was observed in the striatum, septal nucleus, amygdala, ventral thalamus, cerebellum, in cell clusters within the tegmentum and hindbrain, and surrounding the third ventricle. The staining pattern with an antibody against the conserved carboxy terminus overlapped significantly with the *in situ* hybridization expression pattern for *Pax6* transcripts. This suggests that the protein encoded by the novel *Pax6* transcript, if translated, has an expression pattern that overlaps, at least partially, with the conserved Pax6 protein.

Supported by NIDCD # DC00446.

487. Responsive breadth of rat taste cells to four basic taste stimuli

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The breadth of responsiveness of rat taste cells to four basic taste stimuli was studied using the entropy measure (H) proposed by Smith and Travers in 1979. H values range from 0.0 of narrow tuning to 1.0 of broad tuning. Based on depolarizing receptor potentials of 26 rat taste cells to the four basic taste stimuli, taste cells were classified into nine NaCl-best, four (quinine–HCl) Q–HCl-best, 10 HCl-best and three sucrose-best cells. NaCl-best cells were narrowly tuned to the four basic taste stimuli, but the other three stimuli-best cells were broadly tuned to the stimuli. 85% of the taste cells responded to more than one of four basic taste stimuli. The mean H values for NaCl-best, Q–HCl-best, HCl-best and sucrose-best cells were 0.285, 0.832, 0.781 and 0.796 respectively. The mean H value for all 26 taste cells was 0.621. This was larger than H in rat gustatory fibers. Transformation of large H values in taste cells into small H values in taste fibers may be due to a non-random interaction between taste cells and taste fibers during the synaptic formation. Broad tuning properties of rat taste cells suggest that the across-taste cell response pattern may play an important role in taste quality coding mechanisms in rats.

Supported in part by an NSF Grant in USA and a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture in Japan.

488. Tuning specificity of mouse olfactory receptor neurons responsive to *n*-aliphatic odorants

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We previously showed that the sensitivity of the individual olfactory receptor neurons (ORNs) is maximal for one or two odorants with similar carbon chain lengths in two homologous series of *n*-fatty acids (nFA) and *n*-aliphatic alcohol (nAA). This result suggested that the odorant receptor sites were finely tuned to the stereochemical structures of odorants. In the current study, the odorant tuning specificity of ORNs was investigated further with seven homologous series of n-aliphatic odorants with a 7–9 carbon chain respectively; nFA, nAA, *n*-aliphatic amine (nAM), *n*-bromoaliphatic carboxyl acid (nBACA), *n*-aliphatic dicarboxyl acid (nADCA), perfluorinated i-fatty acid (PnFA), *n*-bromoalkyl (nBA). Twenty-seven ORNs were responsive to some odorants in the panel. 85% of them were responsive to nFA, 74% to nBACA, 59% to nAA, 19% to PnFA, 11% to nAM and 11% to nADCA. By comparing sensitivities to odorants, the contribution of the functional group on the receptor–ligand interaction was relatively estimated. In most of receptor neurons (proteins) sensitive to nFA, the replacement of an methyl group of nFA by Br made the sensitivity decrease and the replacement by a carboxyl group was more remarkable. In most case the replacement of a carboxyl group of nFA reduced the sensitivity where the effects were Br > amine > hydroxyl group. All of ORNs responsive to nFA had smaller responses to PnFA than nFA. These results indicate the stereochemical structure such as the hydrocarbon chain and the functional groups including methyl group contributes to activate receptors.

489. Electrophysiological studies of olfactory discrimination of salmonid fishes, lacustrine sockeye, *Oncorhyncus nerka*, and masu salmon, *O. masou*, to various natural water

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It is generally considered that olfactory systems play a major role in mature salmon when they search the home stream for spawning. In this study, we measured salmon olfactory neural responses to various natural water, and examine the ability of olfactory discrimination in river odor differences. The results obtained in this study are summarized as follows. (i) In both lacustrine sockeye and masu salmon, each river water elicited olfactory responses in different magnitudes. The cross-adaptation experiments showed that there were no pair of river waters which the secondary response was completely abolished. The minimum concentration of the river water to induce the response to after adaptation to lake water was between 0.1 and 1.0%. (ii) There was no significant difference of the response profile between male and female, and species as well. Moreover, seasonal changes of magnitudes of responses were not observed. (iii) In sockeye salmon reared at Toya Lake Station showed less olfactory sensitivity to river water than the fish caught in L. Shikotsu. (iv) Nonvolatile and heat-labile componetnts of the river water elicited large olfactory responses, whereas, volatile components had no efficacy of odor for salmon olfactory systems. These results indicated supported partially the olfactory hypothesis on salmon homing migration.

490. Major urinary proteins of the 'strange male' house mouse (*Mus musculus*) confer the specificity required to induce pregnancy block

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The pregnancy of a female mouse (*Mus musculus*) is blocked by olfactory cues contained in the urine from a strange male but not in the urine from the stud male. We investigated the role of Major

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Urinary Proteins (MUPs) in determining these urinary cues by exposing pregnant female mice to one of the following: (A) urine from strange males; (B) chemically purified MUPs derived from strange male urine, with their ligands attached or (C) chemically purified MUPs derived from strange male urine, with their ligands removed via chemical extraction; (D) urine from stud males to which had been added chemically purified MUPs from strange males, with their ligands removed; (E) urine from stud males or (F) chemically purified MUPs from stud males with their ligands attached. Statistical analysis revealed that the exposure of pregnant females to (A) urine from strange males, (B) strange MUPs either with or (C) without the ligand attached or (D) combined with stud urine blocked significantly more pregnancies than exposure of female mice to (E) stud urine or (F) MUPs and ligand extracted from the urine of stud males. These results indicate not only that (i) the MUPs of male mice demonstrate pheromonal activity but also that (ii) the MUPs of strange males confer the specificity required to induce pregnancy block in females.

491. Effect of psychotropic drugs on a range of taste responses in young and elderly persons

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Epidemiological studies indicate that the mean number of medications used by community-dwelling elderly over the age of 65 ranges from 2.9 to 3.7 medications, and this number increases significantly for elderly living in retirement and nursing homes. Over 250 commonly used drugs, including most psychotropic medications, have been reported clinically to affect the sense of taste. While clinical observations are helpful in determining potential chemosensory side effects of drugs, quantitative experimental studies are necessary to obtain scientifically accurate data on chemosensory changes due to pharmaceutical compounds. The purpose of this study was to investigate the effect of six psychotropic drugs (amitriptyline HCl, clomipramine HCl, trifluoperazine, desipramine HCl, imipramine HCl and doxepin HCl) on taste sensitivity. Detection and recognition thresholds were obtained for young and elderly subjects for each psychotropic drug using liquid drops and drug impregnated filter paper. Thresholds applied as a liquid tended to be lower than those applied on filter paper. For example, the threshold for amitriptyline HCl in water delivered as drops was 0.155 mM; on filter paper half tongues, the threshold was 0.216 mM. In addition, the effect of each psychotropic drug on nine different tastants (NaCl, $CaCl_2$, $FeSO_4$, sucrose, citric acid, quinine–HCl, KCl, capsaicin and WS-3) was determined. The nine tastants were evaluated using 23 taste descriptor scales before and after application of a psychotropic drug. Each drug was found to have a bitter taste of its own as well as other unique taste properties such as metallic. In addition, each drug differentially modified the taste of each of the 9 different taste stimuli which may explain complaints of dysgeusia to these drugs.

492. The effects of temperature and pH on sweetness intensity ratings of 15 sweeteners

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The purpose of the present study was to determine the effects of temperature (48–50 and 4–6•C) and pH (pH 3.0, 4.0, 5.0, 6.0, 7.0) on the sweetness intensity ratings of 15 sweeteners. A trained panel evaluated the following 15 sweeteners classified as follows: three sugars (fructose, glucose, sucrose), two polyhydric alcohols (mannitol, sorbitol), three terpenoid glycosides (monoammonium glycyrrhizinate, rebaudioside-A, stevioside), two dipeptide derivatives (alitame, aspartame), one sulfamate (sodium cyclamate), one protein (thaumatin), two *N*-sulfonylamides (acesulfame-K, sodium saccharin) and one dihydrochalcone (neohesperidin dihydrochalcone). Four levels of each sweetener were tested that were isointense with 2.5, 5, 7.5 and 10% sucrose, according to formulae developed by DuBois *et al.* (1991). However, neohesperidin dihydrochalcone, sodium saccharin and the three terpenoid glycosides do not reach the sweetness intensity levels achieved by 10% sucrose, and were therefore not tested at that level. In addition, monoammonium glycyrrhizinate does not reach the 7.5% sucrose intensity level, and so was not tested at this level as well. Manipulation of pH levels had minor effects on sweetness intensity levels for most of the fifteen sweetener compounds in this study. Warm temperatures tended to enhance sweetness intensity ratings for many sweeteners at the two lower concentrations. The effect of cold temperatures was dependent on the intensity and the structure of the sweeteners. Sweeteners with delayed onset of maximum sweetness intensity (monoammonium glycyrrhizinate, neohesperidin dihydrochalcone, rebaudioside-A, stevioside and thaumatin) were almost always enhanced across all concentrations at both temperatures.

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493. Measurement of odor emissions from swine operations: psychophysical and gas chromatographic data

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Large confined hog operations have produced an increase in the number of complaints to state and county health boards regarding odors. Odorous compounds can be released from hog houses and from the lagoon water. The primary objective of this work was to identify the chemical compounds responsible for the odors associated with hog farming activities and to measure the concentrations and perceived intensity of these compounds in air. Identification of the chemical compounds associated with hog farming was performed using gas chromatography and mass spectrometry (GC/MS). These data were compared with previous data from other states in the US and other countries. Over 400 compounds were found in the air, manure, and lagoons on hog farms. These compounds include acids, alcohols, aldehydes,

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amides, amines, aromatics, esters, ethers, inorganic gases, hydrocarbons, halogenated hydrocarbons, ketones, nitrites, nitrogen heterocycles, phenols, sulfides, mercaptans and steroids. The presence of many sulfur-containing compounds was shown using GC/FPD (flame photometric detection) although most of those compounds were not detected by GC/MS and were not identified. The concentrations of the compounds in air on North Carolina swine operations were estimated to be in the mid to sub-parts per billion range. More VOCs were found in North Carolina than have been reported elsewhere. Sensory evaluation was also performed by a human odor panel for air samples obtained at various locations at distances up to 1500 ft from confinement buildings and lagoons. The air samples were obtained in Tedlar® bags using a pump apparatus. The odor of air samples was evaluated on four dimensions (thresholds, intensity of odor and irritation, quality, and hedonics). Thresholds and odor character were dependent on the speed at which the odorant was delivered to the nose.

494. Effect of psychotropic drugs on electrophysiological taste responses in gerbil

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Integrated chorda tympani (CT) recordings were made before and after 5 min applications of six different psychotropic drugs to determine their effect on taste. The effects of these drugs on nineteen taste solutions with salty, sweet, sour and bitter tastes were examined. The compounds with salty or sour components were: NaCl (30 and 100 mM), KCl (500 mM), monosodium glutamate (MSG; 50 and 100 mM), HCl (5 and 10 mM) and citric acid (80 mM). The sweet compounds tested were: sucrose (30 and 100 mM), glucose (300 mM), fructose (300 mM), maltitol (300 mM), dulcin (3.5 mM) and sodium saccharin (10 mM). The compounds with a bitter component were: $CaCl_2$ (300 mM), $MgCl_2$ (100 mM), quinine–HCl (Q-HCl; 30 mM) and urea (2 M). All solutions were tested at room temperature. The six psychotropic drugs tested were: amitriptyline hydrochloride, clomipramine hydrochloride, desipramine hydrochloride, doxepin hydrochloride, fluoxetine hydrochloride and imipramine hydrochloride. The main findings of this study were that 1 and 5 mM amitriptyline blocked responses to most of the taste compounds tested. 1 mM amitriptyline numerically blocked responses to all compounds tested except for 10 mM sodium saccharin. The greatest effect was seen for 30 mM Q-HCl with a reduction in response of 52.2%. 5 mM amitriptyline significantly blocked responses to all compounds except 300 mM glucose, 300 mM fructose and 30 mM maltitol. Taste responses were reduced in a range from 24.6 to 92.3%. 1 mM clomipramine HCl significantly blocked responses to: 300 mM fructose (22.3%), 3.5 mM dulcin (27.9%) and 30 mM Q-HCl (50.6%). 5 mM clomipramine HCl had a greater effect on taste responses by significantly blocking responses to nine taste solutions. Responses were reduced in a range from 36.1 to 92.7%. 1 mM and 5 mM desipramine HCl numerically blocked all taste responses with the greatest effects seen on 30 mM QHCl (65.4%), 5 mM HCl (64.6%) and 10 mM HCl (66.3%). Doxepin HCl had the greatest effect at the 5 mM concentration by reducing the responses of 30 mM QHCl (75.6%), 5 mM HCl (66.2%) and 10 mM HCl (66.9%). When 1 mM fluoxetine HCl was applied to the tongue, two taste responses were enhanced: 30 mM NaCl (37.1%) and 5 mM HCl (18.2%). 5 mM fluoxetine HCl significantly suppressed 14 of the taste responses. 1 mM and 5 mM imipramine HCl had significant effects on many of the taste responses. 5 mM imipramine HCl significantly reduced all taste responses except for 50 and 100 mM MSG. Taste responses were significantly reduced in a range from 27.9 to 71.9%.

495. Introducing CAP: a statistical system for the control of assessor performances

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Descriptive analysis with trained panel of assessors is increasingly used for many different purposes such as basic research in psychophysics, quality control, formulation of new products and consumer research. In any case, the sensory data produced must be reliable and valid as any scientific measure should be. Although the reliability and the validity of the data is a matter of correctly training the assessors, assessing the level of quality achieved by these data can only be done using statistics. The statistical litterature is usually confusing for the panel leader. Further, there are no guidelines or standard methods for assessing judge performances. Even the definition of the different performances is not clear enough. The paper will summarize previous and current works of the author in that topic. It will first define a number of performances worth to control and then propose the CAP (control of assessor performances) system to the panel leader, as a general strategy for checking these performances.

CAP not only clarifies how to use simple techniques such as the analysis of variance in the context of evaluating assessor performances, but it also features a number of statistical techniques very rarely used in the sensory field, such as the tests of contrasts in analysis of variance, the RV coefficient and its analytical permutation tests, the multivariate analysis of variance, the canonical discriminant analysis and the canonical correlation analysis. CAP acknowledges whether the sensory attributes should or should not be considered as similar between assessors (conventional versus free-choice profiling). CAP can deal with an unbalanced design where each assessor has not scored each product equally often. CAP offers two way of treating the replicates, depending whether they need to be seen as a session effect or not. Last but not least, the implementation of the CAP method into the SAS® software, automatizes all the computations and summarizes their results into a few neat tables, from which the screening of the assessor performances by the panel leader becomes a pleasure.

496. Olfactory function and quality of life following endoscopic sinus surgery in HIV disease

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Previous studies have indicated a decreased sensitivity in olfactory function among individuals with human immunodeficiency virus

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(HIV). Little information is available concerning the surgical treatment of sinusitis among these individuals, despite a demonstrated incidence rate of 16–68%. This study examined the benefit of endoscopic sinus surgery (ESS) for HIV^+ patients diagnosed with chronic sinusitis. The study also evaluated olfactory function in three groups: HIV^+ patients presenting to the UCSD Nasal Dysfunction Clinic with nasal or sinus complaints (pre- and post-ESS), HIV^+ patients presenting to the UCSD Owen Clinic without nasal or sinus complaints, and HIV^- controls. All participants underwent ENT examinations, including nasal endoscopy. Olfactory sensitivity was assessed using an ascending threshold method with *n*-butyl alcohol. Patients receiving ESS as treatment were also evaluated for disease-specific symptoms as well as with a general quality of life assessment before and after surgery. Results of odor threshold testing showed significant differences (on ANOVA) among the three groups, such that HIV^+ individuals with nasal or sinus complaints scored poorer than HIV^+ individuals without nasal or sinus complaints, and both HIV^+ groups scored poorer than the HIV^- control group, $P < 0.05$. Significant olfactory sensitivity loss persisted for ESS patients after surgery, suggesting that the impairment in these patients may be due to viral disease rather than to inflammation. Significant improvement in other disease-specific symptom scores (nasal congestion, obstruction, headache, sleep disturbance, etc.) and results of the general quality of well-being assessment showed ESS to be beneficial in the extended health management of HIV.

Supported by NIH grant # AG04085.

497. Functional innervation of dissociated olfactory bulb neurons by co-cultured explants from the olfactory epithelium

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Olfactory sensory neurons project to the olfactory bulb and form synaptic contacts on mitral/tufted cells. The electrical and chemical properties of these synapses are poorly investigated, since mostly the tissue is hardly accessible for electrophysiology in preparations *in vivo*. Therefore we established a co-culture system that allows to study synaptic interactions between the olfactory epithelium and olfactory bulb neurons using the patch-clamp technique.

Olfactory epithelium and olfactory bulb were obtained from embryonic rats (embryonic day 20). Dissociated olfactory bulb neurons were plated into culture dishes together with 3–5 explants of olfactory epithelium. During cultivation, fibers growing out of the explants innervated the dissociated bulb cells within 4–7 days.

In bulb cultures interneurons and mitral/tufted cells can be distinguished by morphological and electrophysiological characteristics. After 7–10 days *in vitro*, olfactory bulb neurons formed small aggregates containing in the center a mitral cell, which was surrounded by several interneurons. These aggregates may represent functional units. Similar structures are known from anatomical studies in the olfactory bulb. The aggregates were strongly innervated by bundles of nerve fibers originating from the epithelium explants.

The electrophysiological characterization of the bulb cells was performed using the whole-cell patch-clamp technique. Under asymmetrical Cl-concentrations, excitatory postsynaptic currents (EPSCs) were recorded at a holding potential of –60 mV and inhibitory postsynaptic currents (IPSCs) at –20 mV. Interneurons exhibited spontaneous, glutamatergic EPSCs, while mitral cells showed additionally spontaneous GABAergic IPSCs, suggesting dendrodentritic synaptic connections with surrounding interneurons.

To demonstrate a functional synaptic connection extracellular electrical stimulation of an explant was installed and EPSCs were recorded in the mitral cells located closely to the explant. Upon stimulation bursts of EPSCs were evoked in the mitral cells indicating a functional synaptic projection from olfactory sensory neurons within the epithelium explant to the bulb-neuron.

In summary we have developed a co-culture system that enabled us to study synaptic interactions between olfactory sensory neurons and mitral cells. This system may be useful to examine odorant responses of sensory neurons by recording synaptic currents in the dissociated mitral cells easily accessible for patch clamping.

498. Neurogenesis in the central olfactory pathway of adult decapod crustaceans

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The olfactory pathway of adult decapod crustaceans—like that of adult vertebrates—shows an unusual structural plasticity. Recently turnover of olfactory receptor neurons coupled to molting was demonstrated in adult crayfish (Sandeman and Sandeman, 1996). In adult shore crabs, *Carcinus maenas*, a continuous increase in the number of olfactory projection neurons was shown as well as mitotic activity in their soma cluster (lateral cluster = LC) and in the soma cluster (HBC) of local interneurons of the secondary olfactory neuropils located in the eyestalks (Schmidt, 1997).

In the current study we used *in vivo* labeling with bromodeoxyuridine (BrdU) in combination with neurobiotin-backfills of the olfactory projection neurons to establish if the newly formed cells in the LC of *Carcinus* obtain neuronal characteristics, i.e. grow an axon. Only with survival times of at least 3 weeks between BrdU-injection and the backfills we observed somata in the LC that were double labeled by neurobiotin and BrdU. This clearly demonstrates that newly formed cells in the LC of adult shore crabs become olfactory projection neurons and suggests that this process takes ~3 weeks.

With *in vivo* BrdU-labeling we studied mitotic activity in the central olfactory pathway of other decapods. In adult crayfish (*Cherax destructor*) and adult spiny lobsters (*Panulirus argus*) a large group of nuclei in the LC was labeled in a location equivalent to the proliferation zone of the *Carcinus* LC. Both species lacked BrdU-labeling in somata of the HBC, but in *Panulirus* a distinct group of nuclei in the medial cluster (MC) containing the somata of local interneurons of the olfactory deutocerebrum was labeled. These findings suggest that in adult decapods neurogenesis of olfactory projection neurons is a common feature, whereas other neuron types of the central olfactory pathway are generated in a species specific manner.

This research was support by DFG grant Schm 738/4-1.

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499. Olfactory and chemo-somatosensory event related potentials (OERP and CSSERP) in clinical practice

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Purpose: to evaluate the feasibility and usefulness of OERP and CSSERP in routine work-up of patients with olfactory disorders. Method: a continuous flow olfactometer was used; flow was 8 l/min; stimulus duration was 200 ms; 15–20 unilateral stimuli (H_2S for OERP and CO_2 for CSSERP) were presented with an interstimulus interval of 30–40s. Results: (i) 54 normal volunteers (aged 20–81 years) were tested using H_2S at 55% and CO_2 at 45%. CSSERP were obtained in 41, OERP in 43 volunteers. Discussion and analysis of these results showed that the concentration of CO_2 had to be increased to 55–66% in order to guarantee adequate trigeminal stimulation, whereas the concentration of H_2S had to be lowered to 10% in order to prevent trigeminal irritation and contamination of OERP with CSSERP. Out of nine normal volunteers tested with H_2S at 10% and CO_2 at 55–66% eight had CSSERP and five had OERP. (ii) From June 1995 to January 1997 35 patients (aged 16–70 years) with olfactory disorders (all confirmed by psychometric testing using the Erlangen Sniffin' Sticks Test Battery) were examined at the ENT Department of the University of Basel. Of the 35 patients 29 had CSSERP but no OERP, a finding that was considered compatible with the clinical diagnosis of anosmia or severe hyposmia. In one patient the presence of OERP and CSSERP was considered proof that at least some olfactory function was preserved. In five patients neither OERP nor CSSERP could be recorded. Conclusions: (i) The recording of OERP and CSSERP is feasible under routine clinical conditions. (ii) The presence of OERP helps to rule out anosmia or severe hyposmia. (iii) The absence of OERP is compatible with but does not prove the diagnosis of anosmia.

500. Olfactory system development in Pax-3 mutant mice

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Transcriptional regulation is an important mechanism utilized during embryonic development to direct morphogenesis. Pax-3 is a paired domain transcription factor critical for the proper development of a number of cell types, particularly neural crest derived cells. Mutations in Pax-3 are responsible for Waardenburg Syndrome in humans and the Splotch phenotype in mice. Sensorineural deafness, hypoplasia of limb muscles, and malformation of facial bones can be attributed to defects in neural crest cell migration in Waardenburg Syndrome. Recent studies have suggested that the protooncogene *c-met* is also required for limb muscle development. Furthermore, *c-met* encodes a member of the receptor tyrosine kinase family that is a receptor for hepatocyte growth factor/scatter factor (HGF/SF). Homozygous *c-met* knockout mice have no limb muscles much like Pax-3 mutants. A potential Pax-3 binding site has been identified in the human c-*MET* promoter and it has been suggested that Pax-3 modulates *c-met* expression. Both Pax-3 and *c-met* are expressed in the olfactory epithelium of mice during early stages of development, E11–15. During this time period we examined the migration of olfactory neurons as well as luteinizing hormone-releasing hormone (LHRH) neurons from the vomeronasal organ and olfactory epithelium to the forebrain. In Splotch-delayed mice, the migration of LHRH neurons across the nasal septum is relatively normal, but as they enter the forebrain, their migration trajectories and positions are severely perturbed. Many LHRH neurons are found in extreme caudal and dorsal positions in the forebrain of Splotch mice compared with control littermates. This may be partially due to the fact that the olfactory bulbs and forebrain are severely malformed in homozygous Splotch-delayed mice. These studies suggest an important role for Pax-3 during critical stages of olfactory system development

Supported by DC00953 from NIDCD.

501. Coding of bitter stimuli in the cortex of the macaque

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Neurons in macaque taste cortex have response thresholds and intensity–response functions similar to those reported by humans in psychophysical studies. The representation of taste quality is broadly similar as well. Thus we can proceed to investigate fine details of gustatory coding in the domain of each basic taste quality. Such studies have been completed for qualities of sweet, salt and sour. This study uses stimuli that humans describe as predominantly bitter. We recorded the activity of 50 taste neurons in insular cortex of two cynomolgus macaques. Stimuli were water, fruit juice, glucose, NaCl, HCl and 16 bitter solutions. Mean spontaneous rate was 3.2 s/s; mean breadth-of-tuning was 0.77. Eighty-two percent of the cells could be categorized into one of four subgroups: glucose (44%), NaCl (14%), HCl (14%) or quinine–HCl (10%). In a multidimensional representation of stimulus similarity, glucose was closely related to fruit juice, and HCl to water. NaCl was isolated from all others. The foci of this study, the 16 bitter stimuli, formed a coherent cluster composed of two main subgroups: (i) QHCl, PHE, THR, caffeine, PROP and PTC, and (ii) $MgCl_2$, $CaCl_2$, NH_4Cl, ARG, CYS, HIS, nicotine, and vitamins B_1 and B_2. Urea, reported by humans to have a sour component, was on the fringe of the bitter group edging toward HCl. We know of no comprehensive study on the taste qualities of bitter stimuli in humans to which these data may be compared. However, several of the same stimuli were applied during semantic differential and adaptation experiments with humans. Where descriptions of taste qualities are available or can be inferred from adaptation, they conform well with the presumed quality in the macaque based on the position of each stimulus in the MDS.

Supported by a research grant IBN-9120611 from the NSF.

502. The diagnosis of conductive olfactory loss

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It is well recognized that among the most common causes of olfactory loss are a viral respiratory infection, and nasal or sinus pathology. In the latter instance the loss is conductive and therefore amenable to therapeutic intervention. It is thus

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important that such a diagnosis not be missed. However, it is not clear that patients who present primarily because of an olfactory deficit, though it be conductive, will necessarily display the typical diagnostic features of inflammatory disease. As a result, the etiology may easily be mistaken to be post-viral or idiopathic.

The present series reviews 53 consecutive patients diagnosed with conductive olfactory loss presenting to the University of Cincinnati Taste and Smell Center between 1987 and 1995. Of these 53 patients, 57% were found to be anosmic and 40% hyposmic, with 34% complaining of dysosmia. Only 32% complained of nasal obstruction, while 59% described a history of chronic sinusitis and/or IgE mediated allergy. Only 45% admitted that their olfactory loss at times seemed to fluctuate in severity. Anterior rhinoscopy failed to diagnose pathology in 48% of cases, whereas nasal endoscopy missed the diagnosis in only 8%. Systemic steroids elicited a temporary reversal of conductive olfactory loss in 84% of patients that received them, offering a useful diagnostic maneuver, whereas topical steroids did so in only 23%.

The clinical diagnosis of a conductive olfactory loss can not reliably be made by history alone, but requires a thorough physical examination that should include nasal endoscopy. The temporary administration of systemic steroids may be helpful to demonstrate that the loss is reversible.

503. Neural processing of plant-associated odors in the antennal lobe of the moth, *Manduca sexta*

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To learn about coding and processing of olfactory information in the primary olfactory center of the brain and about a possible topographical organization among glomeruli ('odotopy'), we are studying the antennal lobe (AL) of female sphinx moths, *Manduca sexta*. The cell bodies of AL neurons in this species are found in three distinct and characteristic groups. Of these, the smallest group, which is situated on the anterior surface of the AL, is the focus of this research. Neutral Red staining showed cell bodies of different sizes distributed in 1–2 clusters. Neuronal staining with DiO and glial cell staining with propidium iodide, followed by laser-scanning confocal microscopy revealed fasciculation of the primary neurites of the anterior AL neurons (ANs) as well as restriction of their arborizations to a small number of AL glomeruli. These glomeruli reside in two distinct 'domains', one posterior-medial and including several glomeruli, and the other relatively lateral and possibly consisting of a single glomerulus.

Intracellular recording from cell bodies or principal neurites of single ANs combined with intracellular staining (Biocytin or Lucifer Yellow) has confirmed that the ANs are uniglomerular projection neurons. Many exhibit regular, bursting activity. Their responses to vegetative and floral odors can be grouped into four broad categories, suggesting response selectivity of individual cells to certain odors, e.g. 'excitatory' to host-plant floral odor and 'inhibitory' to leaf odor. This screening should lead to increased understanding of the functional organization of the AL and to tests of the theory that olfactory glomeruli have odotopic significance for olfactory information processing.

Supported by NIH grant DC-02751 (to J.G.H.).

504. Up-regulation of neurotrophin receptor (p75-NTR) in olfactory bulbs of developing hypothyroid rats shown by immunocytochemical and mRNA studies

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Neurotrophins are critical in many aspects of sensory development. Research from our laboratory has shown that early thyroid deficiency profoundly retards growth and development of olfactory epithelium and bulb (OB): >50% of the olfactory receptor neurons fail to develop or mature (Paternostro and Meisami, 1994, 1996); OB size and growth is reduced together with 25% loss in number of mitral cells number; glomeruli number is unchanged but their size is markedly reduced (Sendera and Meisami, 1995, 1996). Based on the hypothesis that the neurotrophin NGF (nerve growth factor) and its receptor, the common neurotrophin receptor (p75-NTR), are involved in responses of thyroid deficient OB, we determined changes in expression of p75-NTR receptor protein and levels of its mRNA by means of immunocytochemistry and RPA (ribonuclease protection assay) respectively, in OBs of postnatal normal and hypothyroid rats. Hypothyroidism was induced by adding propylthiouracil (PTU) in the litter's drinking water (0.1% v/w) from birth. Immunocytochemistry of p75-NTR was carried out using the monoclonal antibody mAb 192-IgG (Bohringer). In the normal OB expression was patchy and limited to glomerular neuropil where expression intensity varied from none to moderate, depending on the glomeruli. Interestingly, receptor expression in the 25- and 90-day hypothyroid OB was markedly elevated (80%), as measured by intensity of immunoreactivity, compared with controls with most glomeruli showing moderate to high levels of immuno-reactivity. Quantitative determination of mRNA levels for p75-NTR in the OB tissue by the sensitive method of RPA revealed marked and highly significant increases (40–60% depending on age) in the hypothyroid rat OB compared with age-matched controls. These results suggest that the increased expression of p75-NTR receptor protein in the *hypothyroid* rat OB is mediated by corresponding increase in expression of the gene for this receptor. This receptor upregulation presents an interesting paradigm for interaction of thyroid hormones with NGF and its receptor in the olfactory system, in view of our previous finding that NGF levels are *decreased* in developing hypothyroid OB (Sendera and Meisami, 1995, 1995).

Supported by NIH grant GM07143 and UIUC Research Funds.

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505. Sensory specification of olfactory neurons in *C. elegans*

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The nematode *C. elegans* responds to many water-soluble and volatile chemicals using a few defined chemosensory neurons. Using a behavioral screen, we previously identified a gene *odr-7*,

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that is required for the sensory functions of the AWA olfactory neurons. *odr-7* encodes a member of the nuclear receptor family of transcriptional regulators, and is expressed only in the AWA neurons. Thus *odr-7* may define the sensory specificity of the AWA neurons, by regulating the expression of signalling molecules within the AWA neurons.

We have recently shown that a target of *odr-7* regulation is the olfactory receptor encoded by the *odr-10* gene. We now show that *odr-7* also regulates the expression of the *osm-9* gene, which encodes a putative calcium channel. In addition, preliminary evidence suggests that while *odr-7* is sufficient for the expression of *osm-9*, gene(s) in addition to *odr-7* may be required for the expression of *odr-10*. We are currently examining whether regulation of these genes by *odr-7* is direct or indirect.

In order to further understand the role of *odr-7*, we are taking genetic and biochemical approaches to identify additional targets of *odr-7* regulation. In behavioral screens, we have identified additional genes required for the sensory function of the AWA neurons, and which may represent targets of *odr-7* regulation. These genes are being characterized and cloned.

We are also exploring how the sensory functions of other chemosensory neurons are specified. In a forward genetic approach, we are using a combination of behavioral and visual screens to identify mutants where the sensory functions of specific olfactory neurons are affected, and where the expression of putative signalling genes in these neurons are altered. In a reverse genetic approach, we are identifying the roles of genes homologous to *odr-7* in the development and function of chemosensory neurons. We hope that these approaches will allow us to understand the mechanisms that result in the functional specification of chemosensory neuron types.

506. Expression of basic fibroblast growth factor in the taste buds of rat

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Basic fibroblast growth factor (bFGF) is widely distributed in the central nervous system, where it is involved in development, differentiation, and survival of nerve cells. Recent studies have revealed that bFGF plays an important role in development and morphogenesis of the retina and olfactory epithelium. There is far less known of the role of bFGF in the peripheral nervous system. To investigate if bFGF gene expression occurs in the epithelium of rat circumvallate papillae, we used reverse transcriptase–polymerase chain reaction (RT–PCR) and immunohistochemistry in the rat circumvallate papilla. When RT–PCR was performed using mRNA prepared from circumvallate papilla, amplification products of the expected size (405 bp) were obtained with primer sets specific for rat bFGF. BFGF immunoreactivity was confined to some of the taste bud cells, whereas none of the other papillary epithelial elements were reactive. These results suggest that, as in the retina and olfactory epithelium, bFGF has a significant influence on the life cycle of certain taste bud cells.

507. Sodium deprivation affects neuronal responses in the geniculate ganglion to an array of gustatory stimuli

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The activity of single neurons in the geniculate ganglion was recorded from anesthetized rats that had been maintained on either a sodium deficient diet (deplete, $n = 33$) or one to which 1% (w/w) NaCl had been added (replete, $n = 32$). As reported previously, across the entire sample, significant differences between the diet conditions occurred in response to citric acid ($P < 0.02$) and sucrose ($P < 0.02$), but not to NaCl or quinine. Within best-stimulus categories, the acid-best units from the depleted rats responded less to NaCl and acid ($P < 0.02$). In the deprived condition, both NaCl- and sucrose-best neurons responded less to one of the four stimuli, but the responses themselves were miniscule. The activity of these same cells also was recorded during anterior tongue stimulation with an NaCl and a sucrose concentration series (0.01, 0.03, 0.3 and 1.0 M for both), followed by a subset of other chemicals [0.3 M glycine, 0.1 M monosodium glutamate (MSG), 0.1 M polycose, 0.1 M sodium nitrate and 0.1M ammonium chloride]. Overall, concentration response functions existed for NaCl and sucrose in both diet conditions. The sucrose series produced less activity in the depleted rats ($P < 0.01$) but, once again, the average responses were very small (max. < 2.0/s). This effect could not be attributed to a particular category of neurons, because none of those comparisons was significant. In fact, only in the acid-best units did the NaCl series produce significantly lowered responses in rats fed a sodium deficient diet ($P < 0.03$). The other five stimuli all produced smaller responses in cells recorded from rats fed a sodium deplete diet but, of these differences, only glycine ($P < 0.05$) and MSG ($P < 0.03$) were significant. When considered by best-stimulus category, only acid-best units responded differentially with respect to diet, and then only for MSG ($P < 0.01$) and $NaNO_3$ ($P < 0.01$). Thus, in the present study, dietary sodium deprivation was accompanied by a trend toward reduced responsiveness to all stimuli, but the statistically significant effects were carried predominantly by acid-best neurons.

Supported by NIH DC 00240, 02016, MH 43787 and MH 00653.

508. Ultrastructure of antennal sensilla of the female sphinx moth, *Manduca sexta*

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The structure and innervation of sensilla on the antennal flagellum of the female sphinx moth, *Manduca sexta*, were examined using scanning electron, field-emission scanning electron, and transmission electron microscopy. Eight sensillar types were identified: two types of trichoid sensilla, four types of basiconic sensilla, and two types of coeloconic sensilla. The two types of trichoid sensilla consist of either a short hair averaging 26 μm long or a tall hair averaging 34 μm long. One type is innervated by two bipolar neurons, while the other is innervated by one or three bipolar neurons. These sensilla exhibit features characteristic of olfactory chemosensilla. The two types of basiconic sensilla

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consist of either a short peg averaging 15 μm long or a tall peg averaging 22 μm long. Both types are each innervated by three bipolar sensory neurons. These sensilla exhibit features of olfactory chemosensilla. The third type of basiconic sensillum consists of small spoon-shaped peg averaging 4–5 μm long and is innervated by two bipolar sensory neurons. This sensillum exhibits features of olfactory chemosensilla. The fourth type of basiconic sensillum consists of a tall and stubby peg averaging 38–40 μm long. Each peg is composed of several confluent sensilla, the number of which varies depending on the location of the peg on the flagellum. Each of these sensilla is innervated by three bipolar neurons and exhibit features of thermo-hygrosensilla. Both types of coeloconic sensilla consist of a small peg averaging 2 μm long, recessed in a cuticular pit. One type is innervated by five bipolar sensory neurons and exhibits features of olfactory/thermosensilla, while the other is innervated by three bipolar sensory neurons and exhibits features of thermo/hygrosensilla. A thorough count of sensilla on the dorsal, ventral, and leading edge surfaces of an annulus, situated about midway along the flagellum, revealed a total of 2216 sensilla supplied by ~5330 sensory neurons. A female antenna with ~85–90 annuli) provides the flagellar nerve with at least 4×10^5 receptor axons.

Supported by NIH DC-02751.

509. Spatial discrimination of NaCl and citric acid tastes on the different sides of anterior tongue

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We have found that taste cues can be lateralized on gustatory epithelium in the absence of discriminatory tactile cues and that the detection of taste is required for the localization of taste to occur. Discriminations could be based upon: (i) taste intensity cues, (ii) qualitative taste cues or (iii) a combination of both. In order to determine whether the taste localization phenomenon depends on the discrimination of quality, intensity, or some combination of the two, we designed an experiment that could test subjects' ability to lateralize two taste stimuli presented on different sides of the anterior tongue. Subjects always received 0.1 M NaCl on either the left or right of the tip of the tongue while various concentrations (1–32 mM) of citric acid were placed simultaneously on the other side and subjects were requested to answer on which side they felt a salty taste. NaCl was first repeatedly presented alone on one side of the tongue to give the subjects a lateralized, salty search-image. Subjects determined which side was NaCl-stimulated as a U-shaped function of the concentration of citric acid presented at the other side with its minima where the perceived intensity of citric acid was equivalent to that of NaCl. Some subjects could not identify the taste stimuli significantly better than chance at this point. Although all subjects' functions initially dipped when discriminating between NaCl and citric acid, few subjects' minima fell to chance performance. These results suggest that the localization of taste sensation is primarily based on the taste intensity cues, but taste quality information is also sufficient to localize taste cues.

Paul Breslin is a Morley R. Kare Fellow. This work was also funded by NIH grant # R29 DC02995 (P.A.S.B.).

510. Electrophysiological characterization of rat main olfactory bulb (MOB) glomerular layer (GL) cell populations

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Olfactory nerve (ON) terminals interact synaptically with local circuit and projection neurons within the GL of the MOB. The GL contains distinct populations of interneurons and association cells, including periglomerular (PG) cells, short axon (SA) cells and various tufted cell types, collectively termed juxtaglomerular (JG) cells. The electrophysiology of JG cells is obscure, and their specific responses to ON input unknown. We investigate the membrane properties, electrophysiological response to ON input, and morphology of JG neurons, in an *in vitro* MOB slice preparation.

JG cells (n = 30) can be categorized by their extracellular response to ON stimulation. Type I cells (n = 17) generate spontaneous activity at 1–5 Hz, responding to ON stimulation with short-latency excitation, followed by inhibition. Inhibition duration is related to stimulation intensity, but baseline activity is independent of ON stimulation. Type II cells (n = 7) spontaneously generate high-frequency bursts of activity, and respond to ON with inhibition lasting up to 800 ms. Baseline activity is otherwise unaffected by ON stimulation. Type III cells (n = 6) have low spontaneous rates of activity (0.1–0.5 Hz). ON stimulation arrests their activity for 200–400 ms, followed by excitation lasting up to 50 s. The excitatory effect of individual stimuli may be cumulative, if time to recovery exceeds interstimulus interval, but activity is always suppressed for 200–400 ms following each stimulus. The initial inhibition of Type I and Type II cells is synchronous with excitation recorded simultaneously from mitral cells with a second patch pipette. The spatial distributions of the three cell types differ. Type I cells occur all around the glomeruli, Types II and III predominate in the superficial GL. Intracellular labeling with biotin showed distinctive profiles of PG and SA cells. Our immediate goal is to determine how the JG response types identified here correspond to the distinct anatomical forms of JG cell.

Supported by DC29218, DC00347 and NS29218.

511. Olfactory responses of masu salmon (*Oncorhynchus masou*) to stream waters: electrophysiological evaluation of amino acids and bile acids as olfactory cue for homing migration in salmonids

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In salmonid fishes, many behavioral studies have shown that olfactory systems play an important role in searching the home stream in the final stage of homing migration. However, the home stream odors recognized by salmon olfactory systems have not yet been characterized. To characterize the home stream odors, we determined the content of main cations, various amino acids and

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bile acids in the stream water by HPLC and ICP-AES, which are potent odorants for fish. Analyses of amino acid and bile acid in stream waters showed that each natural water contained different species and concentrations of amino acids and bile acids. The stream waters examined contained a large variety of amino acids, whereas they contained one to seven cholic acid derivatives. In addition, we recorded olfactory neural responses of masu salmon to natural stream waters and the artificial stream waters containing amino acids, bile acids and salts with the concentrations determined by the analyses. Natural stream waters elicited large olfactory responses. The cross-adaptation experiments demonstrated that odor of each stream water was discriminated by the olfactory systems. As with the natural stream waters, the artificial stream waters containing amino acids, bile acids and salts elicited large olfactory responses. The cross-adaptation experiments showed that each artificial stream water is well discriminated. The olfactory systems did not discriminate between the artificial stream waters constituted of only salts. The present results suggest that amino acids and bile acids are major components of the home stream water odors for being discriminated by salmon olfactory systems.

512. Nasal irritant provocation using dilute chlorine gas: pilot testing of an exposure system

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Nasal irritation (and related physiologic reflexes) are an important component of so-called 'sick building syndrome'. As a consequence, any variation in nasal irritant sensitivity within the population would be of interest to clinicians and environmental scientists. Based upon published observations, we hypothesized that subjects with a history of seasonal allergic rhinitis (SAR) are more sensitive (i.e. have greater perceptual acuity *and* physiologic reactivity) to airborne chemical irritants than non-rhinitic subjects. As part of pilot testing, six subjects (two with and four without a history of rhinitis) were studied in a climate-controlled chamber with set-points of 22 ± 1°C and 40 ± 3% relative humidity. The test irritant (Cl_2) was presented using a subsystem within the chamber, consisting of a nasal mask with a scavenger system for removal of exhaled gases. We describe pilot data comparing the effect of 15 min exposures to chlorine (at 0.5 and 1.0 ± 0.04 ppm) versus air on measured nasal airway resistance (NAR). In pilot tests, SAR subjects—but not nonrhinitics—showed a trend toward increased NAR after 0.5 ppm chlorine exposure (NS). At 1.0 ppm, on the other hand, the response of the two subgroups to chlorine was indistinguishable, with the pooled group showing a significant NAR response to chlorine versus air measured 15 min post-exposure ($P < 0.05$). These reflex changes occurred with mean nasal irritation ratings of 0.24 (at 0.5 ppm) and 1.75 (at 1.0 ppm) on a 0–5 visual analog scale with verbal descriptors. No inter-group differences were apparent in subjective nasal irritation. The above results were consistent with the *a priori* hypothesis that SAR subjects will react *physiologically* at a lower minimum exposure level than will non-rhinitics. Based upon these preliminary results, an exposure level of 0.5 ppm chlorine was chosen for an ongoing comparison of the nasal irritant response of SAR and non-rhinitic subjects.

Supported by NIDCD 1 K08 DC00121-01A1.

513. Generation of neurons from a non-neuronal precursor in adult rodent olfactory epithelium *in vitro*

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Within the olfactory epithelium is a stem cell which can divide and differentiate to produce new sensory neurons. The identity of the neuronal stem cell is unknown but one candidate is the horizontal basal cell which lies adjacent to the basement membrane and expresses keratin. Previous attempts to generate mature sensory neurons from purified horizontal basal cells *in vitro* were unsuccessful. We show here for the first time that olfactory neurogenesis can be reproduced *in vitro* from partially-purified cultures of adult rat precursor cells cultivated in a serum-free medium. Rat olfactory epithelium was dissected from the nasal septum, separated from the underlying lamina propria, and its cells were dissociated and grown in a medium containing epidermal growth factor for 5 days. Immunochemistry showed that only supporting cells (SUS1-positive) and horizontal basal cells (keratin-positive) survived for this period. At day 6, the cells were stressed either by passaging them or by a simple mechanical stress. In each case, a morphological and immunological differ- entiation was observed within 24 h to 48 h. Newly formed bipolar cells were found to be $S100^-$, $GFAP^-$, $N\text{-}CAM^+$ and/or $MAP5^+$. After passaging 14% of the surviving cells were immature neurons ($MAP5^+$) and 4% were mature olfactory neurons ($MAP5^+$ and OMP^+). In addition the same experiment was conducted on transgenic mice in which the *lacZ* gene was linked to the OMP promoter. Using X-Gal staining we showed that OMP^+ cells disappeared before Day 5 in culture but reappeared after passaging. These results suggest that olfactory sensory neurons can arise from a non-neuronal precursor, probably the keratin-positive horizontal basal cell.

Supported by the CNRS, Marcel Mérieux Foundation, NHMRC and the Garnett Passe and Rodney Williams Foundation.

514. Does arabinose alter in taste during mutarotation?

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Previous work with equilibrium solutions of D- and L-arabinose indicates that the D- and L-enantiomers are identical in taste and solution properties, as also is the D,L-mixture.

In the crystalline state, arabinose exists as the β anomer. Since the β anomer of arabinose possesses two axial and two equatorial hydroxyl groups, it is likely to exist in both the $^4C_1$ and $_4C^1$ conformations. On mutarotation, the β tautomer changes to an equilibrium mixture of predominantly a arabinose. Mutarotation of arabinose therefore involves conformational as well as configurational changes, which means that major differences will occur over time in the shape and orientation of the sugar as it stimulates the receptor. Though the apparent molar volume remains uniform, at 93.2 cm^3/mol for a 10% solution, there is a noticeable decrease in isentropic apparent molar compressibility

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during mutarotation, from −1.632 to −1.691 cm^3/mol.bar. This highlights the increased sensitivity of compressibility as a measure of solute structure effects on the disturbance of water. Again, identical results are found for each enantiomer.

Sensory analysis of 0.7 M solutions of both enantiomers shows no significant change in taste on mutarotation. The structural interpretation of taste therefore remains unresolved.

This research was supported by EC grant PL-94-2107.

515. Molecular basis for an odor map: rt–pcr of olfactory receptors from the olfactory bulb glomerular layer

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Several approaches, including molecular and activity markers, have provided an outline of the how odors are represented in the olfactory bulb (OB). Recent *in situ* hybridization studies have provided evidence that olfactory receptor (OR) mRNA is present in the OB. The studies mapped a small number of previously cloned ORs to punctate sites in the OB believed to correspond to individual glomeruli. To gain insight into the odor map, it is desirable to be able to sequence ORs from chosen locations in the OB and assess relations between ORs from nearby and distant glomeruli. We therefore developed methods to remove small glomerular samples from identified locations in the rat OB and to sequence ORs by RT-PCR. Partial sequences (transmembrane domains 2–7) of 20 novel OR subtypes were obtained from five samples. We tested three hypotheses: (1) that the cloned ORs would resemble previously reported rat ORs; (2) that nearby glomeruli would yield relatively similar sequences; and (3) that distant glomeruli would yield distinct sequences. Comparison of the novel and previously cloned ORs clearly supported Hypothesis (1), providing the first direct evidence for OR mRNA in the OB and making it possible to map these ORs to specific locations in the OB. Support for Hypothesis (2) was seen in one sample, where 4 clones showed 82.7% similarity, compared with the mean between-sample similarity of 46.3% ($P < 0.0001$). However, other samples showed more complex distributions, including distinct clones within the same sample and similar clones in different samples. With regard to Hypothesis (3), the sequence relations between distant sites are obviously complex. Future studies can employ the new methods to sample and clone ORs across the glomerular sheet.

Supported by grants from NIDCD and NINDS (C.A.G.); grants from NIDCD, NIMH, NASA and NIDCD (Human Brain Project; G.M.S.); grants from NEI (T.E.H.); and fellowships from NLM-IAIMS and the Yale University MSTP (M.S.S.).

516. G_i-protein is involved in odorant-induced inhibition of adenylyl cyclase

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We report evidence that a single effector enzyme, adenylyl cyclase, in cilia from rat olfactory epithelium, can be coupled with activating and inhibitory G-proteins. Peptide-specific polyclonal antibodies (Santa Cruz Biotech.) directed against C-terminal sequences of the α-subunit of G_s-protein ($G\alpha_{s/olf}$-subunit) inhibited adenylyl cyclase activity elicited by mixture of odorants containing equal parts of ethyl butyrate, eugenol, (+) and (–) carvones at concentrations of 10–1600 μM. In contrast, antibodies raised against Gαi-subunits produced a strong enhancement of the odorant-induced cAMP accumulation. When IgG was substituted for antibodies, the odorant-induced adenylyl cyclase activity was unchanged. Western blot analysis and immuno-electron microscopy confirmed the presence of $G_{i\alpha}$-subunits in our olfactory cilia preparations. Because the adenylyl cyclase enhancement in the presence of Gαi-antibody is controlled by odorants and GTP, and could not be controlled by βg-subunits and Ca^{2+}-calmodulin, we therefore hypothesize that adenylyl cyclase of olfactory neuron cilia is inhibited functionally by odorants through direct coupling with a G_i-protein pathway. This mechanism of inhibition may be important for understanding the attenuation of odorant-stimulated cAMP levels in olfactory neurons. The evidence of both stimulatory and inhibitory odorant-induced regulation of adenylyl cyclase activity in olfactory cilia may indicate that an initial integration of different odorant stimuli begins at the level of primary reactions in the same effector enzyme.

Supported by FAA 93-G-058 (V.V.) and NIH DC 01532 (E.E.M.).

517. Olfactory receptor database (ORDB): a tool to enhance the cloning and sequencing of a large gene family

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Olfactory receptor (OR) molecules form one of the largest gene families yet identified. Cloning and sequencing members of this family therefore presents unique challenges compared with other gene families. Because of the large numbers involved, laboratories need to know if partial sequences they have isolated and are considering working up are also being worked on by other laboratories. In response to requests from several laboratories, we have constructed a novel database (ORDB: http:/senselab.yale.med.edu/ORDB/) that contains such unpublished OR sequences. Participating laboratories submit their unpublished sequences to a 'private' database. They can then ask the question: 'Is there anyone else working on my sequences' by doing BLAST searches of the database with their submitted sequences. If a similar or identical sequence is detected, the user is notified and given the name of the laboratory that submitted the detected sequence. The identity of the sequence thus remains confidential, but the two laboratories can communicate via hotlinks and email and decide on how they wish to proceed in completing the sequencing. A 'public' part containing published sequences is also provided in ORDB, together with information on source species, tissues, and participating laboratories. To date, over 200 unpublished and 200 published sequences have accumulated, from eight species and 23 laboratories. During the past year, the number

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of hits from participating laboratories has averaged ~30 per month. ORDB has been recognized as one of the Protein Family WWW Sites. The unpublished data tool has been cited as a possible model for databases supporting drug development based on specific receptors. Feedback from users is actively solicited in order to make this tool as interactive and useful as possible.

Supported by NIDCD, NASA and NIMH (Human Brain Project).

518. Development of a systematic nomenclature for the large gene family of olfactory receptors

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Olfactory receptors (ORs) are members of the superfamily of seven transmembrane domain, G-protein-coupled receptors. The number of different OR genes in the mammals has been estimated to be ~1000. Currently ~200 ORs have been cloned and sequenced from a variety of organisms. A recent study, involving cloning and sequencing of 20 ORs from different parts of the olfactory glomerular sheet, raised the need for a nomenclature reflecting similarities and differences in OR sequences that may have significance in relation to olfactory function. Here we discuss guidelines for such a scheme using derived protein sequences. This system comprises a hierarchical classification of sets and subsets of ORs, based on residue sites potentially involved in ligand binding as indicated by correlated mutation analysis (Singer *et al.*, 1995). The resulting names convey information of species and putative function (i.e. olfactory) followed by a triplet of amino acid single letter code that defines an OR set. If a set contains more than one member, additional letter and number combinations are used to define subsets and their individual members. The scheme is novel in that a new receptor can readily be named by the sequencer; new receptors fit easily and logically into any pre-existing hierarchy; and the nomenclature reflects directly specific residue sites of potential functional significance. The nomenclature can easily be modified as more specific data such as from expression studies becomes available. We will discuss relations to the phylogeny of G-protein-coupled receptors, and to more general schemes for classification of eukaryotic genes (Lonsdale and Price, 1996).

Supported by NIDCD, NASA and NIMH (Human Brain Project).

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519. Odors can potentiate taste-cued aversion learning

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It is generally assumed that taste enjoys a privileged role in aversion learning and that odors can serve as effective cues only if they are associated with taste during conditioning. In the present study various concentrations of odors that had no gustatory properties or a taste (saccharin) were paired with an illness-producing injection of LiCl and rats were later tested for their intake of the conditioning stimulus. Odor and taste were found to be equally effective as conditioning agents. Further, a relatively low ineffective concentration of a taste became an effective conditioning stimulus if paired with a high concentration of an odor on the conditioning trial (potentiation of taste by odor). Our results clearly favor a within event learning rather than a stimulus gating mechanism as an explanation for the potentiation effect in taste or odor aversion learning.

520. A role for the right anterior temporal lobe in taste quality recognition

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We conducted two experiments to examine central processing of the tastant citric acid. In the first experiment, elevated citric acid recognition thresholds but normal detection thresholds were observed in a group of patients who had undergone a right anterior temporal lobectomy for the treatment of pharmacologically intractable epilepsy compared with both a control group and a group who had undergone the same operation in the left hemisphere. In the second study using positron emission tomography (PET), we report increased regional cerebral blood flow (rCBF) bilaterally in the caudolateral orbitofrontal cortex, in the right anterior temporal lobe, and in the right caudomedial orbitofrontal cortex, in a condition where citric acid was presented compared with a condition in which water was presented, with similar somatosensory stimulation across both conditions. We propose that the elevated recognition thresholds exhibited in patients with resection of the right anterior temporal lobe may be accounted for by damage in an area corresponding to that of rCBF increase isolated in the PET study. These results suggest that while taste sensation may be computed in the primary taste cortex, taste recognition requires further processing by structures located in the anterior temporal lobe. Further, they point to preferential processing of this higher-order gustatory function by the right cerebral hemisphere.

This work was supported by MRC grant MT-10314.

521. Cobalt uptake induced by excitatory amino acids in cells of the primary gustatory nucleus in goldfish

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The vagal lobe in goldfish is a prominent laminated medullary structure that contains superficial cell layers equivalent to the gustatory part of the nucleus of the solitary tract. Deeper layers are homologous to part of the nucleus ambiguus of mammals. Previous electrophysiological studies on *in vitro* slice preparations of the vagal lobe have shown that both NMDA and non-NMDA (AMPA/kainate) receptors mediate evoked responses in the sensory layers, and preliminary results suggest that excitatory amino acids (EAA) are also involved in the motor region.

Agonists that stimulate EAA ionotropic receptors activate

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channels that are permeable to divalent cations, including Co^{2+}. Cobalt influx into neurons by agonist activation then can be visualized by sulfide precipitation thereby revealing the distribution of agonist-activated receptors in the vagal lobe. Vagal lobe slices, 300–700 μm thick, were placed in oxygenated artificial CSF. Slices were incubated in oxygenated uptake buffer, then bathed in uptake buffer containing 5 mM $CoCl_2$ and 500 μM kainate, a non-NMDA agonist. Control slices were bathed in medium containing only uptake buffer and cobalt (no agonist). After rinsing the slices in uptake buffer and EDTA, the cobalt was precipitated with ammonium sulfide and the tissue rinsed again. After fixation with 4% paraformaldehyde, the slices were sectioned on a vibratome and the cobalt sulfide precipitate intensified using a variation of Timm's method.

Cells activated by the agonist kainate appeared darkly filled after precipitation and intensification. Cobalt-filled small cells were found scattered within the sensory layers and within the motor layer. Large motoneurons in the deeper layers also loaded with cobalt upon kainate stimulation. Control slices did not contain any reactive cells in either the sensory or motor layers. These results indicate that cobalt permeable kainate-activated (non-NMDA) receptors are present in the sensory layer and in nucleus ambiguus motoneurons. Studies are continuing on the pharmacology of the excitatory amino acid receptors utilized by this system.

Supported by NIH grant DC00147.

522. Pharmacological disruption of inhibition in the honey bee antennal lobes modulates behavioral generalization between structurally related odorants but not between unrelated odorants

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Fast GABA-mediated inhibition influences the formation and processing of olfactory representations in the first order neuropils of invertebrates (antennal lobes) and vertebrates (olfactory bulbs). In both groups, local interneurons transmit inhibition within and among olfactory glomeruli. This inhibition influences the response patterns of Projection Neurons (PN) and gives rise to assemblies of PNs that are phase locked in their responses to particular odors. In the latter case, these oscillations are thought to be involved in recognition of and discrimination among different odors. It therefore follows that disruption of lateral inhibitory transmission, which blocks oscillation of neural assemblies, should also measurably disrupt behavioral discrimination.

We used proboscis extension conditioning of the honey bee to test this hypothesis. An odor is briefly forward-paired with sucrose reinforcement during four acquisition trials. Three pure odorants were chosen based on similarity or dissimilarity of molecular structure (1-hexanol, 1-octanol and geraniol). Different subject groups received injection of saline, picrotoxin or muscimol into both antennal lobes 45, 60 or 90 min *prior to* conditioning. The response measure for a subject group was the probability of mouthpart extension to odor presented without reinforcement. A series of three such extinction trials were performed 60 min *after* conditioning. The sequence of the trials was randomized and consisted of presentations of the conditioned odorant (CS), an odorant that is structurally similar to the conditioned odor (SIMILAR), and with an odor that is less similar (DISSIMILAR). The critical measure for hypothesis testing involved the degree to which subjects generalize, that is, respond to, the SIMILAR and DISSIMILAR odorants, to which subjects were exposed during acquisition trials.

In saline and muscimol injected groups, responses were strongest to CS and equally low levels of generalization were detected to SIMILAR and DISSIMILAR. In groups that received injection of picrotoxin 45 min prior to conditioning, responses were equally strong toward CS and SIMILAR, and each was higher than the response level to DISSIMILAR. Thus, disruption of inhibition increases generalization (i.e. decreases discriminability) of two odors, but only when those odors are structurally related. The representations of distinct odors appears to be less affected.

523. Symposium: Synaptic processing of chemosensory signals

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As we acquire new tools to investigate the central processing of information in taste and olfactory pathways, we are beginning to reveal mechanisms by which these systems shape and modulate their inputs. Far from being passive relays of information originating in taste and olfactory receptors, neurons in the central nervous system actively process input from the periphery and significantly alter its character. Speakers in this symposium are applying several new methods to the electrophysiological investigation of these systems, including the use of *in vitro* slice preparations, patch-clamp recording, and microinjection of neurotransmitter agonists and antagonists *in vivo*. These studies show that glutamate is likely the neurotransmitter at the first central synapse in both taste and olfactory systems, that chemosensory responses can be modulated by both excitatory and inhibitory neuroactive substances, and that synapses in these systems show experience-induced changes in their response properties. They further demonstrate parallels in these processes across a wide range of species, from insects to mammals. This symposium will provide a forum for a comparison of these mechanisms across sensory systems and across species that will allow us more clearly to formulate future directions of research.

524. Inferences about the taste of sucrose and fat emulsions to the laboratory rat

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It has been shown that laboratory rats will consume large quantities of an emulsion of sucrose and corn oil resulting in significant body weight increases. The present studies were designed to gain information about the salient taste qualities of the sweet/fat mixtures. A LiCl induced taste aversion procedure was used. On conditioning day, for 10 min rats were allowed to drink sucrose 25 M blended with corn oil (84 parts of sucrose and 16 parts of corn oil using Tween 80) followed by the LiCl injection

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(L). Control animals were given a saline injection (S) following the 10 min drinking period. Four 90 min post-conditioning day aversion tests were given. On post-conditioning day 1 the rats received a preference test with the sucrose-fat mixture versus water. On day 2 the rats were tested with sucrose versus water, on day 3 the test was between corn oil and water and on day 4 the test was between sucrose and corn oil. On day 1 the L group showed a significant aversion to the sucrose-fat mixture when compared with the S group. On day 2, the L group showed only a slight aversion to sucrose. On day 3, the L group showed a profound aversion to the corn oil as compared with the S group. On the final test, the S group drank both corn oil and sucrose while the L group drank only the sucrose, showing a strong aversion to the corn oil. In this and other subsequent conditioning studies the conclusion is that the rats generalize to the taste of the fat, not the sucrose in the mixture.

525. The galvanic skin response as a non-verbal measure of olfactory function

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Most tests of olfactory ability require that a subject have some level of verbal ability. This study was designed to test the hypothesis that, with proper controls, the extinction of the galvanic skin response (GSR) can serve as an effective nonverbal measure of olfactory ability. In a series of experiments, the GSR was measured in response to a repeated battery of odorants and sounds. In normosmic subjects, the GSR extinction (defined as the rate of reduction in the height of the response as a function of the trial number) was more rapid for sounds as compared with smells. Although the smell battery included odorants which varied in their trigeminal levels, no repeatable difference in the rate of GSR extinction between the odorants from the smell battery was seen. A comparison of the GSR response to the smell battery in anosmic subjects to that of normosmic subjects will allow for a test of the possibility that the extinction of the GSR could be an effective non-verbal measure of olfactory ability. This comparison will also allow the possibility of differentiating between malingers and non-malingers in a group of people claiming to be anosmic.

Supported in part by NIH grant number 9-PO1 DC00220.

526. Analysis of pheromone induced behavioral responses in transgenic *Drosophila* expressing moth pheromone binding protein in a subset of olfactory sensilla

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Seven members of the odorant binding protein family have been identified to date in *Drosophila*. These proteins are expressed in overlapping patterns in the sensilla of the antenna. Odorant-binding proteins play a significant role in determining the chemical specificity of individual olfactory neurons in *Drosophila*. I have previously identified and characterized OBP-1, a *Drosophila* odorant binding protein family member required for normal behavioral responses to ethanol. Mutants lacking this protein have specific defects in avoidance of high concentrations of a subset of odorants, including ethanol. I propose that some olfactory neurons, including those in the OBP-1 positive sensilla, specifically mediate avoidance responses. This data suggests that the ability to concentrate specific odorants in the peri-dendritic fluid is an important determinant of chemical specificity of olfactory neurons in *Drosophila*.

To further explore the relative contribution of odorant-binding proteins to chemical specificity, I expressed a moth pheromone-binding protein in a subset of *Drosophila* sensilla. If odorant-binding proteins are the sole determinant of odorant specificity in *Drosophila* the transgenic flies should display abnormal chemosensory behavior in response to pheromone. I expressed the moth pheromone-binding protein (PBP) from *Antheraea polyphemus* in a subset of *Drosophila* sensilla using the OBP-1 promoter. Transgenic flies were created and expression of the moth protein was confirmed by western blot and immunofluorescence to frozen tissue sections using antibodies specific to the moth pheromone-binding protein (gift of Gunde Ziegelberger). Behavioral responses induced in wildtype and PBP expressing transgenic flies by chemically synthesized pheromone (gift of Glen Prestwich) will be presented with a model of olfactory discrimination in *Drosophila*.

527. Excitatory and inhibitory modulation of taste responses in the hamster brainstem

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The rostral portion of the nucleus of the solitary tract (NST) contains second-order gustatory neurons, sends projections to the parabrachial nuclei and brainstem reticular formation, and receives descending projections from several nuclei of the ascending gustatory pathway. Electrophysiological responses of NST neurons can be modulated by several factors, including blood glucose and insulin levels and taste aversion conditioning. We are using extracellular electrophysiological recording *in vivo*, combined with local microinjection of neurotransmitter agonists and antagonists, to study the mechanisms by which taste responses of cells in the hamster NST can be modulated. Afferent fibers of the chorda tympani (CT) nerve make excitatory synaptic contact with NST neurons; this excitation is mediated by an excitatory amino acid, probably glutamate. Microinjection of kynurenic acid, a nonspecific glutamate receptor antagonist, into the NST completely and reversibly blocks afferent input from the CT nerve, produced by either anodal electrical or chemical stimulation of the anterior tongue. The non-NMDA (AMPA/kainate) receptor antagonist CNQX also completely blocks gustatory input to these cells, whereas the NMDA antagonist APV produces only a small effect. There are many GABA-containing neurons within the NST and taste-responsive NST cells are maintained under a tonic GABAergic inhibition. Microinjection of the $GABA_A$ receptor antagonist bicuculline methiodide increases the taste responsiveness of NST neurons, whereas application of GABA inhibits taste responses in these cells. Preliminary data show that GABAergic inhibition can be produced by stimulation of the gustatory cortex. There are both intrinsic substance P

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(SP)-containing neurons and extrinsic SP-immunoreactive fibers in the rostral NST. Microinjection of SP into the NST enhances the responses of many NST cells to gustatory stimulation; NaCl-best neurons are preferentially excited by SP.

Supported in part by NIDCD DC00066 (D.V.S.) and DC00207 (B.J.D.).

528. Localized expression of inositol 1,4,5-trisphosphate receptors in rat odontoblasts suggests a role for IP_3 in calcium-mediated secretion during dentin formation

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The cellular distribution of inositol 1,4,5-trisphosphate (IP_3) receptors was examined in rodent maxillary incisor teeth. *In situ* hybridization studies with a conserved transmembrane probe of type I IP_3 receptor indicated that this receptor/channel was highly expressed in odontoblasts. Odontoblasts are polarized secretory cells that are responsible for the mineralization of dentin. In contrast, very low labeling was observed in dental pulp. Northern analysis with this transmembrane probe showed a single message size of ~9.5 kb. Northern analysis also demonstrated that type III IP_3 receptor was expressed in incisor teeth. Immunocytochemical studies confirmed that types I and III IP_3 receptor proteins were both highly expressed in odontoblasts with very low expression in dental pulp. Immunocytochemical studies also suggested a cytoplasmic membrane localization for IP_3 receptors within odontoblasts. Finally, antibodies that recognized alpha subunits of the G_q class of regulatory GTP binding proteins also stained odontoblasts, and stained some arterioles within dental pulp. These results indicate that receptor-mediated regulation of calcium release through IP_3 receptors may occur in odontoblasts of rat incisor teeth. These findings also suggest that IP_3 receptor/channels may regulate calcium flux in odontoblasts during mineralization of dentin, or in growth and differentiation of odontoblasts. Finally, these results suggest that odontoblasts should be a promising model for the identification of a proposed role for IP_3 and calcium in the regulation of cellular secretory activity in mammalian olfactory tissue.

This work was supported by the Johnson & Johnson Focused Giving Program, the Scottish Rite Benevolent Foundation's Schizophrenia Research Program, N.M.J., POI-00161 from NIDCD and the NIMH.

529. The effects of perinatal salt intake on adult salt behavior in rats: a replication and adjustments

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We are investigating whether or not salt (NaCl) intake early in life influences NaCl behavior in adulthood. In 1983 Contreras and Kosten showed that offspring raised on a high salt diet during prenatal and early postnatal development (i.e. the perinatal period) produced elevated adult NaCl preferences over those raised with medium and basal salt exposure. This increase was correlated with decreased water and not increased NaCl intake. In a replication of this study, the robustness of this effect was examined. Male Sprague–Dawley rats ($n = 60$) were maintained on diets containing 0.1, 1.0 or 3.0% NaCl (BA, MD, HI) from conception to postnatal day 30; standard chow (~1.0% NaCl) was administered thereafter. At postnatal day ~120, 48 h two-bottle preference testing began with dH_2O and several concentrations of NaCl, glucose, and KCl. Analysis of absolute consumptions revealed significant group differences in tastant and water intakes associated with NaCl; for example, the HI group showed increased NaCl and reduced water intake. However, in the HI group, there was a significant elevation in preference for glucose, but not NaCl or KCl. Changes in water intake with NaCl ingestion may represent a mechanism for fluid homeostasis. Thus, intake amounts may reveal differences not apparent in preference scores. Rats were sacrificed and selected organs excised and weighed; significant group differences were found for adrenal (BA > MDandHI), kidney (HI > MD > BA), and liver (HI > MD > BA) measurements. We conclude that dietary exposure to NaCl during the perinatal period has a direct and robust influence on adult salt intake.

Supported by NIH grant DC 02641.

530. Sniff-driven activation in the piriform cortex of *humans*

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Cytoarchitectural evidence points to an extension of the piriform cortex from the temporal in to the frontal lobe in humans and monkeys. In search for functional evidence to support this finding, we took advantage of a known phenomenon characterizing piriform cortex in mammals: sniff driving. Electrophysiological findings in mammals indicate that sniffing induces an oscillation of activity in the olfactory bulb, which in turn drives the piriform cortex. We sought to test for this phenomenon in humans, and if present, to use it as a functional criterion in delineating piriform cortex. Methods of air dilution olfactometry were adopted for the fMRI environment. The olfactometer enabled switching from odor to no-odor conditions with rise time under 500 ms, while providing no visual, tactile, auditory, or thermal cues to the subject. To test for sniff-driving, four subjects were each scanned four times during the performance of a detection task while sniffing at a different rate in each scan. Sniffing rates were held constant at once every 8 (0.125Hz), 10 (0.1Hz), 15 (0.066Hz) or 25 (0.04Hz) s throughout 320 s scans. The odor used was decanoic acid. To test for activity induced by odor stimulation regardless of sniffing we scanned eight subjects during two odor detection tasks and an odor recognition task. In these tasks the number of sniffs was balanced over eight alternating 40 s odor and no-odor conditions, thus sniff-induced activation did not correlate with the task. Sniffing induced a characteristic pattern of activation in all four subjects in the piriform cortex of the temporal lobe and in the posterior and medial orbitofrontal region in the frontal lobe. In contrast, the presence of odor regardless of sniffing rate induced significant activation in the lateral and anterior orbitofrontal cortex, ventromedial frontal, insula, and superior temporal as well as

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significant but modest amounts of activation in the piriform, posterior orbital and medial orbital. Activation induced by odor in the lateral and anterior orbital cortex was greater in the right than in the left hemisphere. Our findings show that sniff-driven activation is conserved in humans where it extends from the temporal to the frontal lobe. The dissociation between regions primarily driven by olfactory exploration versus regions primarily driven by olfactory content may pose as a functional distinction between primary and secondary olfactory cortex, thus pointing to a region of primary olfactory cortex in the frontal lobe.

531. Lateralized versus bilateral measurement of olfactory thresholds: comparison of two methods

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This study investigated whether bilaterally determined (dirhinal) olfactory thresholds differ from unilaterally determined (monorhinal) ones. In other words, whether bilateral spatial summation occurs at the threshold level.

Twenty-four healthy volunteers participated (13 women and 11 men; age range 18–37 years; mean 21.5 years). Subjects had normal olfactory function, as measured by the UPSIT. Butanol thresholds were established using a staircase procedure in two sessions separated by 1 to 7 days. On one occasion 'Sniffin' Sticks' (pen-like odor dispensing devices) were used, on the other sniff bottles. Order of testing was counterbalanced, and volume and minimal cross-sectional area of the anterior nasal cavity was assessed using acoustic rhinometry.

Thresholds were significantly higher for dirhinal presentations than monorhinal presentations for both methods ['Sniffin' Sticks': $F(2,46) = 5.88$, $P = 0.005$; sniff bottles: $F(2,46) = 6.72$, $P = 0.003$]. However, when compared with the respective best nostril this difference was no longer present ['Sniffin' Sticks': $F(1,23) = 0.46$, n.s.; sniff bottles: $F(1,23) = 1.32$, n.s.]. Sniff bottles produced consistantly lower thresholds than 'Sniffin' Sticks' [$F(1,23) = 26.22$, $P < 0.0001$].

Although the results suggest that spatial summation at threshold levels may not occur, additional studies on this topic are warranted.

Supported by NIDCD grant PO1 00161, NIH.

532. Spawning stream selection by free-ranging migratory sea lamprey is dependent upon olfactory cues of which conspecific bile acids appear to be an important component

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Although it has long been suspected that many species of fish locate spawning habitats using olfactory cues, this has only been clearly demonstrated for Pacific salmon which return to natal streams using unidentified, learned cues. In the present study we tested the possibility that the sea lamprey (*Petromyzon marinus*), an ancient agnathan fish with a well-developed olfactory system, locates spawning streams from large lakes and oceans using innately recognized olfactory cues which include two unique bile acids (petromyzonol sulfate and allocholic acid) released by larval conspecifics living in these systems. Our earlier studies have found that adult lamprey are attracted to odor of conspecific larvae, larvae release these bile acids in great quantity, and the adult olfactory system detects both bile acids with high (picomolar) sensitivity and specificity. In the present study we tested the behavioral relevance of these findings. Adult sea lamprey were caught migrating in the Cheboygan River (MI), their nasal cavities filled with either plastic dental impression material (blocking their olfactory sense), or gelatin (control), and then released 4 km outside the river's mouth. Nearly 47% of the control animals were recaptured at the trap site within 3 weeks, while only 4% of the animals with blocked noses were recaptured. The importance of the olfactory system was verified using a 5 m long and 2 m wide Y-maze supplied with lake water. In these experiments, control animals were strongly attracted to Cheboygan River water even when it was diluted 20 times. In contrast, animals with blocked noses were not attracted to river water but exhibited activity patterns otherwise similar to the control animals. Other experiments found a 50/50 mixture of petromyzonol sulfate and allocholic acid to be attractive, although less so than river water. We conclude that while adult lamprey locate spawning streams based on an odor which includes conspecific bile acids, the cue is likely more complex than the simple mixture of bile acids we tested. Presently we are testing the activity of other odorants, various bile acid mixtures, and temperature.

Supported by the Great Lakes Fishery Commission.

533. Effect of concentration, pH and anion species on the sourness and astringency of organic acids

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The effect of concentration, pH and anion species on the perceived sourness and astringency of aqueous solutions of lactic, malic, tartaric and citric acids was investigated. Trained subjects rated sourness and astringency intensity in acid solutions at three levels of constant pH varying in normality and at three levels of constant concentration varying in pH. To assess the comparative sourness and astringency of the organic acid anions of study, binary acid solutions matched in pH and titratable acidity were similarly rated. As solution pH was decreased at constant acid normality, both sourness and astringency increased significantly ($P < 0.001$). By contrast, as the normality of the acid solutions was increased at constant pH, only sourness demonstrated significant increases ($P < 0.001$) while astringency remained constant or decreased very slightly. At the lowest normality levels tested, all solutions were judged as significantly more astringent than sour ($P < 0.05$). Although lactic acid was judged to be significantly more sour than citric acid ($P < 0.05$), no other sourness or astringency differences among the organic acid anions were noted. This study demonstrates that for organic acids sourness is independently influenced by the concentration, pH and anion species of the acid, whereas astringency is a function of pH which can be modulated by concentration-related sourness.

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534. Memory, and brain systems

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Studies of animals with complex nervous systems, including humans, have provided new insights about how memory is organized in the brain. One new development is the possibility of studying the anatomy of memory in humans using magnetic resonance imaging. In addition, work with monkeys, using an animal model of human amnesia, has led to identification of the major components of the medial temporal lobe memory system. The system includes the hippocampal formation and adjacent, anatomically related cortex, especially perirhinal and parahippocampal cortex. Neuropsychological analysis of patients with circumscribed memory impairment (amnesia) has led to a distinction between declarative (conscious) memory, which is dependent on the structures damaged in amnesia, and nondeclarative (nonconscious) memory, which is independent of these structures. The brain system damaged in amnesia is essential for the formation and storage of declarative memory, and for its retrieval during a lengthy period of consolidation and reorganization. As time passes, the role of the hippocampal formation in memory diminishes, and a more permanent memory gradually develops elsewhere, probably in neocortex.

535. The effect of gustatory nerve transection on taste discriminations involving quinine in rats

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Previous studies in rats have demonstrated that glossopharyngeal nerve (GL) transection has little or no effect on quinine preference–aversion functions, licking behavior to a range of suprathreshold quinine concentrations in brief-access taste trials or the quinine detection threshold. Electrophysiological studies, however, have demonstrated that the GL is uniquely sensitive to quinine, and contains fibers that are narrowly tuned to respond to this stimulus relative to sugars, salts and acids. The chorda tympani nerve (CT) and superior laryngeal nerve, although quinine-responsive to varying degrees, do not appear to contain such narrowly tuned quinine units. Whole nerve records of the rat greater superficial petrosal nerve (GSP) suggest that this nerve responds only modestly to quinine. We hypothesized that the GL might be critical in taste discriminations involving this stimulus. Water-deprived rats were trained to press one of two levers after a 10 lick sample of quinine (0.068, 0.16, 0.32 or 0.75 mM) and to press the other lever after a 10 lick sample of citric acid (0.0031, 0.011, 0.034 and 0.12 M). Correct responses were rewarded with a 40 lick access to distilled water; incorrect responses were punished with a 30 s time out. Rats averaged 84% correct responses before surgery, though most rats did not respond better than chance to.0031 M citric acid. Following combined GL and CT section, the percentage correct dropped significantly to 66%. Less severe but significant decreases in performance were found in rats with transection of the CT alone or transection of the GSP in combination with the CT (73% correct responses; both groups). Surprisingly, GL transection did not significantly reduce performance. Because citric acid is a complex stimulus that may have a detectable odor and trigeminal sensations at higher concentrations, it is possible that rats may have used these nongustatory cues to perform the discrimination. We are currently examining quinine–KCl discrimination to further elucidate the roles of these nerves, as KCl should be less likely to provide salient nongustatory cues.

Supported in part by R01-DC-01628, K04-DC-00104 (NIH), NSF Graduate Research Fellowship.

536. Expression of Rek7 and its ligand in the olfactory nerve pathway of rat

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Primary olfactory neurons exhibit a mosaic topographical projection from the olfactory neuroepithelium to the olfactory bulb. Axons expressing a single odorant receptor selectively fasciculate in the nerve fibre layer before targeting specific glomeruli. Receptor tyrosine kinases and their ligands are involved in both axon fasciculation and axon targeting in other systems (Winslow *et al.*, Neuron 14: 973–981, 1995). In the present study, we examined the expression of Rek7, a member of the EPH family of receptor tyrosine kinases, and its ligand in the developing olfactory pathway. Rabbit anti-Rek7 antibodies and recombinant Rek7-IgG (human) fusion protein were used to localize receptor and ligand distribution, respectively. Immunostaining for both Rek7 and its ligand were present in all nerve fibre bundles coursing between the olfactory neuroepithelium and olfactory bulb from as early as E13.5. The cellular localization of these molecules was examined in cultures of olfactory Schwann cells and explant cultures of olfactory neuroepithelium. Rek7 was present both on primary olfactory axons and Schwann cells. In contrast, Rek7 ligands were present on primary olfactory axons but were restricted to a subset of Schwann cells. The uniform distribution of Rek7 and its ligands on primary olfactory axons suggests that these molecules are not involved in the selective targeting of subpopulations of axons to specific glomeruli. However, because these molecules have been implicated in fasciculation and repulsion in other systems they may play an important role in regulating axon fasciculation in the olfactory nerve pathway, particularly in the nerve fibre layer where axon sorting occurs.

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537. Topochemistry of olfactory transduction in moths

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In insects, as in vertebrates, an increasing number of proteins is being characterized, which are assumed to play a role either in the second messenger cascade triggered by stimulus recognition or in perireceptor events, such as stimulus transport and deactivation. The localization of such proteins with the resolution of the electron microscope builds a bridge between biochemical data, usually obtained from homogenized antennae, and the complex and diverse ultrastructure of antennal sensory organs. Moreover, as different functional specificities often reside with

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different morphological types of insect sensilla, topochemistry may also allow for additional conclusions on the function of these proteins.

Using antibodies cross-reacting with vertebrate G-proteins we suc-ceeded in the localization of a G-protein of the Gq-family at the membrane of olfactory receptor cells of *Antheraea polyphemus* and *Bombyx mori*. The label is found on the outer segments of the receptive dendrites, just where the so far elusive olfactory receptor molecules are assum-ed. An IP_3 receptor could be labelled in the same location. Calmodulin was detected in the cytoplasm of olfactory dendrites, while Calcineurin, a Ca^{2+}/Calmodulin regulated phosphatase, was found to be associated with the dendritic cytosceleton. Thus, proteins directly or indirectly involved in second messenger chains could be immunolocalized for the first time in insect olfactory receptor cells.

Specific antibodies against pheromone-binding protein (PBP) and general-odorant binding protein 2 (GOBP2) of *A. polyphemus* revealed that these proteins are concentrated in the aqueous sensillum lymph around the receptor dendrites. The two classes of odorant-binding proteins are never co-expressed in the same sensillum. PBP is found mostly in pheromone-sensitive sensilla trichodea, whereas GOBP could be detected in sensilla sensitive for plant odours and other 'general odours'. Moreover, comparison of the cross-reactivity of the PBP anti-serum with antennae of eight moth species showed that positive labeling was correlated with the use of similar pheromones rather than with the systematic relatedness of those species. Thus, odorant-binding proteins might not only have a general transporter and deactivator function but also take part in stimulus recognition, at least in the pheromone-sensitive sensilla of moths.

538. Odorant-binding proteins—expression and function

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While much is known about the structure and chemistry of odorant-binding proteins in both insects and vertebrates, precise data about their physiological role scanty and sparse. Nevertheless, there is a wealth of interesting and challenging concepts which slowly are being supported by data. In particular, immuno-cytochemical methods have helped to provide information about the local expression of these proteins, which in turn may be correlated with physiological data from local electrophysiological recording.

Perhaps the most intriguing aspect of our present knowledge on odorant-binding proteins is that these small soluble proteins must have been invented by nature several times, as indicated by their totally different structure in insects and mammals. If a common function of odorant-binding proteins can be established, this would represent an impressive example of convergent evolution. On the other hand, evidence is accumulating that odorant-binding proteins are serving not only one but multiple functions among which the following are quoted most often: (i) OBPs as solubilizers and carriers of odorants in the aqueous peri-receptor compartments, (ii) OBP-ligand complexes as receptor activators, (iii) OBPs as odorant deactivators and (iv) OBPs as odorant-discriminators.

539. Event related somatic manifestations reliably indicate hedonics and intensity of tastes and odors

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Sensations and feelings are inaccessible to direct measurements. Sensory testing is dependent on reliable indirect, but quantifiable indicators of the sensory experience. Psychophysical testing methods are applicable only to collaborative verbal human subjects. In contrast, recording and critical evaluation of stimulus-dependent reflectory somatic manifestations were found to present alternative avenues of testing for both man and many animals. Tastes and odors are known to induce hedonically polarized sensations in many living organisms. For man this polarity is reflected by the metaphoric use of verbal labels of tastes and odors as equivalents of 'good' and 'bad'. Among the investigated somatic manifestations encephalographic arousal response, autonomous reactions s.a. changes in salivary flow-rate, in heart- and respiratory rate were found to reflect on hedonics and intensity of chemosensations. Among sensory–motor coordinations masticatory activity and in particular the stimulus-dependent, expressive orofacial motor coordinations were found to mirror hedonic polarity and intensity differences of taste- and smell sensations. The event-related expressive oral-facial responses were found to be innate, probably inherited and controlled primarily by brainstem structures. They were found to be independent of visual reinforcement, and termed as gustofacial and nasofacial reflexes. These reflectory reactions were extensively studied in the last three decades in the perinatal human infant, in normal and diseased adolescents and adults as well as in several animal species. Human studies evinced sizable correlation between sets of psychophysical and behavioral data. Among the tested animals findings on nonhuman primates revealed striking similarity between taste-induced facial expressions of the neonate human infant and that of apes.

540. Whole cell currents in olfactory receptor neurones of bullhead catfish

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Voltage-dependent and extracellular calcium-regulated whole cell currents were studied in olfactory receptor neurons (ORNs) of brown bullhead catfish (*Ameiurus nebulosus*). ORNs, dissociated in divalent free papain solution at 37°C for 15 min and containing 0–3 preserved dendritic cilia, responded to step depolarizations with time- and voltage-dependent inward and outward currents. Transient inward currents, activated between –60 and –40 mV, peaked at –40 to –20 mV and rapidly inactivated. Outward currents activated at –40 to –20 mV and displayed transient and sustained components. The possible distribution of chemical stimuli was determined by using pressure-injected high density liquid from a 2–5 μm tip diameter pipette into water under phase contrast microscope. Laminar, fast stimulus flow, extending from the pipette tip to the location of the cell, was observed. Voltage-clamped ORNs did not respond to amino acid stimuli (1 mM L-norvaline and L-arginine–HCl) suggesting that the amino acid transduction mechanisms on the cilia were damaged.

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Application of calcium-free Ringer's solution to deciliated ORN's that were voltage clamped at −70 to −50 mV elicited small sustained currents which developed 20–30 ms after the onset of stimulation and reversed around 0 mV. These results indicate that extracellular calcium regulated nonselective soma conductances were not damaged in papain isolated ORNs.

Supported by MZT J1-7127-0487 (T.V.) and SZF SPS-0050-95 (M.S.).

541. A context effect critical to determining taste-mixture phenomena

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This study addresses a context effect involved in the tasting single-component sugar solutions with binary mixtures of them on the psychophysical functions for single-component solutions of those sugars.

In Experiment 1, volunteers were trained in the method of magnitude estimation by judging the intesity of hue of colored water and then gave magnitude estimtes of the sweetness of solutions of fructose (0.125, 0.25, 0.50, 1.00 M) and sucrose (0.075, 0.15, 0.30, 0.60 M) under two conditions. In the UNMIXED context, three sets of each of the long series of concentrations (a total of 24 samples) were tasted in a single session. In the MIXED context, the participants tasted the 16 binary mixtures made by combining each of the four concentrations of sucrose as well as the four unmixed concentrations of each sugar, for a total of 24 samples. These were tasted in a single session, with the samples given in random orders. The order of interaction of treatment × concentration, but no differential effects for the two sugars. The estimates were greater and slope was steeper for the MIXED context than for the UNMIXED. For Experiment 2, the study was repeated but with equal-interval, rather than log, series of concentrations covering the same ranges as Experiment 1. No context effects were found, suggesting that the difference between the experiments in concentration series, which affected the asymmetry of the disributions of concentrations, was critical.

These results have implications for the interpretation of studies of mixtures of tastants where baselines determined from unmixed components are used to determine suppression of energy.

542. Detection of very complex taste mixtures: generous integration across constituent compounds

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Mixtures can often be tasted when all components are too weak to be tasted separately. Such mixtures are said to be integrative. Integration was demonstrated by mixing compounds in concentrations proportional to their separate detection thresholds and then measuring the detection threshold of the mixture as a whole by forced choice with plain water. Mixtures of 3, 6, 12 and 24 compounds were thus evaluated. With earlier data on 2-, 3- and 4-component mixtures, the results show that the concentration of any constituent compound goes down in approximate proportion to the number of compounds with which it is in mixture. This nearly complete integration describes mixtures of like-quality compounds, of unlike-quality compounds, and of both like and unlike quality compounds together. Integrative mixtures provide a model for the detection of the ultra-complex stimuli of everyday life, such as foods and drinking waters. Although the degree of integration may trail off slightly with mixtures of high complexity, the results proffer no limit on the number of compounds that can be at least partially integrated. In principle, integration permits detection of natural substances whose myriad components could all be far below threshold. The mechanism of taste integration is speculative, but the facts are congenial to the hypothesis of multiple parallel channels for the processing of intensity and quality.

Supported by NIH Grant AG 04287.

543. An artificial neural network for analyzing co-channel sensory data from insect taste cells

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We describe the signal processing (SP) and analysis subsystem of an operational model of an insect central nervous system feeding decision center capable of predicting caterpillar responses to specific chemical mixtures. Our model includes: (i) a demultiplexer; (ii) a neuron activity encoder; and (iii) a predictor of the feeding behavior. Detection of a chemical mixture is performed by two taste sensilla each containing four taste neurons. The insect uses this information to choose among acceptable and unacceptable plants. Extracellular electrophysiological recordings obtained from the taste sensilla, quantify the activity of the constituent taste neurons in response to chemical mixtures. Recordings may contain action potentials (AP) produced by all four of the constituent taste neurons of each sensillum. Known chemical reference compounds activate specific neurons. A different shaped AP is produced by each of the four neurons of a sensillum in response to its reference compound. Thus shape can be used to estimate the origin of each AP. The demultiplexer employs conventional SP techniques and an expert system to detect the presence of an action potential within the signal. Once an AP has been detected, the demultiplexer module uses an artificial neural network (ANN) to estimate which of the taste neurons is active. The ANN classifier is trained with spikes elicited by reference compounds. The trained classifier is highly successful in identifying spikes, evoked by reference compounds, that where not part of the training set. The ANN classifier's results are compared with two traditional methods of spike classification: (i) template matching; and (ii) principal components (Wheeler, 1997). The ANN outperformed both traditional methods when applied to spikes elicited during the tonic phase of response.

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544. Temporal representation of odors by synchronous neural assemblies

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Stimulus evoked oscillatory synchronization of neural assemblies and temporal patterns in neuronal activity have long been

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observed in many sensory systems (Adrian, 1942) in both vertebrates, such as rats, and invertebrates, such as insects. Our work on insect olfaction suggests that complex temporal patterns and neural synchrony may play important roles in sensory coding.

In insects such as the locust and the honeybee, odor presentations elicit bouts of cell synchrony detected extracellularly as an oscillating local field potential, and intracellularly as synchronized subthreshold membrane oscillations, and synchronous action potentials. Olfactory interneurons often respond to more than one odorant, but with slow temporal patterns (consistent sequences of excitation and inhibition) that are odor specific (Laurent and Davidowitz, 1994). We have shown that information about odor identity is contained in the identities of the activated neurons, the temporal patterns with which these neurons respond to odors, and the precise timing of their action potentials (Laurent *et al.*, 1996; Wehr and Laurent, 1996).

Synchrony among olfactory interneurons can be specifically abolished by application of the GABA antagonist picrotoxin without affecting the odor specific, slow temporal patterns (MacLeod and Laurent, 1996). In a recent collaboration with Brian Smith and Seetha Bhagavan, we have shown that abolishing cell synchrony impairs the ability to distinguish similar odorants (such as alcohols of different chain length) while sparing the ability to distinguish dissimilar odorants (such as alcohols from terpenes). Thus, temporal information may provide a critical additional dimension for encoding fine sensory distinctions in cases where spatial representations may greatly overlap.

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545. Influences of membrane-permeable cyclic nucleotides on membrane conductance of murine taste bud cells

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Recent studies have suggested that cyclic nucleotide plays an important role in taste transduction, especially for sweet and bitter tastes. In the present study, therefore, the change in membrane conductance of mouse and rat taste bud cells after application of membrane-permeable cyclic nucleotide was investigated in order to elucidate the effects of raise in intracellular cyclic nucleotides.

The taste bud cells were dissociated mechanically from circumvallate and foliate papillae after serial treatments of isolated tongue epithelium with collagenase and low Ca^{2+}. The taste bud cells were identified by their spindle-like cell body and long cell process. Whole cell currents of taste bud cells were measured by perforated patch recording using amphotericin B and conventional whole-cell recording with CsCl pipette solution. Membrane-permeable cyclic nucleotide analogues such as 8-bromo-cAMP (8-br-cAMP), dibutyryl cAMP (db-cAMP) and 8-br-cGMP as well as saccharin Na were added to the bath solution.

Some taste bud cells demonstrated significant reduction of voltage-dependent K^+ outward currents after application of 125–500 mM 8-br-cAMP, 250 mM db-cAMP and 250–500 μM 8-br-cGMP in perforated patch recording. Bath application of 5 mM saccharin Na as well as 8-br-cAMP suppressed K^+ outward currents and thereby increased voltage-gated Na^+ currents in single cells. When K^+ currents were almost completely blocked with CsCl pipette solution, 125–250 mM 8-br-cAMP, 250 mM db-cAMP and 250 μM 8-br-cGMP remarkably increased the whole cell conductance. Cyclic nucleotide induced currents were reversed at –10 to +10 mV, indicating that the non-specific cation channels may have been activated. In a few taste bud cells a transient enhancement of voltage-gated Na^+ current was observed prior to the increase in whole cell conductance after addition of 8-br-cAMP.

These results indicate that raise in intracellular cAMP and cGMP results in not only suppression of K^+ channels but also increase in membrane conductance of mouse and rat taste bud cells.

546. Taste-modifying triterpene glycosides from *Staurogyne merguensis*

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We have found a plant having a new type of taste-modifying activity on Penang island of Malaysia. After chewing the leaves of *Staurogyne merguensis*, water elicits a sweet taste. The plant grows wild and native people often cook rice with its leaves to give a sweet taste to the rice. In spite of this interesting property, no study on the active principle has been carried out. Now, we purified five new oleanane-type triterpene glycosides named strogins 1–5 from leaves of *Staurogyne merguensis*. Their structures were determined on the basis of chemical and spectral evidence (Hiura *et al.*, 1996). Strogins 1–5 have an oleanene skelton and glucuronic acid at C-3 of the aglycone. Strogin 1 itself showed a sweet taste and a sweetness-inducing activity. Strogin 1 (1 mM) elicited sweetness equivalent to that of 0.15 M sucrose. Cold water elicited sweetness equivalent to that of 0.3 M sucrose after 1 mM strogin 1 was held in the mouth for 3 min. The activities of strogins 2–5 were also assayed. The activity and stability of strogin were examined.

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547. Infantile olfactory memories: good memories of bad events

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Neonatal rats are capable of learning to avoid or approach an odor. However, under most circumstances, the neonatal rat will learn a preference for an odor, even when the reinforcer used during conditioning is clearly aversive to the pup. For example, pairing an odor with a tailpinch or a moderate shock produces a subsequent preference for the conditioned odor. During the later part of the second week of life, conditioning becomes more

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similar to that seen in the adult, so that odor and 1 mA shock pairing now produces an aversion to the odor. This series of studies further explores this developmental change in olfactory learning.

Pups were given odor (2 µl citral) and shock (0.7 mA, 1 s, ITI 3 min) pairings for a single 30 min training session. Pups were tested in a two-odor choice test (citral versus familiar clean bedding odor). Experiment 1 assessed the age at which pups change from learning a preference to learning an aversion from odor-shock conditioning. The results show that PN9 pups learn/express an odor preference whereas PN10 pups learn/express an aversion, illustrating a developmental change in the representation of this training from appetitive to aversive learning. Experiment 2 assessed whether pups would express a preference (typical of PN9) or express an aversion (typical of PN10) when trained on PN9 but tested on PN10. The results showed that pups trained on PN9 exhibit a preference when tested on either PN9 or PN10. Experiment 3 assessed whether the olfactory memory could be changed by extending the training from PN9 (preference learning) to PN10 (aversion learning). The results showed that when learning began during the period of preference learning and extended into the period of aversion learning, pups continued to express a preference. Experiment 4 assessed the neural correlates of PN9–10 olfactory memories. These data are currently being analyzed. In summary, these results suggests that once an olfactory memory is formed, the original memory trace continues to be strengthened by subsequent experience. In fact, the original memory appears to be strengthened in spite of dramatic developmental changes in the representation of this training from appetitive to aversive learning.

Supported by NIH grant HD33402.

548. Cell death, phagocytosis and neurogenesis in mouse olfactory epithelium and vomeronasal organ after colchicine treatment

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The olfactory epithelium is characterized by the continuous proliferation of neuronal precursor cells (basal cells) followed by the death of olfactory neurons. In this study we observed massive cell death that occurred in the middle to basal region of the olfactory epithelium and vomeronasal organ near the boundary of the respiratory epithelium after colchicine treatment. The TUNEL method revealed the presence of many apoptotic bodies at 20 h after injection of colchicine (4 mg/kg), and lectin (UEA-I) staining showed that apoptotic cells to be globose basal cells and immature olfactory neurons. Electron micrographs taken at 20 h after colchicine treatment showed that many apoptotic bodies, which showed nuclear and cytoplasmic condensation, had been phagocytized in the foot processes of supporting cells. A small number of macrophages also had phagocytized dead cells. By 3 days after colchicine treatment, a large number of immature olfactory cells and globose basal cells had disappeared from the olfactory epithelium and vomeronasal organ. For examination of proliferating cells, mice of post colchicine-treatment days 2, 3, 4, 5, 6, 7, 10 and 14 were injected with bromodeoxyuridine (BrdU). BrdU-labeled cells in the olfactory epithelium were globose basal cells (not horizontal basal cells) and increased in number at 6 days after colchicine treatment. In the vomeronasal organ BrdU-labeled cells increased in number at 4 days. These results indicate that when large numbers of precursor and immature olfactory cells die by apoptosis, the rest of the precursors may generate new olfactory neurons.

549. A patch-clamp study on isolated olfactory receptor neurons from the rainbow trout

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To elucidate functional roles of ciliated and microvillous receptor neurons in fish olfaction, their basic response properties to electrical and chemical stimulation were studied with patch-clamp techniques in the rainbow trout, *Oncorhynchus mykiss*. The two types of receptor neurons which were discernible with their knob structure were isolated from the predissected epithelial tissues from individual olfactory lamellae with or without using proteolytic enzymes. A mixture of four amino acids (glutamate, arginine, alanine and norvaline at 1 or 10 mM concentration) filled in a stimulating pipette (tip opening, ~1.5 µm diameter) was applied to receptor neurons in normal Ringer's solution by a pressure ejection system. Voltage-gated current responses of both types of neurons held at –60 mV to depolarizing and hyperpolarizing voltage steps were essentially similar in properties. Spiking responses recorded with cell-attached condition to the mixture were more robust in the neurons isolated without using proteolytic enzymes. Whole-cell responses of ciliated neurons held at –60 mV to the mixture, which were recorded with the conventional membrane rupture or Nystatin-*N*-methyl-D-glucamine perforation method, were monophasic inward currents, of which peak amplitude increased with an increase in concentration of the mixture of amino acids. The latencies were very short (<20 ms), suggesting that the directly gated-ion channels by amino acids may be involved in fish olfactory transduction.

Supported in part by JMESC 07044172.

550. Effects of acute administration of haloperidol and methylphenidate on olfactory performance

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The influence of dopaminergic drugs on olfactory function in humans is unknown. In rats, it has been shown that a dopamine D1 agonist enhances odor detection performance, while a D2 agonist depresses performance. As part of a pilot project to assess the impact of acute administration of methylphenidate (Ritalin) and haloperidol (Haldol) on several measures of neurobehavioral performance and cerebral blood flow, eight subjects (five men, three women) were examined before and after these two medications. Baseline [Tc–99m] ECD SPECT scans were acquired. During the uptake interval, neuropsychological tests, including the University of Pennsylvania Smell Identification Test (UPSIT), were administered. The UPSIT consists of four booklets of 10

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odors each, administered bilaterally. For each condition, two booklets were given in a counter balanced fashion. The SPECT scan and UPSIT were then repeated 1 h after methylphenidate or haloperidol administration. The protocol was repeated on a second day with the next medication in a counter balanced design. No significant change in olfactory performance as measured by the UPSIT occurred with either medication. However, bilateral dorsolateral prefrontal cortex region to whole brain uptake measured by SPECT decreased post methylphenidate but increased post haloperidol (medication by pre–post interaction, $F = 10.97$, $P = 0.02$). There were no significant changes observed in region to whole brain values in other regions associated with olfactory performance (orbital and medial frontal, superior and inferior temporal, and the amygdala). However, the absolute values for the amygdala showed large changes from baseline. The results suggest that acute systemic manipulation of CNS dopamine activity by Ritalin and Haldol have no influence on the ability to correctly identify odors.

Supported by NIMH grant MH43880, NIDCD grant P01-00161, National Institutes of Health.

551. Immunolocalization of TrkB in mammalian nasal chemosensory mucosae

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To understand neurotrophic interaction of brain-derived neurotrophic factor (BDNF) and its high-affinity receptor, TrkB, in mammalian chemosensory mucosae, immunolocalization of TrkB was examined in developing rats. Under deep anesthesia, developing (after postnatal day 1, P1) and adult Sprague–Dawley rats were transcardially perfused with Zamboni's fixative and cryostat sections of nasal regions containing olfactory and vomeronasal mucosae were collected for immunohistochemistry. In adult rats, intense immunoreactivity for TrkB was localized in olfactory and vomeronasal nerve bundles, and moderate immunoreactivity in dendrites and somas of olfactory and vomeronasal receptor neurons. Moreover, the apical surface of the olfactory mucosa, which is called 'mucociliary complex', exhibited the greatest intensity for TrkB immunoreactivity, whereas no such immunoreactivity was observed in the apical surface of the vomeronasal sensory epithelium, 'mucomicrovillar complex'. In contrast, immunoreactivity for TrkB was very weak in olfactory and vomeronasal mucosae at P1 rats and its intensity was gradually increased during development. Since immunoreactivity for BDNF in adult olfactory and vomeronasal system is much greater that that in P1 rats, the above results suggests that increased amount of TrkB is produced by olfactory and vomeronasal receptor neurons in adult rats and BDNF is a very important neurotrophin in adult olfactory and vomeronasal sensory epithelia.

Supported by a grant for medical science and health care for the elderly from Sasakawa Health Science Foundation and a grant from the Naitoh Foundation to S.T.

552. Zonal expression of OCAM by mouse olfactory axons: a developmental study

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Olfactory sensory neurons located in one of the four odorant receptor expression zones of the olfactory epithelium (OE) project their axons to a corresponding zone in the main olfactory bulb (OB). Neurons in the ventrolateral three zones of the OE express a novel cell adhesion molecule, OCAM (R4B12 antigen), on their axons projecting to the caudoventral zones of the main OB. To reveal roles of OCAM in formation of the zone-to-zone projection, we investigated developmental expression of OCAM in the mouse main olfactory system by immunohistochemical analysis.

OCAM-positive axons were detected from embryonic day 10 (E10), when early axons have grown out of the OE but have not yet reached the primordial OB. At E11, when early axons have already penetrated into the primordial OB, OCAM-positive axons were also observed in the primordial OB. At E13, when the OB has already evaginated, OCAM was expressed by subsets of olfactory axons that originated from the ventrolateral regions of the OE and projected to the caudoventral OB. After E14, when the main and accessory OB could be distinguished, OCAM-positive and OCAM-negative olfactory axons became more clearly segregated in the main OB. At early postnatal days, individual immature glomeruli were either OCAM-positve or OCAM-negative.

These results indicate that OCAM is expressed by growing olfactory axons and that zonal expression pattern of OCAM appears around at the time when the olfactory axons just reach the target. Such zonal expression at early developmental stages suggests that OCAM may play roles in formation of the zone-to-zone projection of the olfactory axons.

553. Response of snake vomeronasal receptor neurons induced by intracellular dialysis of inositol 1,4,5-trisphosphate

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A previous study has demonstrated the presence of G-proteins (G_s, G_i and G_o) in the vomeronasal epithelium of garter snakes. These G-proteins appear to be coupled with chemoattractant receptors which, when bound to ligand, result in an increase in IP_3 accumulation and a reduction of the basal level of cAMP. These results suggest that the chemosignal transduction in vomeronasal receptor neurons is mediated by these second messenger pathways.

To investigate the role of these second messengers in signal transduction, studies were conducted with snake vomeronasal receptor neurons in slice preparations using the patch-clamp technique in the whole-cell configuration. The mean resting potential was ~ –70 mV, and the average input resistance was 3 GW. An injected current of 1–10 pA was required to depolarize the neuron. In response to depolarizing voltage steps, voltage-clamped vomeronasal receptor neurons displayed transient inward currents followed by sustained outward currents which were blocked by internal Cs^+ ions.

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Intracellular application of 0.1 mM 3-deoxy-3-fluoro-D-myo-inositol 1,4,5-triphosphate, a derivative of IP_3, evoked an inward current in 4/8 vomeronasal neurons. Similar application of 0.1 mM IP_3 also evoked an inward current in 6/16 (38%) neurons. The present results demonstrate the existence of an IP_3-activated conductance in snake vomeronasal receptor neurons. In contrast, intracellular dialysis of 1 mM cAMP elicited no detectable current response in any neuron ($n = 10$).

Supported by NIDCD Grant # DC 02531 to M.H.

554. Olfactory event-related potentials (OERP) change as a function of stimulus concentration

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The study investigated OERP in relation to changes in stimulus concentration. Sixteen subjects participated (eight women, eight men; age 17–34 years). Normal olfactory function was ascertained prior to the experiment using odor threshold measures, odor identification, and odor discrimination. OERP were recorded in response to olfactory stimulation with four concentrations of vanillin (7, 28, 56, 84% v/v; stimulus duration 200 ms, interval 40 s, flow 8 l/min); 16 stimuli of each concentration were applied in a randomized order. EEG recordings were made at sites Fz, Cz and Pz (bandpass 0.2–15 Hz; sampling frequency 250 Hz; pre-stimulus period 500 ms). Amplitudes and latencies of the following four peaks were measured (latencies in ms at Pz after 84% v/v): P1 (277), N1 (348), P2 (412) and P3 (496). After each stimulus subjects rated ist intensity on a computerized visual analogue scale in comparison to a standard (54% v/v). Butanol thresholds were determined immediately after each session (single staircase, triple-forced choice). In addition, the volume of the anterior nasal cavity was assessed using acoustic rhinometry.

All subjects were able to discriminate the 4 different concentrations of vanillin. With increasing stimulus concentration amplitudes became significantly larger; this was most pronounced for P3 (P1N1: $F = 2.90$, $P = 0.046$; N1P2: $F = 5.15$, $P = 0.004$; N1P3: $F = 35.7$, $P < 0.001$; P3: $F = 38.6$, $P < 0.001$). Correspondingly, latencies shortened with increasing concentra- tions (P1: $F = 25.2$; N1: $F = 17.51$; P2: $F = 14.8$; P3: $F = 13.4$; all $P < 0.001$). While there was no correlation between OERP amplitudes and thresholds, latencies were the shorter the lower the subjects' thresholds (coefficients of correlations for peak latencies at Cz for 84% v/v: P1, $r_{15} = 0.59$; N1, $r_{15} = 0.58$; P2, $r_{15} = 0.55$; P3, $r_{15} = 0.45$). These results demonstrate that OERP are bioresponses which exhibit a clear relation to stimulus concentration.

Supported by DFG grant Ko812/5-1, NIDCD grant PO1 DC00161:NIH.

555. Pheromone receptors are expressed in testis

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Previous studies have demonstrated that germ cells as well as sperm express odorant receptors (ORs), leading to the hypothesis that odorant receptors are involved in guiding sperms to eggs by means of chemoattraction. By analogy, we speculated that the germ cells and sperm also express pheromone receptors (PRs).

By RT–PCR technique using degenerate primers based on the sequences conserved among the previously reported PR cDNAs in rat, we obtained cDNA fragments of PRs in mouse testis. The sequence analysis revealed that the predicted amino acid sequences of these fragments were highly homologous to the known PRs. This result indicates that PRs are expressed in testis. To identify which cell types of testis express PRs, *in situ* hybridization analysis was performed on sections of testis using the cloned PR and OR cRNA probes. The result of these experiments will be discussed in relation to the possible functional role of PRs in sperm guidance and chemoattraction.

556. Responses of mouse taste cells to glutamate

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There is considerable interest in the mechanisms underlying the unique taste of glutamate. However, the nature of the receptors involved in recognition of glutamate by taste receptor cells, as well as the subsequent cellular events leading to modulation of neurotransmitter release at synapses with afferent taste fibers, remain unclear. We have proposed on the basis of patch-clamp, fluorescence imaging and reconstitution studies with mouse (C3H/HeJ) taste tissue that ionotropic receptors with many characteristics of NMDA-type receptors from brain may be involved in glutamate taste transduction. Although responses to the metabotropic receptor agonist L-AP4 were observed in these studies, they appeared to be inhibitory, whereas responses mediated by ionotropic receptors resulted in membrane depolarization and increases in intracellular calcium. Molecular biological, immunohistochemical, electrophysiological and behavioral studies have demonstrated the presence of metabotropic, G-protein-coupled glutamate receptors (mGluR4) in taste buds from glutamate-sensitive regions of rat tongues, suggesting their involvement in glutamate taste. Recent patch-clamp and imaging studies with a different strain of glutamate-sensitive mouse (C57BL/6J) also implicate several different mechanisms in taste cell responses to glutamate. These results also suggest that there may be significant differences in taste cell responses to glutamate and glutamate receptor agonists between the two strains of mice.

Supported by Monbusho International Scientific Research Program, National Institutes of Health grants DC001838 and DC00566 and Department of Veterans Affairs.

557. Patterns of axonal arborization in olfactory glomeruli during development

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The axons of primary sensory olfactory neurons terminate in a

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single glomerulus in adult rat olfactory bulb. Each axon gives rise to a terminal arborization which is confined to a discrete region of the glomerulus. The arborization has a mean length of ~158 mm, an area of 880 mm^2 and a mean number of branches of ~7 mm (Halasz and Greer, 1993). In order to determine whether there is exuberant branching and growth of terminal arbors, followed by subsequent remodelling during development we have assessed changes in the structure of arbors in late embryonic and early postnatal rats. The olfactory bulb and nasal cavities were removed en bloc and small localized injections of horseradish peroxidase were made into the olfactory nerve fibre layer of the dorsal olfactory bulb. Tissue was immersed in modified Eagle's medium, incubated at 37°C for 5 h and then fixed in 4% paraformaldehyde. Frozen coronal sections (100 mm) of the olfactory bulb were cut and reacted with diaminobenzidine and hydrogen peroxidase to demonstrate impregnated fibers. Terminal arbors of individual olfactory axons were reconstructed at 2400× magnification. At postnatal day 3 (P3) arbor length had reached adult-like proportions (147.7 ± 45.8 mm) whereas the branch area (450.8 ± 166.2 mm^2) was only ~50% adult size. Interestingly the arbors were markedly more complex at P3 with (14 ± 4.7) branch points/arbor which is ~50% greater than in adults. These results indicate that there is considerable postnatal remodelling of terminal arbors within glomeruli. In particular there is a reduction in terminal branching concomitant with an increase in branch area. Interestingly there is no change in overall arbor length suggesting that the increase in arbor area is achieved by growth of main branches, while minor branches are retracted.

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558. PROP taster status is related to the perception and preference for fat

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Individuals who are sensitive to the bitter compound 6-*n*-propylthiouracil (PROP) are also more sensitive to selected bitter and sweet substances, to sharp foods and to the trigeminal irritant capsaicin. PROP tasters have a greater density of fungiform papillae and it is speculated that they also have more trigeminal innervation. Since oral texture perception is also mediated, in part, by trigeminal fibers, it has been proposed that individual differences in fat perception might also be linked to PROP taster status and taste bud density. This work tests the hypothesis that individuals who are PROP tasters: (i) have a higher density of fungiform papillae; (ii) are more sensitive to capsaicin; and (iii) have increased ability to discriminate differences in fat content in salad dressing. Hedonic ratings for the salad dressing were also collected and related to the perceptual judgements. Individual subjects were classified as PROP nontasters, medium tasters or supertasters (n = 25/group) by comparing their psychophysical function for PROP to that of NaCl. Papillae densities (papillae/cm^2) were significantly different among the three taster groups ($P < 0.0001$), and were highest among the supertasters. Both medium tasters and supertasters perceived more oral burn from capsaicin than did nontasters at concentrations of 50, 70 and 100 ppm ($P < 0.0001$). Medium tasters and supertasters could also discriminate differences in fat content between 40% fat and 10% fat salad dressings ($P < 0.005$), but the nontasters could not. Although medium and supertasters showed no preference for either dressing, the nontasters preferred the 40% fat sample. The reasons for these latter findings are unclear at present. These data support the hypothesis that fat perception and preference can be linked to genetic and anatomical differences between individuals.

559. Behavioral taste responses of developmentally NaCl restricted rats to various concentrations of sucrose and NaCl

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A variety of neurophysiological and morphological alterations are observed in rats whose dietary sodium is restricted during pre- and postnatal development. The physiological effects are most notable when NaCl concentrations are used as the stimuli. However, there is relatively little data available to indicate if these alterations affect the behavioral taste perception of these same rats. The challenge is to present each rat with a concentration of NaCl for a brief period of time so as to not allow so much NaCl to be ingested that the rat becomes replete, but long enough to determine the rats taste response. Fortunately, a brief exposure taste apparatus has been recently developed that allows for just such an examination.

This project was aimed at determining the behavioral taste responses to concentrations of sucrose and NaCl of rats whose dietary sodium has been restricted during pre- and postnatal development. Control and NaCl restricted subjects were initially trained to lick from a bank of 16 individually presented sipper tubes to obtain water or a solution of 0.25M sucrose. Once the subjects were responding immediately to the presentation of each tube, testing was initiated. Each subject received four days of testing using a sucrose series (0.5, 0.25, 0.125, 0.06 and 0.03 M concentrations) to determine their taste response. This was followed by 4 days of testing using a NaCl series (0.5, 0.25, 0.125, 0.06 and 0.03 M concentrations). Results will determine if the NaCl restriction and subsequent neurophysiological and morphological alterations affect the rats behavioral taste response as well.

This research was supported by NIH grant # DC00407.

560. The effect of mouth movements and adaptation on the differential sensitivity to sucrose

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Mouth movements diminish the degree of adaptation (Theunissen and Kroeze, 1996). In this study we tested the hypothesis that mouth movements lead to a decreased differential sensitivity, compared with a condition in which no movements are made. The rationale for this hypothesis lies in the findings of McBurney *et al.* (1967). They found that the jnd for NaCl was lower after adaptation. As mouth movements diminish adaptation, one would expect that they would also diminish this increase in sensitivity.

The jnd for sucrose was established in three conditions: one with no mouth movements, one with a standardized rate of mouth movements, and one in which the subject was free to choose her

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own rate of mouth movements. No differences between the jnd's were found. The reason for this might be that in this study subjects were not completely adapted. To test this hypothesis the experiment was repeated with two subjects who were completely adapted to the stimulus. However, still no differences in jnd's were found.

Therefore McBurney *et al.* (1967) experiment was replicated with sucrose solutions instead of NaCl. The differential sensitivity was established in two conditions: one in which subjects were completely adapted to the standard sucrose solution, and one in which the subjects were adapted to water. It was found that when subjects were adapted to the standard solutions they had a higher differential sensitivity than when they were adapted to water. These results are in agreement with the findings of McBurney *et al.* (1967).

The main difference between these experiments is that in the two first experiments a sip-and-spit procedure was used, whereas in the last one a gravitational flow method was applied.

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561. Discrimination and hedonic properties of R- and S-nicotine by smokers and non-smokers

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The aim of the current study was to investigate whether smokers and non-smokers are able (i) to discriminate between R- and S-nicotine and (ii) to characterize their hedonic properties. Thirty healthy male subjects—15 smokers and 15 non-smokers—were given a pseudorandomized sequence of R- and S-nicotine stimuli (seven R- and seven S-nicotine stimuli) in the right nostril using a Kobal-olfactometer. Subjects were instructed to group the perceived stimuli into two categories (R- and S-nicotine). The individual thresholds for the odors and burning sensation of both enantiomers were determined. In order to test the role of the olfactory and the trigeminal system in this discrimination task nicotine enantiomers were presented at two different concentration levels: (i) concentrations above the individual threshold for the odors but below the threshold for the burning sensation and (ii) concentrations above the individual threshold for the burning sensation. Additionally, the subjects evaluated the hedonic properties using a bipolar visual analog scale (very unpleasant . . . very pleasant).

Statistical evaluation (*t*-test, $P < 0.01$) revealed that subjects were able to identify R- and S-nicotine in both concentration ranges tested. No threshold differences were observed between smokers and non-smokers. At low concentrations smokers perceived both enantiomers as more pleasant than non-smokers (*t*-test, $P < 0.05$). At higher concentrations smokers perceived only the S(–)-isomer to be less unpleasant than non-smokers (*t*-test, $P < 0.001$). This difference at higher nicotine concentrations might be explained by smokers' experience with S-nicotine, which is the main nicotine enantiomer in cigarettes.

Supported by Philip Morris, USA.

562. Gustatory preference of ants (*Lasius niger*) towards >100 natural and artificial compounds known to be sweet in man

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A thousand or more compounds are known to elicit a sweet taste in man, with sweetness potencies reaching up to 225 000 times that of sucrose. From detailed structure–activity relationships, a sophisticated model, the multipoint attachment (MPA) theory, was proposed for the human sweetness receptor. Sweetness reception has been investigated in many vertebrates (primates, rodents, etc.), but in insects, especially in ants, little information is available. The present work describes taste preference in ants (*Lasius niger*) towards more than one hundred compounds sweet in man. Natural compounds such as carbohydrates with monosaccharides (glucose, galactose or fructose), disaccharides (sucrose, trehalose or melibiose), trisaccharides (raffinose or melezitose), but also polyols (xylitol, ribitol), L- and D-amino acids (phenylalanine or tryptophan), proteins (monellin or thaumatin) and many synthetic sweeteners such as saccharin, acesulfame, dulcin, sweet dipeptide derivatives (aspartame or alitame), superaspartame or hyperpotent guanidine sweeteners (lugduname or sucrononate) were experimented. By using a specific procedure (duration of contact with compounds combined with behavior), the taste preference and the sweet quality of compounds were ascertained. Results are presented and commented with respect to the MPA theory proposed for humans.

563. Characterization and role of Schwann cells in the olfactory nerve pathway

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We have previously shown that FGF-1 and HSPG cause differentiation of Schwann cells in dissociated cultures of embryonic olfactory neuroepithelium (Key *et al.*, 1996). FGF-1 and HSPG induce spreading of olfactory nerve Schwann cells (OSC), and produce a phenotype conducive for axon growth from peripheral olfactory neurons (PON). In this study, we have isolated Schwann cells from the olfactory nerve and characterized their expression of FGF receptors and several glial markers. Both RT–PCR and immunohistochemical analysis revealed that Schwann cells express FGF receptors 1–4 together with FGF-1 and a specific perlecan variant (Joseph *et al.*, 1996). *In vitro*, OSC express S-100, GFAP and p75 by immunohistochemistry. In order to examine the role of p75, the low affinity nerve growth receptor, we examined the primary olfactory system in p75 null mutant mice (Lee *et al.*, Cell 69: 737–749, 1992) by immunohistochemistry. OSC in mutant mice did not express p75 or GFAP, and weakly expressed S–100. Interestingly a subset of PON axons displayed an aberrant projection in the olfactory nerve pathway. These results suggest that expression of p75 by OSC is important for both the normal differentiation of these cells as well as the growth of primary olfactory axons.

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564. Olfactory development in invertebrates

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Invertebrate olfactory systems offer many advantages for cellular and molecular studies of development and for functional studies of developmental plasticity. For example, nematodes have chemical senses that can be studied using genetic approaches. Arthropods, which include insects and crustacea, have the advantages that certain neurons can be reliably identified from one individual to another, and that olfactory receptor neurons are located on peripheral appendages and thus can be manipulated independently of their brain targets, even very early in development. Among the insects, olfactory learning can be displayed and used as a basis for studying olfactory plasticity in bees; genes are especially tractable in flies; individual growth cones can be visualized in the grasshopper embryo; and receptor neurons and glomeruli of known olfactory specificity and behavioral significance can be followed during early development in moths. In addition, many insect nervous systems are amenable to organ culture and dissociated-cell culture, opening the door to experimental studies of cellular interactions that can not be performed *in situ*. Recent research in the moth *Manduca sexta* attempts to identify the nature of the interactions between olfactory sensory axons, olfactory neurons of the brain, and glial cells in the creation of the array of glomeruli that underlie olfaction in the adult. Results indicate that timing of the ingrowth of olfactory receptor axons is critical for normal glomerulus development, that a subset of axons expresses a fasciclin II-like molecule that may play a role in guidance of their growth, and that glial cells must surround developing glomeruli in order to stabilize the 'protoglomerular' template made by receptor axon terminals. Moreover, glial cells are dye-coupled to each other early in glomerulus development and gradually become uncoupled. Electrical activity in neurons is not necessary for glomerulus formation; and some intercellular interactions, perhaps involving soluble factors, appear to involve tyrosine phosphorylation. In sum, a detailed picture is emerging of the cellular interactions that lead to the formation of glomeruli.

565. Effects of pleasantness/unpleasantness on the event-related potentials elicited by mismatch between an odorant stimulus and a word stimulus

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The purpose of this study is to examine whether an odorant stimulus can deviate from a semantic context or the emotion such as pleasantness/unpleasantness can elicit the event-related potentials (ERPs) as a word or the emotion. ERPs were recorded while 14 students juged the veracity of a simple statement (e.g. Lemon/is/pleasant) presented with the order of subject (S), object (O) and verb (V), which is normal in Japanese grammar with S–O–V paradigm. In the first experiment, an odor was given as subject (S) and a word of pleasantness/unpleasantness was used as object (O). In contrast, in the second experiment a word of pleasaneness/unpleasanteness was used as subject (S) and odor was given as object (O). In both experiments five different odors were administered randomly using four types of Japanese statements (true-affirmative, false-affirmative, true-negative and false-negative).

The students judged the statement's truth or falsity by pushing button. In both conditions, a late negative potential was elicited by the object (O) stimulus when it was mismatched with the subject (S) in the condition in which the unpleasant odor was given. In addition, the negativities elicited by the incongruous odorant stimulus and word or the emotion had the same morphology and scalp distribution.

These results indicate that not only a word but a physical stimulus such as an odor or the emotion of pleasantness/unpleasantness which deviates from a semantic context or the emotion can also elicit the negative potentials as the same N400 component as a color patch already shown by J. Katayama and A. Yagi.

566. Ipsilateral dominance of human olfactory active centers estimated from the event-related magnetic fields measured by a 122-channel whole-head neuromagnetometer using odorant stimuli synchronized with respirations

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Olfactory event-related magnetic fields were measured by the blast method which was controlled under the synchronization with subject's respirations. Though our blast method is considered to give the trigeminal responses evoked by the puffing effects within the olfactory responses, we could clearly detect a sharp and large olfactory peak (latency: ~350–450 ms) which was different from the trigeminal response obtained by only the pulse stimulation of a fresh air. The location of an estimated olfactory nervous center is currently under debate. In this experiment six volunteer subjects who gave informed consent were participated and olfactory magnetic fields were measured by magneto-encephalography (MEG) using a 122-channel whole-head neuromagnetometer (Neuromag-122TM) in Osaka-Lerc in ETL Japan. An odorant pulse (300 ms duration) of amyl acetate was given into one (right or left) nostril of a subject ~40 times at one session of the experiments at random every 3–12 respirations. The generators of olfactory magnetic fields were estimated at two regions located fairly nonsymmetrically near the bilateral frontal areas. In almost all subjects the latency and intensity of ipsilateral olfactory MEG responses were shorter and larger than those of the contralateral olfactory MEG responses, respectively. These results suggest that the olfactory MEG responses on the ipsilateral side are generally larger and dominant than that on the contra-lateral side in the human olfactory system.

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567. Hypothalamic control of amino acid appetite

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Preference for umami taste materials, such as monosodium L-glutamate (MSG) and the 5'-ribonucleotides, IMP and GMP, varies as a consequence of protein nutrition. Rats fed diets deficient in dietary protein or an essential L-amino acid (AA), L-lysine (Lys), avidly consumed Lys, glycine and NaCl but not umami substances. However, when the rat's protein nutrition was normal or when they were recovering from deficiency, a preference for umami substances was evident. These data suggest that the central mechanism for recognition of protein malnutrition may be coupled with umami taste preference. To test this, Lys-deficient and normal rats were employed as a model for taste preference changes. AA levels in plasma and brain remain essentially unchanged throughout the day while the rat is on standard chow but are altered during Lys deficiency. The recognition site for the deficit in rats' brains was localized to the ventromedial (VMH) and lateral (LHA) hypothalamus as determined by functional MRI (4.7 Telsa). Studies of single neuron activity in the LHA of Lys-deficient rats suggested that neuronal plasticity occurred. Following Lys deficiency, cells responded specifically to Lys, both iontophoretically applied and during ingestion of AA. Other LHA neurons of non-deficient rats differentially responded to MSG. The present results suggest that the LHA and probably the VMH play important roles in recognition of deficient nutrients. Neural plasticity of hypothalamic cells helps maintain AA homeostasis. Furthermore, a preference for umami substances may be an indicator that the organism (rat or person) is free of protein malnutrition.

568. Embryonic development of taste buds in the rabbit

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Taste buds of mammals are very complex, containing a number of cells of verying morphology and function. Although numerous ultrastructural studies have examined taste buds of mammals, controversy still exists regarding the classification of cell types, and of the lineage of taste bud cells. We examined the embryonic development of rabbit taste buds by electron microscopy and immunohistochemistry. At prenatal day 27, relatively larger and lighter cells appeared in the basal portion of the epithelium. These cells were immunoreactive for CK 20, and characterized by the presence of numerous dense-cored granules, resembling those of type III cells of the adult taste bud and of Merkel cells in the epithelium. The emergence of these cells in the epithelium preceded the arrival of nerve fibers. By prenatal day 28 the glossopharyngeal nerves entered the epithelium and became associated with type III-like cells. Occasionally Schwann-like cells, resembling type II cells, accompanied by numerous nerve fibers were found to penetrate *via* the basal lamina into the epithelium at prenatal day 28. Type I cells differentiated from the surrounding keratinocytes, probably by neural induction, after prenatal day 30. Our results thus indicated that three distinct cell types (types I, II and III) in rabbit taste buds, according to the classification by R.G. Murray, are separate cell lines and never transform into one another.

569. Enhancement of odorant clearance from the olfactory receptor environment may be related to second-messenger-mediated transporter phosphorylation

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The olfactory organ of the spiny lobster, *Panulirus argus*, consists of a dense array of aesthetasc sensilla on the lateral branch of the antennule. These sensilla contain primary chemosensory neurons that are bathed in a proteinaceous sensillar lymph. Among these neurons are populations that are responsive to a variety of amino acids, including glycine and taurine. Previous work has shown that the presence of one amino acid (taurine) in the sensillar lymph can enhance the clearance of another amino acid (glycine) from the receptor environment. Analysis of the kinetics of glycine transport showed that taurine increased the rate of removal of this amino acid from the receptor environment without affecting the affinity of the transporter for its substrate. Here I describe results of the examination of the hypothesis that odorant-induced changes in the intracellular concentrations of second messengers such as diacylglycerol (DAG) and cyclic AMP (cAMP) may be responsible for phosphorylation-mediated regulation of transporter activity. Treatment of sensilla with 100 nM concentrations of the phosphatase inhibitor okadaic acid led to a 5-fold increase in glycine transport relative to untreated controls; simultaneous treatment with 1 mM taurine and 100 nM okadaic acid led to a 7-fold increase in glycine transport. In contrast, glycine transport following treatment of sensilla with phorbol esters known to stimulate protein kinase C was no different than transport by control sensilla. These results corroborate the hypothesis that amino acid transport can be up-regulated by conditions that enhance the phosphorylation state of proteins. The results further suggest that the enzymes that bring about such phosphorylation are not DAG-activated protein kinases, while leaving open the possibility they are protein kinases that are activated by cAMP.

Supported by NIDR R03 DE11916-02.

570. Efferent connections of rNST neurons expressing quinine-elicited Fos

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Gustatory stimulation with sucrose or quinine elicits expression of the Fos protein in the rostral division of the nucleus of the solitary tract (rNST) and some of these cells project to PBN. The aim of the current study was to further characterize rNST neurons expressing gustatory-elicited Fos-like immunoreactivity (FLI) by comparing FLI cells that project to the PBN or reticular formation (RF). In anesthetized male rats, injections of the retrograde tracer, Fluorogold (FG), were made into the PBN (n = 12) or medullary RF (n = 4) and intraoral cannulas were implanted. After 10–14 days, rats were placed in a testing chamber and stimulated with 0.003 M quinine–HCl (n = 9) or left undisturbed (n = 7). After

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testing, animals were anesthetized and perfused, and frozen brainstem sections were cut and reacted immunohistochemically for Fos using nickel-enhanced DAB as the chromogen, and for FG using naphthol/pyronin. Consistent with previous results, the subnuclear distributions of PBN- and RF-projection neurons were distinct. Both were present in all subnuclei, but PBN-projection cells were most numerous in the rostral central (RC) subnucleus; RF-projection cells in the ventral subnucleus (V). Gustatory stimulation induced a subnuclear pattern of FLI that resembled that of PBN-projection cells; i.e. FLI neurons were most numerous in RC, though notable numbers occurred in V. Cell counts across 4 levels of rNST for quinine-stimulated animals with PBN (n = 7) or RF injections (n = 2) revealed similar numbers of FLI neurons (231 ± 40 versus 265 ± 63) but more PBN (823 ± 87.6) than RF (221 ± 176.6) projection cells. Subsets of both efferent neuron types were double-labeled but a smaller proportion of RF- than PBN-projection neurons (2.3 ± 0 versus 5.1 ± .9%) expressed FLI, suggesting a greater representation of FLI cells in the ascending pathway. Further, only a minority of FLI neurons were double-labeled after injections into either PBN or RF (maximal percentages = 30.9 and 4.5% respectively), suggesting that yet other rNST neurons that express gustatory-elicited FLI may be interneurons with local connections or that project to the caudal NST. Supported by DC-00416.

571. Distribution of Fos-like immunoreactivity following stimulation with Q–HCl in decerebrate rats

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Previous work in our laboratory has demonstrated a consistent pattern of Fos-like immunoreactivity (FLI) in awake rats following intraoral stimulation with quinine–HCl (Q–HCl). Labeled neurons concentrated in the medial portion of the central subnucleus of the rostral nucleus of the solitary tract (rNST) are contiguous with a distribution of labeled neurons arcing ventrally into the parvocellular (PCRt) and intermediate zones of the reticular formation, and may constitute the 'early' sensory components of a polysynaptic pathway mediating taste driven rejection behavior intrinsic to the brainstem. We predicted that a similar distribution of neurons should thus be evident in a chronic decerebrate (CD) preparation stimulated with Q–HCl. To test this hypothesis, eight rats were decerebrated at the supracollicular level. Four CD rats were stimulated with 7.2 ml of 0.003 M Q–HCl over a 30 min period and four CD rats served as unstimulated controls. Following an additional 30 min, the animals were anesthetized and perfused and the brain was removed, sectioned and treated with conventional FOS immunohistochemistry. Additional control groups of non-decerebrate rats received either Q–HCl or no stimulation. Overall, CD rats showed a 57% decrease in FLI in the medulla compared with intact rats, however there was label in the nucleus prepositus and raphe obscurus, structures not labeled in the intact preparations. Despite this reduction and difference in pattern of label in CD rats, the distribution of label in other structures was highly similar to intact preparations. Thus, there was more FLI in the rNST following Q–HCl stimulation (CD, X = 50; intact, X = 70) compared with non-stimulated rats (CD, X = 2; intact, X = 9) and it too was concentrated in the medial aspect of the central subdivision of the rNST. Although not as pronounced as intact preparations, Q–HCl stimulated CD rats also had more FLI in each of six subdivisions of the RF, particularly in the PCRt. In conclusion, despite some differences in the pattern and absolute amount of FLI between CD and intact rats, medullary areas associated with oral sensory and orosensori-motor processing were similarly labeled in all rats receiving Q–HCl stimulation.

Supported by DC 00417 to J.B.T. and DK 21397 to H.J.G.

572. Measurement of odour emissions from industrial plants

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The industrial processing of food and beverages causes odour emissions, which are hardly accepted by the population or the neighbourhood of industrial plants. The evaluation of odour emissions as a legal issue is very difficult.

The most common measuring method of odour emissions is olfactometry. Based on the human sense of smell the aim is to determine the odour potential of industrial plants. Odour emissions are quantified by the odorant concentration, which results from the dilution factor of the gas sample with neutral air down to the odour treshold of the panelist.

The scope of research is to compare the olfactometric results with analytical measuring methods of odours.

Gas chromatography and mass spectroscopy (GC/MS) are used to identify and quantify the single compounds of the odour mixtures. The objective is to find indicator compounds, which correlate with the human odour sensation. Because of the splitting of the gas sample synergetic effects of the various compounds are not recognized.

Therefore a new measuring technique with sensor-arrays (electronic nose) is applied. In advantage the whole odour mixture is analysed. Using non selective, mass sensitve sensors the analysis is based on pattern recognition and training of odour recognition.

These analytical methods will be compared with the olfactometric measuring method. The topics of research are the odour emissions of breweries during the mash and whort boiling. The changing composition of the odour mixture during the boiling, e.g. varying malt and hop ingredients, are taken into account.

573. Expression of 7-TMD olfactory receptor proteins in olfactory receptor neuron cell lines

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Cell lines of primary olfactory neurons were generated by c-myc oncogene transformation of primary cultures of olfactory neuroepithlium (MacDonald *et al.*, 1996). We were interested in determining whether these olfactory receptor neuron cell lines express mRNA for members of the multigene family of seven-transmembrane-domain odorant receptor proteins. Two cell

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lines, 4.4.1 and 8.1, expressed primary olfactory neuron markers and exhibited a neuronal phenotype upon serum starvation. We used the reverse transcription polymerase chain reaction (RT–PCR) and a modified version of 3'RACE to determine whether odorants receptors were expressed in either differentiated or undifferentiated 4.4.1 and 8.1 cell lines. We adopted this methodological approach to try and overcome the difficulties associated with RT–PCR from mRNA derived from genes which do not contain introns. To control for any possible genomic contamination, all PCR reactions were carried out in tandem with a no reverse transcriptase control. PCR products were Southern blotted and probed with an internal oligonucleotide specific for odorant receptors. Message RNA from rat olfactory neuroepithelium was used as a positive control. The identity of receptors from cell lines remains to be determined by DNA sequencing.

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574. Changes in olfactory behavior of male mice after intranasal injection of GABA

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It is well known that GABA is one of the most important neurotransmitters in the brain and particularly in the olfactory bulb. This compound does not pass through the blood–brain barrier. In our previous experiments (Gladysheva and Troitskaya, 1994) it was shown that intranasal injection of GABA caused suppression of aggressive behavior of male mice in comparison to mice which have got the same or even higher doses of GABA intraperitoneally. The difference between effective doses was at least 1000-fold. One of the reasons of such an effect is that GABA, when injected into the nasal cavity, can be transported to the olfactory bulb through the olfactory epithelium and the olfactory nerve. In this case it has not to pass the blood-brain barrier. This work presents results of behavioral experiments in which the possible changes of olfactory behavior and olfactory sensitivity to isovaleric acid (IVA) were tested in male mice after intranasal injection of GABA. Two concentrations of IVA were examined (10^{-2} and 10^{-5} M). The first is repellent to male mice, the second is attractive. The behavioral reaction to both concentrations of IVA disappeared within 15 min after intranasal injection of 0.5 mg/kg GABA. The same dose, when injected intraperitoneally, was ineffective. The effect of GABA lasted for >7 days. In the second series of experiments changes in olfactory sensitivity were tested. In odor aversion learning tests mice which were intranasally treated with GABA (1 mg/kg) could not distinguish within 15 min the threshold concentration and the concentrations above the threshold of IVA. The possible reason of this effect might be that the GABA receptors in the olfactory bulb are blocked at high concentrations of GABA.

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575. Electrical coupling between supporting cells in frog olfactory epithelium

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Supporting cells were recorded from slices of frog olfactory epithelium using patch-clamp methods. Single channel recordings in the cell-attached mode with 4 mM K^+ in the bath (K^+_o) indicated an intracellular potential (IP) of –68 ± 7 mV (n = 22). The input resistance was 32 ± 14 MΩ (n = 15). With 100 mM KCl in the pipette IP was –67 ± 4 mV (n = 32) in the whole-cell mode. Decreasing K^+_o to 2.5 mM increased IP to –73 ± 8 mV whereas increasing K^+_o to 8.5 mM decreased IP to –54 ± 5 mV (n = 12). Both the low input resistance and the dependence of IP upon changes in K^+_o support the hypothesis of glial-like properties of olfactory supporting cells, as proposed from intracellular recordings *in vivo* (Trotier and MacLeod, 1986; Khayari *et al.*, 1991).

When K^+ was replaced by Cs^+ in the recording pipette, to block K^+ channels and to set E_K = 0 mV, the IP was not altered significantly (n = 12). Depolarizing responses were still recorded during odorant stimulations (n = 5). The addition of 1 mM octanol in the bath induced a large increase of the input resistance (>500 MΩ) and a complete depolarization of the recorded cell loaded with CsCl (n = 8). As octanol is known to block intercellular connections through gap-junctions in other preparations we interpret these observations by the electrical uncoupling of the recorded cell with the neighboring supporting cells.

The electrical coupling between supporting cells suggested by these experiments would facilitate the clearance of potassium ions released by olfactory receptor neurons in the extracellular medium during odorant stimulations.

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576. Scanning electron microscopy of the rat vomeronasal organ and its isolated receptor neurones

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The application of patch clamp methods to isolated receptor neurones from the vomeronasal organ of amphibia and mammals has become a powerful tool for analysis of the membrane processes in these cells. As cell morphology can indicate the functionality of cells we have performed a scanning electron microscopical study of the morphology of the receptor neurones in (i) the intact vomeronasal organ and (ii) of the isolated receptor neurones.

Cross sections of the vomeronasal organ showed the tightly packed receptor neurones in the medial wall. The cell soma was ~12 times 15 mm. The dendrite was slightly less than 1 mm in diameter and extended from the cell body to the surface of the organ. A characteristic feature of the microvillous receptor neurones was their long dendrites varying between 30 and 100 mm.

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The dendrite ended in a club-shaped terminal vesicle 7 mm long and 3 mm in diameter. The distal portion of the terminal vesicle contained a large number (>50) of microvilli. The isolated receptor neurones were mainly of the same morphology as the ones observed *in situ*, but their dendrites were often bended. The length of the microvilli of the isolated receptor neurones were shorter than those observed *in situ*. By recording from cells with long dendrites we hope to avoid cells where e.g. the chloride channels are activated due to changed morphology (Lascola and Kraig, 1996).

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577. Membrane properties of the microvillous receptor neurones of the rat vomeronasal organ

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The vomeronasal organ of rats was dissected free from its bone capsule and the sensory epithelium separated from the non-sensory tissue, placed in a Ca^{2+}-free solution and dissociated mechanically after treatment with papain. The suspension of isolated cells were placed in a recording chamber perfused with Ringer-solution containing 140 mM NaCl and 4 mM KCl. The temperature of the recording chamber was kept between 32 and 35°C by blowing hot air onto the chamber. Recordings were made with patch pipettes in gramicidin-perforated whole-cell configuration from isolated vomeronasal receptor neurones (VRNs) with a characteristic bipolar shape and a dendrite >60 μm. In voltage-clamp recordings depolarizing voltage pulses evoked a fast and transient inward current. Both the transient inward and the slowly inactivated outward currents diminished in recordings where the pipette solution contained 140 mM NaCl and 4 mM KCl. In current-clamp recordings, many receptor neurones showed discrete depolarizations which sometimes could induce action potentials without any overt stimulation. Application of a Ringer solution containing 4 μM K^+ or dihydroouabain decreased the sodium pump current, induced depolarization of cells having membrane potentials negative to –60 mV and induced action potentials. Both observations suggest that the sodium pump current contributed to set the resting membrane potential of rat VRNs as it does in the frog VRNs (Trotter and Døving, 1996).

This study indicates that microvillous receptor neurones isolated from rat vomeronasal organs are amenable to patch-clamp experiments and present membrane currents similar to those observed in frog vomeronasal microvillous receptor neurones.

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578. Noise analysis of the quinine-induced current in frog taste receptor cells

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It has been shown that quinine, a bitter tasting substance, induces a cationic membrane current in frog taste receptor cells. We analyzed the quinine-induced current fluctuation to further characterize the cationic channel conducting the current. Taste cells were enzymatically isolated from fungiform papillae of the bullfrog tongue. Membrane currents were recorded under voltage-clamp by using the whole-cell patch clamp technique. A Cs^+-containing pipette solution was used to block K^+ currents. When quinine was bath applied, it induced a steady inward current in cells (n = 11) held at –60 mV. The current data were digitized at a sampling frequency of 2 kHz after low-pass filtered (cut-off frequency, 1 kHz). The spectral density distribution was calculated by 1024 points FFT, and was fitted by a sum of two Lorentzian functions, with corner frequencies at 10–20 Hz and at 70–120 Hz. The single-channel conductance calculated from the variance versus mean current plot was 4 pS in a normal saline solution containing 1.8 mM Ca^{2+}. In a nominally Ca^{2+}-free solution, the single-channel current was doubled. Our previous study has shown that the quinine-induced current is enhanced in low extracellular [Ca^{2+}]. This enhancement could be partly attributable to the Ca^{2+} suppression of the single-channel conductance.

Supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (No. 08458272).

579. Rainbow trout (*Oncorhynchus mykiss*) alevins respond to amino acids with snapping behavior and to pH changes with dispersal behavior

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The behavior of rainbow trout larvae changes at the transition from the alevin larval stage, which feeds on nutrients from its yolk sac, to the fry stage, which feeds on exogenous resources. In daylight, alevins rest on the aquarium bottom and do not respond to organic stimuli with feeding behavior, whereas fry swim most of the time and eat food. The question of whether taste and olfaction are used by alevins to control different behaviors was investigated. Histological evidence suggests that the peripheral olfactory and taste systems are fully-developed five days prior to the onset of exogenous feeding. Behavioral responses of alevins to highly potent amino acids determined physiologically and to changes in pH were studied. Two behaviors were observed: (i) biting/snapping responses to amino acids (e.g. L-proline and L-alanine) that were not accompanied by food search; and (ii) swimming activity released by changes of >0.5 pH unit. In adult rainbow trout, swimming, turning and biting/snapping behaviors are released in response to visual and olfactory stimuli, whereas taste stimuli alone in adult anosmic rainbow trout are ineffective. The biting/snapping actions and other feeding behavior patterns in adult rainbow trout are thus not the result of simple taste mediated reflexes. In contrast, amino acids do release taste mediated reflexive biting/snapping actions in alevins, but not other

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feeding behavior patterns. Upon detecting a change of >0.5 pH unit, either greater or less than ambient level (within the range of pH 7–9), alevins respond with vigorous swimming behavior from 1–8 days before first exogenous feeding. The behavioral responses to the pH change ceased to occur immediately after the larvae started to swim in daylight. The biting/snapping reflex and swimming behavior to pH changes, which enables dispersal, were never observed in fry and adult rainbow trout.

Supported by MZT J1-7127-0487 (T.V.) and NSF IBN-9221891 (J.C.).

580. Olfactory receptor sensitivity in malaria mosquitoes with different host preferences: *Anopheles gambiae* s.s., *A. arabiensis* and *A. atroparvus*

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The responses of antennal olfactory cells to host-specific odours were investigated in three Anopheline species (Diptera, Culicidae), which differ as to host preference. *Anopheles gambiae* s.s. is strictly anthropophilic, *A. arabiensis* is opportunistic and *A. atroparvus* is mainly zoophilic. The host odours we applied were five short-chain fatty acids found in human sweat: acetic, propionic, butyric, iso-butyric and iso-valeric acid, and three cattle-derived odours: 1-octen-3-ol, and 3- and 4-methylphenol.

Receptor cells of the sensilla trichodea of all three species responded to these odours. Fatty acids evoked both increase and decrease of spontaneous spike activity, whereas stimulation with 1-octen-3-ol and the phenols resulted in excitation only. A difference in sensitivity between the species was found: the cells of *A. gambiae* s.s. and *A. arabiensis* reacted more strongly to fatty acids than the cells of *A. atroparvus*, whereas the latter were more susceptible to 1-octen-3-ol.

Currently we are measuring the response to five different doses of propionic acid, 1-octen-3-ol and 3-methylphenol. Further experiments on the antennal response to specific host odours and on CO_2 sensitivity are planned.

581. Recalling sweet taste intensities measured by *ad libitum* mixing

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The purpose of this study was to clarify methodological issues raised and discussed in earlier reports on taste memory with *ad libitum* mixing as the testing method. The potential effects of (i) the between-subjects design, (ii) prefills and (iii) training were investigated. Aqueous solutions of sucrose (4.1 or 14.6%) served as standard stimuli. In the first of the two sessions the subjects (six groups, 10–13 subjects in each) produced the standard twice by mixing low (0%) and high (33.9%) concentrations of taste substance to prefilled comparison stimuli. In the second session the subjects produced the same concentration as in the first session, ten times without the standard. The intervals between the first and second session were 1, 25 or 125 h. Different groups were used for each interval and concentration. Five prefills below and five above (range 2.0–26.4%) the standard were used. Two additional groups (one for both standards) produced the standard ten times in the first session, reproduce them after 25 h and were compared with the corresponding less trained groups. Results: (i) After the 25 h time interval, the produced concentrations were higher at 4.1% concentration than in the first, session, but not at 14.6% concentration. This finding replicates earlier results from within-subjects designs for memory of sucrose intensity in water, water-citric acid mixture and in red currant juice. (ii) The prefills had no effect on reproduced concentrations. The only substance in the solution was the one to be recalled and thus, the relative ease of the task may explain the unbiased performance. The lack of the effect of prefills suggests that the subjects do not get misled by the prefills at least when a simple medium is used. (iii) The extent of training in the first session has no effect on the produced concentrations after 25 h the training effect observed in earlier studies could be due to the more complex tasting task used than in the present study. Overall, the results support the view that *ad libitum* mixing is a reliable method in taste memory research.

582. Dopamine modulates a hyperpolarization-activated inward rectifying current (I_h) in rat olfactory receptor neurons (rORNs)

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Inward rectification is a property of cell membranes whereby an increase in membrane conductance is induced by hyperpolarization from the normal resting potential. This hyperpolarization-activated current (I_h) has been described in a variety of cell preparations including rORNs (Lynch and Barry, 1991). Its maximum conductance is regulated by the basal activity of adenylate cyclase: intracellular cAMP enhances the current by shifting its voltage dependence of activation to more positive potentials (Tokimasa and Akasu, 1990). Recent studies have implied the presence of functional dopamine D_2 receptors in rORNs (Mania-Farnell *et al.*, 1993). Since D_2 receptors act through an inhibitory G protein to inhibit adenylate cyclase, we used whole-cell patch clamp techniques to test whether dopamine can modulate I_h in cultured rORNs. Upon hyperpolarizing from a holding potential of −50 mV, a small Cs^+-sensitive inwardly rectifying current (I_h) was observed. Addition of 1 mM dopamine to the external solution reversibly decreased I_h. The average inhibition of I_h at membrane potentials between −170 and −100 mV ranged from 31 to 38%. We propose that dopamine is modulating I_h by shifting I_h voltage dependence of activation to more negative potentials. This effect of dopamine may be mediated by activation of a D_2 dopamine receptor which will result in inhibition of adenylate cyclase and a decrease in intracellular cAMP. Since I_h has been suggested to contribute to the modulation of cell excitability and to spike frequency adaptation (Lynch and Barry, 1991), the results of this study suggest that dopamine may play a role in modulating the sensitivity of ORNs to odorants.

Supported by a Ford Foundation Fellowship to G.V. and NIH NIDCD DC02994-01 to M.T.L.

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583. IL-1β elicited activity changes of forebrain gustatory neurons in the rat and rhesus monkey

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Feeding behavior has been reported to be altered after lateral hypothyalamic or, recently, pallidal microinjections of an immunomodulator, the interleukin-1beta (IL-1β). To elucidate its direct neuromodulatory capacity, in the present experiments, action potentials in the globus pallidus (GP) or orbitofrontal cortex (OBF; a key structure of the central feeding control, functionally and morphologically interrelated with the GP, and known as a secondary taste cortex in the primate) of anesthetized rats and anesthetized or alert rhesus monkeys were extracellularly recorded by means of carbon fiber multibarreled glass microelectrodes during: (i) intraoral infusion of various tastants and (ii) microelectrophoretic administration of IL-1β, glucose and 'classical' neurotransmitters. Of the GP and OBF neurons tested, 8–15% was found to respond to gustatory stimulations. Pallidal taste cells were mainly broadly tuned, whereas OBF gustatory neurons were likely to change in firing rate only to one or two taste qualities. IL-1β-responsive neurons were identified in the GP and OBF in all kinds of preparations. These cells with no exception, were also sensitive to microelectrophoretically applied glucose, i.e. proved to be the elements of the previously discovered, hierarchically organized glucose-monitoring neural network. In both structures, a high majority of gustatory neurons proved to be either facilitated or suppressed by the cytokine. The neurotransmitters elicited differential discharge rate changes of the neurons tested. Our results provided evidence for that IL-1β may exert a direct modulatory role in the central taste information processing.

584. Evidence for a role of protein kinase C in sweet taste transduction

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Recent biochemical studies revealed that sugars and synthetic sweeteners activate different second messenger systems. Sugars activate adenylyl cyclase, producing cAMP, while synthetic sweeteners activate phospholipase C (PLC), producing IP_3 and DAG (Bernhardt *et al.*, 1996). Previously, we showed that cAMP blocks a resting K^+ current in sweet-sensitive taste cells (Cummings *et al.*, 1996). The downstream targets of PLC activation are not known, but a likely candidate is Protein Kinase C (PKC). We have begun to investigate the role of PKC in sweet transduction, using a loose patch technique for recording from taste buds *in situ*. Two questions were addressed: (1) Does activation of PKC mimic the sweet response? (2) Does inhibition of PKC selectively block the response to synthetic sweeteners? Hamster fungiform taste buds were tested for their response to sucrose (200 mM), NC-01 (200 μM), a synthetic sweetener, and PDBu (10 μM), an activator of PKC. Of the 35 taste buds tested, 12 responded to both sucrose and NC-01 with trains of action potentials, and all of these taste buds also responded to PDBu. Of the 19 sweet-unresponsive taste buds tested, only 4 responded to PDBu. In another set of experiments sweet-responsive taste buds were perfused with the PKC inhibitor Bisindolylmaleimide I (BIS; 5 μM) and tested for their response to sucrose and NC-01. The response of NC-01 was reduced to 25% while—in some cases—enhancing the response to sucrose, suggesting that the two second messengers may inhibit each other. These data provide evidence for a role of PKC in the transduction of synthetic sweeteners, and furthermore, suggest that the two second messengers may interact during sweet transduction.

Supported by NIDCD 00244.

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585. Influence of a predator odor on reproductive success in golden hamsters

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This study was conducted to reveal a possible influence of a predator odor on some parameters of reproductive success (fertility, rate of juveniles' sexual maturation) in golden hamsters. In Experiment 1 female hamsters were exposed for 3 weeks prior to mating either to the urine of cats (CUF) or to water (control, CF). The mean duration of mating did not differ significantly between CUF and CF. No treatment was conducted during gestation. The number of CUF giving birth as well as CUFs' litter size were significantly reduced in comparison with CF. These findings show that: (i) exposure to cats' urine prior to mating has a negative influence on reproduction resulting in a decline in fertility; (ii) the effect is apparently not due to deficits in mating. In Experiment 2 pups were exposed either to cat's urine from postnatal day 1 (D1) until day 20 (D20) (CUJ1–20) or to water (CJ1–20). There was no exposure from D21 to D40. In Experiment 3 pups were not treated from D1 to D20 but were exposed to cats' urine or to water from D21 to D40 (CUJ21–40 and CJ21–40 respectively). At D40 juveniles of both experimental groups were unilaterally gonadectomized and reproductive organ weights (ROW) were obtained. ROW was significantly reduced in CUJ1–20 in comparison with CJ1–20 in both sexes. However, there were no differences in ROW between CUJ21–40 and CJ21–40. These findings indicate that: (i) sensitivity to olfactory stimulation differs significantly during ontogenesis; and (ii) cats' urine can suppress the reproductive development of juvenile hamsters.

586. Globular cells in the peripheral olfactory system of *Xenopus laevis* are an origin of extra-bulbar olfactory projections

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Most olfactory axons in *Xenopus laevis* terminate in the olfactory

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bulb. Some afferent axons, however, bypass the olfactory bulb and form extra-bulbar olfactory projections (EBOP) mainly into the hypothalamus. Recently, NADPH-diaphorase, a histochemical marker for EBOP, was shown to be located in receptor cells, ganglion cells of the terminal nerve (GNT), and in a new neuronal cell type, the 'Globular Cell' (Venus, 1996). All of these cells gave rise to axons joining the olfactory nerve, and thus potentially contribute to EBOP.

We performed retrograde DiI tracing to check (i) for EBOP-targets in the diencephalon (DIE), the mesencephalon (MES), the metencephalon (MET) and in the rostral myelencephalon (MYE), and (ii) for correlations between different cell types and their EBOP.

At stage 46/48, most receptor cells and globular cells labeled from MET and MYE were located medially in the principal cavity. From stage 57 onwards, globular cells could only be traced from DIE, whereas receptor cells were also labeled from MES. At stage 52, several cells were labeled in the 'Anlage' of the middle cavity after dye-application to DIE. From stage 56 onwards GNT-cells were labeled from MES. In juveniles, tracing from MES and DIE led to receptor cell labeling in the middle cavity and in the oral cupola of the principal cavity, whereas globular cells were traced only from DIE.

Our results reveal, that EBOP are complex, consisting of at least three different cell types projecting to various brain areas, thus indicating involvement of EBOP in different functional circuits.

Sponsored by SFB 406, GRK 225/2-97 and NIDCD # DC00446.

Reference

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587. A combinatorial model of odor discrimination using a small array of contiguous, chemically defined glomeruli

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The most recognizable anatomical feature of the primary central olfactory regions in vertebrate and invertebrate brains is the organization of the neuropil into glomeruli. Male moths typically possess a sexually dimorphic array of contiguous olfactory glomeruli known as the macroglomerular complex (MGC). These specialized glomeruli appear to be dedicated to the processing of pheromonal volatiles released by females. Using a reconstruction technique that allows visualization of the MGC in three dimensions we have defined the architecture and spatial relationships of MGC glomeruli in two species of Heliothine moth. Furthermore we have determined the tuning characteristics of projection interneurons (PNs) that innervate the MGC glomeruli and project to higher centers in the brain. In *Helicoverpa zea* males, PNs tuned to each of the two components required for upwind flight arborize specifically in two separate glomeruli. The third MGC glomerulus in this species houses PNs which are tuned to compounds antagonistic to upwind flight in behavioral bioassays. We hypothesize that a combination of PNs innervating glomeruli 1 and 2 have to be activated for upwind flight to occur, but activation of glomerulus 3 in combination with the other two has a disruptive influence on upwind flight.

Similarly in *Heliothis virescens*, cells responsive to the two essential components of the pheromone blend arborize in two anatomically distinct glomeruli, which we predict must be activated in combination for upwind flight to occur. At least one of the two other glomeruli in the MGC appears tuned (based upon receptor neuron recordings and fills) specifically to an antagonistic compound. Thus, we believe that activation of different glomerular arrays within the MGC may provide a means for discriminating attractant versus antagonist chemical signals.

Supported by USDA/NRICGP grant 95-37302-1833 to T.A.C and T.C. Baker.

588. Steroid regulation of olfactory development: ecdysteroid action in olfactory epithelia of larval and adult *Manduca sexta*

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Postembryonic development of moth olfactory epithelia requires the persistence of olfactory sensilla through each larval molt and the establishment of a numerous and diverse olfactory sensilla during adult development. Temporal coordination is hypothesized to be under the control of the insect steroid ecdysteroid hormones and the sesquiterpenoid juvenile hormone (JH). In larvae, olfactory sensilla are recycled during each molt; many molecular and cellular events occurring throughout the animal during the molt cycle are regulated by ecdysteroids and JH. When the animal molts from larva to pupa, adult development and metamorphosis initiates; the adult antenna imaginal discs evert to establish the adult olfactory epithelium. Many cellular events which occur in the developing CNS have been shown to be regulated by changing levels of ecdysteroid during this period.

Adult expression of the odorant binding proteins PBP and GOBP2 is triggered by a decline in ecdysteroids late during adult development. We now report that GOBP2 but not PBP is expressed in the larval olfactory sensilla; expression fluctuates during the molt in a manner suggesting ecdysteroid regulation. Adult sensory neurogenesis is sensitive to ecdysteroid treatment, and spatial patterns of ecdysone receptor correlate with patterns of neurogenesis. We propose that GOBP2 and PBP are expressed in response to declining levels of ecdysteroids; that larval expression of PBP is inhibited by the presence of JH; and that larval expression of GOBP2 is inhibited by increasing levels of ecdysteroid which precede each molt and reactivated by declining ecdysteroids which finalize the molt. Neurogenesis of different populations of adult sensilla is temporally coordinated by an increasing level of pupal ecdysteroids and by complex expression patterns of ecdysteroid receptors.

Supported by NIH-NIDCD DC-00588 and USDA-CSRS 94-37302-0615.

589. Chemoreceptor cell responses to odor gradients

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The American lobster (*Homarus americanus*) uses chemoreceptor cells in its lateral antennules to orient to distant odor sources in a patchy odor environment. Within an odor plume, these patches are formed by turbulent odor dispersal and have different sizes and

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concentrations. Odor onset slopes form a strong gradient within a plume that could help a lobster to find the odor source. In previous studies of temporal filter properties of chemoreceptor cells, stimulus pulses ('patches') of different amplitude and duration with very steep onset slopes resulted in rapid adaptation. In the present study, we measured the response of taurine (Tau)-sensitive cells to stimulus pulses with different onset slopes, a parameter not previously tested for its effect on response patterns. The chemoreceptor responses were recorded extracellularly, and the stimulus profile was monitored in real time. Antennular Tau-sensitive chemoreceptor cells showed different thresholds (10^{-9}–10^{-7} M) and different adaptation and/or disadaptation (recovery) rates. The shallowest slope failed to elicit a response. With intermediate stimulus onset slopes, Tau-sensitive cells continued to respond as long as the stimulus concentration increased. Steeper onset slopes caused stronger adaptation, i.e. cells did not respond beyond the initial stimulus onset. Theoretically, a population of such cells could encode long stimulus events. These properties allow receptor cells to respond best to the edges of arriving odor patches and to classify patches by their onset slopes. These cellular feature extraction capabilities may be the basis of gradient search in turbulent plumes.

Supported by a grant from National Science Foundation (IBN9222774).

590. Is there a critical period during neonatal development for imprinting odor?

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Neonatal odor exposure produces changes in olfaction. Adult CBA/J (CBA) mice are highly sensitive to androstenone (AND). Young CBA mice were exposed to 0.1% AND during days 1–14 (n = 8), 14–28 (n = 12) or 28–42 (n = 8) after birth. Similarly exposed adult CBA mice (9–11 weeks of age; n = 8) served as controls. CBA mice exposed to the diluent during days 14–28 (n = 8) were additional controls. Y-maze training and testing commenced 5 weeks after exposures. Fluid-deprived mice were trained to avoid the AND-containing Y-maze arm. Quinine-tainted water, paired with AND, produced the aversion. Sweet water was the reward for correct choices during training and testing trials. AND exposures during days 1–14 did not affect AND sensitivity; thresholds did not differ significantly from either control group. Relative to the age-matched controls, AND exposures during days 14–28 produced >600-fold increase in sensitivity to AND, while the same exposures during adulthood produced a 16-fold increase in sensitivity, relative to the same control group. AND exposures during days 28–42 also produced a 16-fold increase in sensitivity. These results differ from previous findings wherein a much greater induced sensitivity in adults was noted, perhaps because different procedures were employed herein. The present results, however, are consistent with previous findings with rats and mice, which revealed a similar critical period during days 14–28 for affecting sensitivity to and recognition of individual-specific urine in adulthood. Hence, it appears that there is a critical period 2 weeks after birth, just after the eyes open, during which odor imprinting can be modulated. The mechanism(s) underlying this phenomenon have yet to be determined.

Supported, in part, by NIH TW00495, DC00298 and DC00214, and the Russian Foundation for Basic Research # 960451060a.

591. Use of olfactometry and substantivity data in improving performance of a perfume

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In perfumery, the need for an objective evaluation of the performance of a perfume composition implies the development of increasingly sophisticated techniques for the measurement of the diffusion and organoleptic activity of each component on skin, clothing or other surfaces. To answer this need, we have developed an olfactometric system which allows the measurement of the properties of either one or a mixture of components. From a practical point of view, the odorant under study is injected, by means of a syringe pump, into the heated, lower part of an olfactometer under an atmosphere of nitrogen. These conditions result in the continual evaporation of the odorant into the higher part of the instrument where dilution with humidified air occurs, and guarantees perfect control of the gas-phase concentration at the sniffing port of the system. The organoleptic evaluation, with a panel of thirty people, uses the triangle test method in which decreasing (factor of two) concentrations are utilized to measure the ability of the panelists to detect the odorant under study. The panel also quantifies the perceived intensity by choosing between five distinct levels. This allow the graphical representation of the relationship between the perceived intensity and the gas-phase concentration of the odorant. In parallel, our method for the measurement of the diffusion in air of odorants applied onto skin, using the Headspace technique, allows us to express the measured vapor phase concentration as a function of time (Scentwaves™). It is thus possible to combine these data with the olfactometric graphs to evaluate the olfactive impact of the odorant under the realistic conditions of its application. We will illustrate these measurements using a series of different perfume accords.

592. Functional regionalization within lobster olfactory glomeruli

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Four lines of evidence that we heretofore reported separately, together strongly imply that three anatomically distinct regions of lobster olfactory glomeruli (cap, subcap, base) are also functionally distinct. First, receptor cells penetrate the entire length of the glomerulus, presumably providing primary afferent input to all three regions. They arborize most heavily in the outermost cap region. Second, three subtypes of multiglomerular local interneurons each branch exclusively in a different one of the three glomerular regions. Each subtype shows a distinct physiological response to afferent nerve stimulation. Third, two subtypes of output neurons that project to different brain regions arise in different glomerular regions. Those going to the protocerebrum arborize primarily in the base region, while those going to the accessory lobe of the deutocerebrum branch primarily in the subcap region. Finally, GABA and histamine, two neurotransmitters mediating inhibitory glomerular processing, are different-

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ially distributed across the three glomerular regions. We are now beginning to characterize the three subtypes of local inter- neurons in more detail in order to understand the nature of sub-glomerular processing. These findings, along with emerging evidence from other systems, are consistent with the idea that olfactory glomeruli are functionally heterogeneous structures and imply that this organizational feature is fundamental to how information is analyzed at the glomerular level of the olfactory pathway.

Supported by grant # IBN 95-15307 from the National Science Foundation.

593. Various bitter compounds are effective in reducing the taste nerve responses to CO_2 in 5% alcohol solution in rats

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It has been observed that when either CO_2 or alcohol are presented in aqueous solution, they evoke responses not only in the lingual trigeminal, but also in the taste nerves [chorda tympani (CT) and glossopharyngeal nerves] in rats. We are investigating the interaction between the CT nerve responses to these substances and those to basic tastants. In this study, we recorded from the CT nerve in SD rats that were exposed to various taste solutions kept at tongue temperature (28°C) or low temperature (5°C). The whole-nerve response was assessed by calculating the ratio of peak height: background noise height. The stimuli were 0.5 M sucrose, 0.1 M NaCl, 1 mM citric acid, 0.035 M monosodium glutamate (MSG) and various bitter compounds (0.1 mM quinine sulfate, 30 mM caffeine, 0.17 g/l of isohumulones, 0.17 g/l of reduced isohumulones, 0.17 g/l of tetrahydro-isohumulones and 0.17 g/l of hexahydro-isohumulones). The isohumulone derivatives were generously gifted from Kalsec Inc., MI, USA. Each of the above tastants was presented in four different test solutions: (i) aqueous solution, (ii) 800 ppm CO_2 solution, (iii) 5% alcohol (ethanol) solution and (iv) 800 ppm CO_2 + 5% ethanol solution.

The presentation in CO_2 solution of sucrose, citric acid, or MSG led to an additive effect (between the response to CO_2 solution alone and that to the aqueous solution of the tastant). However, this was not true for NaCl or for any of the bitter compounds tested. All the bitter compounds induced either a very slight CT nerve response or a moderate response (quinine sulfate) when in aqeous solution. However, when they were presented in CO_2 + ethanol solution, the response was much smaller than that to CO_2 + ethanol solution alone.

Supported by Kirin Brewery Co., Ltd.

594. Canine olfactory sensitivity to cocaine hydrochloride and methyl benzoate

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Methyl benzoate is a consistent product of cocaine hydrochloride exposed to humid air. The detection responses of canines trained to detect illicit cocaine hydrochloride may be controlled by vapor from cocaine, methyl benzoate, or other constituents of illicit cocaine. The present study addressed the following questions: (i) How capable are dogs of detecting methyl benzoate compared with cocaine hydrochloride? (ii) When dogs are trained to detect methyl benzoate, do they respond to cocaine hydrochloride as being the same or different from methyl benzoate? These questions were investigated using random source dogs trained and tested under laboratory conditions. Odor stimuli were generated and delivered by a vapor generation systems, the outputs from which were characterized by thermal desorption GC/MS. One group of dogs was trained to discriminate pharmaceutical grade and illicit cocaine hydrochloride from clean air and tested using a two lever procedure to determine their sensitivity to these substances. A second group of dogs was trained to discriminate between methyl benzoate and clean air and tested for their sensitivity to the substance. The dogs in this second group were then tested using a three lever procedure to determine whether they responded to cocaine hydrochloride as the same or different from methyl benzoate.

Supported by DAAD05-93-D07021 and DAAD05-96-D7019.

595. An artificial olfactory system based on optical sensor arrays and temporal coding

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The primary receptor cells of the olfactory system generate a complex set of response patterns upon exposure to an odorant. These cells are broadly cross-reactive yet their responses encode information sufficient for final odorant identification which is carried out by processing in the neural circuits of the olfactory bulb and higher cortical regions of the brain. We have employed similar functional principles found in the olfactory system to create a cross-reactive sensor array for broad-band odorant sensing in an artificial device. Optical sensors containing fluorescent indicators are used to generate response patterns that are analyzed by both computational neural and neuronal networks. The key to successful odorant identification with such arrays appears to be incorporation of both the temporal profiles of response as well as response differences across the array after exposure to short, defined odorant pulses. The resulting information-rich signals enable the system to discriminate among odors on the basis of overall chemical structure as well as on their constituent properties. We have begun to test the effects of manipulating the temporal structure of the stimulus by observing sensor array responses in nasal cavity models having a number of different geometries.

596. Mood benefits of fragrance

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Fragrance evokes pleasant images and memories. Fragrance enhances a person's mood, facilitates social interaction and increases visual attention to detail. It is curious that the methods currently used to evaluate the benefit of fragrance measure only its

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intensity, pleasantness and quality. Until now methods have not been developed to measure what may be its most important property—its unique ability to evoke positive mood associations.

Over the past 12 years IFF has developed methods to measure and map the mood associations evoked by fragrance ingredients and finished fragrances. These methods will be illustrated for IFF's living flower and fruit aromas along with some of the essential oils used for the practice of aromatherapy. Our work shows that odor evokes stable mood associations whose nature is related to the quality of the odor.

597. Proliferation in the olfactory epithelium: changes during postnatal life in the rat

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Vertebrate olfactory sensory neurons are continuously replaced throughout life by the division of basal cells and differentiation of the daughter cells into neurons. In contrast with other mammals, the rat's olfactory receptor sheet continues to grow and increase in size even in the adult (Hinds and McNelly, 1981). The question arises: Is there an increase in proliferation because of the increased number of neurons that have to be replaced?

We used the BrdU-method to label dividing cells in the olfactory epithelium, and counted proliferating cells in 10 μm paraffin sections of male and female Sprague–Dawley rats aged P1 (birth) to P333. The proliferation density (number of labeled cells per mm olfactory epithelium = 'cells' in table) in the basal compartment decreases dramatically and continuously with age:

Age	P1	P11	P21	P40	P66	P105	P181	P333
Cells	151	121	70	37	24	17	10	8

Thus, although there is a pronounced increase in total number of neurons, the density of proliferating cells decreases with age. With the increase in size of the olfactory sheet the expectation is that the number of proliferating cells would also increase, to replace dying cells in a larger neuronal population, assuming that the life span of individual neurons did not change. Our results indicate that the life span of mature cells must increase in the older animals. This finding is consistent with those in an earlier report, that the relative number of mature neurons increases with age whereas the number of immature neurons decreases (Verhaagen *et al.*, 1989, J. Neurosci., 9:683).

Supported by NIH grant # DC 00347.

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598. Spectral tuning and sex-specific sensitivity in the feeding claws of male and female fiddler crabs

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We investigated responses of single chemosensory neurons in the claw of male and female *Uca pugnax* to a suite of 10 single compounds. These compounds represent a range of behaviorally active, naturally occurring feeding stimulants, consisting of: the sugars galactose, glucose, maltose, sucrose and the L-amino acids, α-aminobutyric acid, betaine, glutamic acid, proline, serine and valine. We have previously shown that mixtures of these compounds elicit sex-specific responses in fiddler crabs, with female neurons exhibiting stronger responses and lower thresholds than in male neurons. The results of this investigation revealed that chemosensors in the claws of fiddler crabs have high thresholds ($\sim 10^{-5}$), show little evidence of response plateaus even with intensely concentrated stimuli (0.1–1 M) and are rather broadly tuned. Most compounds elicited appreciable spiking activity in a single neuron, and some cells responded nearly equally to all 10 stimuli. Sugars, the dominant naturally occurring stimulus, elicited the greatest activity of all compounds except for the amino acids betaine and glutamic acid. These response patterns are not surprising in light of the chemical stimulus environment, which is characterized by high levels of sugars and other organic compounds. Sex-specificity in the response profiles was also evident. Dose-response functions at concentrations from 10^{-5} to 1 M show all of the compounds elicited greater activity in female than in male neurons. Female neurons generally showed lower thresholds than their male counterparts as well. Differences in tuning were not evident. Initial data indicate that the AC-cAMP potentiator IBMX decreases responses when presented with odorants at concentrations between 10^{-6} and 10^{-8} M, suggesting this pathway is inhibitory. Future efforts will be directed towards examining if differences in 2nd messenger systems can explain the broad sensitivity differences seen in males versus females.

Supported by NIH-NIDCD 1R29-DC02731 to M.J.W.

599. Signal transduction pathway in the mammalian vomeronasal organ: the role of IP_3

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Chemical cues from urine and glandular secretions of conspecifics act upon the dendritic microvilli of chemosensory neurons in the vomeronasal organ (VNO). Projections from the VNO ultimately regulate neurosecretions in the hypothalamus, enabling pheromones to influence reproductive physiology and behavior.

Although behavioral and physiological effects of pheromones have been studied extensively, little is known about their signal transduction pathways in mammals. This has been due partly to the small size of the VNO in most common lab animals. For this reason, we chose the pig as our model system. We detached and partially purified microvillar membranes from porcine vomeronasal neuroepithelium. This microvillar membrane preparation is enriched in adenylate cyclase and phospholipase C. Stimulation of microvillar membranes from female VNOs with boar urine and seminal fluid results in the production of inositol 1,4,5-trisphosphate (IP_3). The dose–response curve for activation of IP_3 is biphasic with a specific GTP-dependent component at low concentrations of seminal fluid and a non-specific GTP-independent component at higher concentrations. Stimulation of microvillar membranes with 1.5% (v/v) seminal fluid can be mimicked by GTPgS and is blocked by GDPbS. The

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GTP-dependent component of the IP_3 response is specific to VNO membranes.

Western blotting with antibodies specific to G protein a subunits reveals that these membranes contain Ga_s, Ga_{i2}, Ga_{i3}, Ga_o and a 40 kDa polypeptide related to $Ga_{q/11}$. The localization of the $Ga_{q/11}$ protein to the dendritic microvilli of the vomeronasal neurons suggests that this G protein may mediate pheromone signaling via the IP_3 pathway.

Supported by grants from the US Army Research Office and the North Carolina Biotechnology Center.

600. Functional expression of odorant receptors in HEK 293 cells

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The ability of the mammalian olfactory system to detect and discriminate thousands of different odorants has led to intensive efforts to understand the underlying molecular mechanisms. The first members of the olfactory receptor gene superfamily were cloned from rat (Buck and Axel, 1991), later nearly hundred additional olfactory receptor genes were identified in several species. The candidate olfactory receptors contain all the characteristic feature of G-protein coupled receptors with seven transmembrane domains. Evidence that this family encodes odorant receptors causes from the exclusive expression in the olfactory epithelium. However, the crucial information that the new gene family indeed encodes the diverse array of odorant receptors can only be achieved by functional expression of the receptor encoding DNA in surrogate cells.

Here we describe Ca-imaging experiments, immunohistochemical and western blot results indicating that we were able to express odorant receptors of the zebrafish functionally in human embryonic kidney cell line (HEK 293).

We constructed an eukaryotic expression vector, pSMyc, which contains a CMV promoter and a membrane import sequence followed by a myc-tag. The receptor encoding DNA has been cloned into this vector to reveal a fusionprotein tagged at the extracellular N-terminal site. HEK 293 cells have been transfected with these plasmids. For further investigations stable cell lines have been established.

Ca-imaging has been performed by a dual wavelength measurement of fura-2 using a set up based on a Zeiss axiovert microscope to which a custom-made dual wavelength exitation device without moving optical components was adapted. Application of TetraMin fish food solution elicits a transient increase in intracellular [Ca] at stable or transient transfected HEK 293 cells. In contrast mock and un-transfected cells showed no changes in intracellular [Ca]. Application of various amino acids and bile acids produced no effect.

In immunohistochemical experiments transfected cells have been treated with the 9E10 antibody against the extracellular myc-tag. These cells showed a distinct staining of the cell membrane, indicating the receptor proteins are localized in a physiological orientation in the cell membrane. Transfected cells have been harvested and analyzed on a SDS–PAGE and in Western blot experiments. Treatment with the 9E10 antibody revealed a 50 kDa band representing the olfactory receptors. These data are confirm with previous examinations on native receptor proteins from the rat olfactory epithelium.

We thank S. Korsching for providing the olfactory receptors.

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601. Evidence for genotoxicity, but not regenerative cell proliferation, by alachlor in rat olfactory mucosa

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Alachlor [2-chloro–2′6′-diethyl-*N*-(methoxymethyl)acetanilide)], a pre-emergent herbicide and ground and surface water contaminant, is used extensively in the production of a variety of important food crops. Chronic rodent bioassays resulted in a dose-dependent incidence of nasal turbinate tumors in rats over 14–126 mg/kg body wt/day. Although previous data indicate that alachlor is nonmutagenic even in the presence of liver S9, this observation does not address target tissue bioactivation and potential cytotoxic and regenerative responses. This study examined whether histopathological lesions and proliferative changes occur in olfactory mucosa of male Long–Evans rats (6–8 weeks old) dosed i.p. with alachlor. No discernible histopathological olfactory lesions were detected in rats dosed with 14, 42 and 126 mg/kg for 1 and 4 days. Lack of cytotoxicity was also observed with the high dose of alachlor (126 mg/kg) administered for up to 28 days; results consistent with the absence of treatment-related, regenerative cell proliferation as assessed with BrdU labeling. In addition, immunohistochemical analysis of p53, a regulatory protein of cell cycle check-point control, showed no evidence of a treatment-related increase in protein levels or nuclear translocalization. *In vitro* genotoxicity studies using *Salmonella typhimurium* strain TA100 showed that alachlor was clearly nonmutagenic without metabolic activation. In the presence of olfactory mucosal S9 activation system, alachlor induced a dose-dependent mutagenic response at 500–1250 µg/ plate, with toxicity at higher doses. Importantly, alachlor with a nasal respiratory mucosal S9 activating system did not produce a mutagenic response, suggesting site specificity of alachlor activation consistent with the target site of carcinogenicity. This study demonstrates that target tissue bioactivation of alachlor is responsible for the formation of a mutagenic metabolite, which may be critical in alachlor-induced nasal tumorigenesis.

Supported by ES00044, NCARS and AG13837.

602. The olfactory event-related potential in individuals with Down's syndrome

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Recent studies have demonstrated that individuals with Down's syndrome (DS) develop plaques and tangles in the brain similar to

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people with Alzheimer's disease. As a result, they show increased dementia and decreased olfactory functioning with age. The olfactory event-related potential (OERP) has been used as an objective quantitative measure of olfactory functioning in normal and clinical populations. Previous research has also demonstrated that DS individuals show irregularities in auditory and visual event-related potentials. The present study attempts to reveal the utility of the OERP components (latency and amplitude) in examining olfactory dysfunction in DS individuals. OERPs were recorded monopolarly at the Fz, Cz and Pz electrode sites, using amyl acetate at a 60-second inter-stimulus interval, for older and younger DS subjects and age-matched normal controls. Participants were screened for nasal health and odor thresholds were assessed. Results indicate that DS subjects have significantly lower amplitudes and longer latencies in the sensory and cognitive components of the OERP than normal controls. In addition, older DS subjects showed significantly longer latencies than the younger DS subjects. This suggests that it takes longer to detect odor stimuli and further process odor information as a DS individual ages. The study demonstrates that the OERP may be a useful measure of olfactory dysfunction in DS which may precede developing dementia in this population.

Supported by NIH grant # DC 02064 (C.M.) and training grant # DC 00032 (M.W.G.).

603. Improved vapor recognition in an artificial nose by spatio-temporal processing using an olfactory analytical network

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We previously described an artificial nose based on principles of distributed processing in the olfactory system (White *et al.*, 1996). In the present study, signals from an array of fiber-optic chemosensors are used as receptor cell input to a computational model of the olfactory bulb (OB) (White *et al.*, 1992). Given this artificial input, the computer model generates a spatio-temporal spiking pattern across simulated mitral cells that varies with vapor identity. Increases inconcentration (simulated by scaling the fiber sensor input signals) result in spike patterns with shorter latencies. To characterize the spike patterns due to different vapors and concentrations, we have developed a 'delay line neural network' (DLNN) that uses the spike timing differences among mitral cells as the basis for discrimination.The network consists of a single layer of units receiving inputs from OB mitral cells via delay lines (DLs). Spikes arrive at a DL unit with latencies that are the sum of the mitral firing latency plus theline delay. DL units receiving coincident input spikes are strongly activated, indicating an appropriate match between mitral latencies and line delays. A DL unit is initialized with mitral latencies produced by a known vapor input pattern. A DLNN therefore contains a number of DL units, each 'tuned' to a vapor of interest. Similar to mitral cells, higher concentrations elicit shorter latency DL unit activity. Unlike mitral cells, however, temporal activity related to vapor identity is no longer a component of the DL unit response. The fiber sensor input patterns are thus converted to a simple spatial code representing vapor identity and a latency code representing concentration. The OB–DLNN combination represents a biologically plausible neuronal network that discriminates among vapors, encodes vapor concentration, and performs better than standard neural networks in discriminating among vapors delivered to the sensor array.

Supported by NIDCD, NSF and ONR.

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604. Verbal and perceptual components of short-term memory for odors

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Just as a written word can be encoded and retained in memory either verbally or in a visual form, so it might seem that an odor might be retained as either a verbal description/name or as a perceptual (olfactory) code. However, one view has it that olfactory memory in the short term does not exist as a separate perceptual code. This was examined in an experimental paradigm in which errors in memory could be recognized as deriving from the substitution of similar verbal codes or of similar olfactory codes. The set of odorants presented for recall was divided into three groups: (i) base odorants (odorants that might be replaced in memory either by similar verbal or similar olfactory representations); (ii) verbal foils (stimuli dissimilar to the base stimuli in odor but which is similar in name); and (iii) odor foils (the reverse). The substitution errors made when attempting to recall test odorants were classified as verbal errors or olfactory errors. A substantial proportion of the errors were olfactory, but verbal errors also occurred. These results support the presence of short-term perceptual olfactory memory, rather than simply verbal encoding of olfactory perceptions.

Supported in part by NIH grant number 9-PO1 DC00220. The odors in this study were generously donated by International Flavors and Fragrances.

605. Synaptic organization of GABAergic dendrites in the gustatory nucleus of the solitary tract in hamster

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Neurons of the nucleus of the solitary tract (NST) exhibit more complex response characteristics than peripheral fibers. A dramatic example is the inhibitory modulation of gustatory NST neurons. Neurons containing gamma-aminobutyric acid (GABA) are a likely source of inhibition in the NST. The present study evaluates GABAergic neurons in the gustatory NST with electron microscopy to identify the axonal endings that provide their synaptic input. Sections of the NST from five hamsters were processed for pre-embedding immunocytochemistry using rabbit anti-GABA (Chemicon) and the ABC reaction. Thin sections of

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the rostral central subdivision containing 400 immunoreactive dendritic profiles were examined and their inputs classified in relation to previous descriptions of axonal ending types. GABAergic neurons were densely supplied with a variety of axonal inputs. Nearly 75% of labelled dendrites received synapses. The most prevalent input (~35% of synapses) was from MP endings packed with medium-sized pleomorphic vesicles (35–40 nm diameter) that form symmetrical and slights asymmetrical synaptic functions with labelled dendrites. Endings with small pleomorphic vesicles (30–40 nm) and symmetrical junctions accounted for ~25% of inputs. Primary-like endings with large vesicles (40–50 nm diameter) and markedly asymmetrical synapses supplied ~20% of inputs. The proportion of the three predominant ending types was similar for small caliber dendrites (<1.5 μm) and spines, medium-sized dendrites, and large dendrites (>3 μm). Other ending types were infrequent. A few labelled dendrites received inputs from GABAergic axon endings with small-medium sized pleomorphic vesicles and symmetrical synaptic junctions. The results suggest that inhibitory neurons in the gustatory NST are subject to both inhibitory and excitatory synaptic influences, the latter derive from primary afferent axons from the tongue.

Supported by NIH grant DC02045.

606. A transient class of neurons pioneer the primary olfactory pathway in the developing zebrafish *Danio rerio*

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The mechanisms that guide the first axons from the olfactory placode to the olfactory bulb in the central nervous system (CNS) are unknown. We show that there are two morphologically distinct populations of neurons in the developing olfactory placode: the first population is present at 24 h and the second at 48 h of development.

We have analyzed the initial outgrowth of axons from the placode in the zebrafish, and found that there is an early class of pioneer neurons that pre figure the primary olfactory pathway. This early group of neurons is located in the basal part of the olfactory placode, they have large, round cell bodies and lack dendrites. These neurons label with an antibody, zns-2 (Dr Bill Trevarrow, University of Oregon). At 24 h after fertilization the axons of these neurons have grown onto the differentiating telencephalon in the region of *emx-1* gene expression. The second group of neurons is evident through DiI labeling at 48 h and they have a morphology characteristic of sensory neurons with oval cell bodies and axons extending to the nare of the nose. The axons of this second wave of neurons co localize in the CNS with those of the pioneer neurons.

Using TUNL assay for cell death we have shown that the early group of pioneer neurons die as the primary olfactory sensory neurons extend their axons into the bulb. We propose that these pioneer neurons provide a pathway from the differentiating olfactory placode to the developing telencephalon where they initiate the formation of olfactory glomeruli within the CNS. And, as the later developing olfactory sensory neurons enter the CNS these early pioneers die.

607. Semaphorin expression in developing and regenerating olfactory neurons

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The regenerating olfactory receptor neurons (ORNs) have demonstrated the capacity to not only extend new axons, but to relocate and successfully reinnervate their target with functional synaptic contacts. What is not known, however, is whether the adult ORN axons use a mechanism for regeneration that is comparable to that used by developing ORN axons for ontogenetic pathfinding.

Semaphorins, a newly discovered gene family, have been shown to function in growth cone guidance by providing signals that guide growing axons to their appropriate destinations. A murine diffusible Semaphorin, M-sema III, has been shown to mediate inhibitory effects on the axons from a subclass of primary sensory neurons in E14 rat embryos (E.K. Messersmith *et al.*, Neuron 14: 949–959, 1995). We localized M-sema III expression in E12-E17 rat embryos by *in situ* hybridization techniques using ^{35}S-labeled and digoxigenin-labeled cRNA probes. M-sema III expression is seen in the olfactory epithelium and surrounding the nasal septum. We also localized low levels of M-sema III transcript expression in the ORN's of normal adult rats. The transcript expression appears to increase in the ORN's of rats that survived three days, one week, two weeks and three weeks following a unilateral bulbectomy. These findings suggest the possibility that M-sema III may play a role in directing ORNs to their target during both development and regeneration.

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608. Effects of abnormal development on the glomerular organization of the olfactory processing center of moth brains

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Virtually all vertebrates and higher invertebrates have the first synapses between olfactory receptor cells and central neurons organized into discrete spherical knots called glomeruli. In some animals a small subset of glomeruli are specialized for processing information about specific social odors, pheromones. The experiments presented here have used a sexual dimorphism in the olfactory processing center (antennal lobes) of the moth *Manduca sexta* to study the effects on adult behavior and brain anatomy of surgically reducing the number of sensory neurons growing from the antenna into the antennal lobes during metamorphosis. Our studies show that the normal glomerular structure of the female antennal lobes can be essentially eliminated. However, female moths possessing these abnormal brains exhibit odor-guided flight behavior indistinguishable from that of normal females. Florescent labeling of the sensory neurons revealed a heterogeneous, but aglomerular pattern of projections in the antennal lobes. When similar experiments are performed on developing male moths, similar results were observed, with one

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important exception. Male antennal lobes showed the same anatomical disruption in the array of 'normal glomeruli' that we observed in females, but the male specific macroglomerular complex (MGC), known to process only the female-emitted sex-attractant pheromone, continues to develop sub-structure similar to normal moths. The normal sub-structure of the MGC persists even after surgically removing over 90% of the antenna early in metamorphosis. Taken together, these results suggest that the heterogeneity observed in the sensory projection in abnormally developed female antennal lobes may underlie functional, but not anatomical glomeruli.

This study was supported by NIH grants NS-20040 and DC-00348, and NSF grants IBN-9216532 and IBN-9511742.

609. Bi-naral interactions in rat piriform cortex

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Convergence of inputs from bilateral receptive fields is a common feature of vertebrate sensory systems. This bilateral convergence allows for relative comparisons of spatially disparate inputs which can result in higher order sensory processes. In the olfactory system, the anterior piriform cortex (aPCX) receives input from the ipsilateral naris via the ipsilateral olfactory bulb and lateral olfactory tract (LOT). The most direct input from the contralateral naris reaches the aPCX from the contralateral anterior olfactory nucleus (AON) via the anterior commissure. These bilateral inputs are segregated in aPCX layer I, with ipsilateral LOT terminals on distal portions of pyramidal cell dendrites (layer Ia) and commissural terminals (along with intra-cortical fibers) on proximal portions of the dendrites (layer Ib).

In the present study, single-unit recordings were made from layer II/III aPCX neurons in adult Wistar rats to examine odor response patterns to unilaterally and bilaterally delivered stimuli. Isoamyl acetate odor stimulation was presented either unilaterally through tubes inserted into the external nares, or bilaterally during unilateral olfactory bulb lidocaine infusions. Olfactory bulb multi-unit or slow-wave activity was recorded simultaneously bilaterally to monitor selectivity of unilateral odor stimulation. The results demonstrate that (i) commissural input to aPCX neurons is sufficient to drive odor responses and (ii) aPCX neurons can be classified on the basis of spatial receptive field type. These receptive fields include cells that respond: (i) selectively to ipsilateral stimulation, (ii) selectively to contralateral stimulation, (iii) to either ipsilateral or contralateral stimulation and (iv) selectively to bilateral stimulation. Thus, in addition to odor/molecular receptive fields, aPCX single-units have spatially defined (ipsilateral/contralateral) receptive fields.

This research was supported by NIH grant DC01674.

610. Latency to discriminate as a measure of similarity of odor quality

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Latency to discriminate may offer a quantitative measure of similarity of odor quality that would permit construction of an adequate map of odor quality space where other techniques have failed to do so. Such a measure would aid research in odor quality coding by allowing comparisons of physiological results with human performance. To this end, we measured latencies of samedifferent judgments to various pairs of odors. Latencies were analyzed using a signal detection model, with the distribution of reaction times to same pairs (i.e. A versus A) being defined as noise, and the distribution of reaction times to different pairs (i.e. A versus B or A versus the binary mixture A–B) being defined as signal plus noise. In this case, d' is a measure of the sensitivity of latency to differences in odor quality. We tested a small number ofsubjects intensively on the principle that any individual's discriminative 'odor space' should resemble any other's. To a first approximation, this held true. As a check on validity, we tested for additivity of discriminability. In other words, we asked whether the sum of the d's for mixtures versus unmixed components equaled the d's for the corresponding unmixed odors. Our data did show additivity, suggesting that latency to discriminate meets an important criterion of measurement. Moreover, odor qualities (mixtures) deliberately formulated to differ did prove different from their unmixed components. Unmixed qualities also differed from one another to varying degrees. We see latency to discriminate as a very promising way to explore the organization of perceived odor quality.

Supported by grant DC00284 from The National Institute of Deafness and Other Communication Disorders.

611. Nerve fibers, intermediate filaments, connexin 43, and CD44 in developing human taste buds

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The development of human taste buds has been monitored immunohistochemically from postovulatory week 6 until adulthood. The innervation pattern of gustatory epithelia has been studied using antiser against PGP 9.5. tubulin and neural cell adhesion molecules (N-CAM, Leu 7). Autonomic and somato-sensory nerve supply has been investigated with antisera against SP, CGRP, NPY and neuronal NOS. In order to obtain information on developmental alterations of the content of taste cell-specific intermediate filaments, we used antibodiesa against keratins 7, 8, 13, 18, 19, 20 and vimentin. The hyaluronate receptor CD44 has been investigated using antibodies against the standard molecule and the two epithelial isoforms CD44 v6 and CD44 v9. Moreover, we studied the distribution of connexin 43 (Cx43) in taste buds and surrounding epithelium.

Nerve fibers invade the epithelium of papilla-like structures but some also penetrate the basal membrane of non-papllary epithelium (week 7–8). They are in close contact with slender epithelial cells that are considered to be the taste bud's progenitor cells. At the same time, CD44 and its isoforms are expressed on apical membranes of epithelial cells covering prospective taste bud primordia. This demonstrates the early determination of taste bud cells before corium papillae develop. In the adulthood, however, only the standard variant of CD44 persists on the taste bud cell surfaces. CD44 v6 and v9 label surrounding non-gustatory epithelium, but no taste buds. Cx43 indicating a possible key role of these cells as ion reservoirs.

Vimentin is located in endothelial cells, fibroblasts and

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(epithelial) marginal cells between taste bud cells and adjacent epithelial cells. Generally, the expression of keratins in lingual epithelium tends to increase from the 8th to 15th week and diminishes thereafter. However, taste bud primordia are constantly labelled throughout all developmental stages and how often the only keratin-positvie structure of the neuropeptides and vasoactive substances rendered negative results for developmental stages postovulatory weeks 7–15, elder stages were positive only for SP in dermal papillae. A secialized autonomic and somatosensory innervation of gustatofy papillae including taste buds in humans has not been observed before the 18th week of gestation.

612. Pheromone degradation in scarab beetles

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Scarab beetles utilize a very diverse repertoire of pheromones; in addition to such unusual fatty acid derivatives as g-lactones and even-number ketones, they include examples of terpenoids, phenolics, amino acid derivatives and alkaloids.

We analyzed extracts of soluble antennal proteins from several species and detected significant degradation of the γ-lactones (buibuilactone, japonilure) essentially in all of them, even in species that do not use this group of compounds as their pheromones. Activity-staining of native polyacrylamide gels showed that species possessing high levels of antennae-specific esterases show significant preference for the *R* stereoisomer. However, this specificity seems one-sided, i.e. species that utilize the *S* enantiomer do not degrade it more quickly. The antennal esterase activity was highly resistant to classical esterase inhibitors, which is consistent with results obtained previously in lepidopteran species.

Recently we isolated a peculiar pheromone with a diamide moiety [1,3-dimethyl-2,4-(1*H*,3*H*)-quinazolinedione] from *Phyllopertha diversa*, which was rapidly degraded by antennal enzymes from this species. Beetles that utilize lactones as their pheromones possess little or no ability to metabolize this compound. The structure of this molecule is very unusual for a pheromone. It is therefore not surprising that only the species that uses it as a semiochemical have developed the ability to degrade it. Lactones and esters, on the other hand, are common odorants, and therefore the presence of appropriate enzymes in a large number of species is expected. Our data point out that the antennae-specific enzymes have evolved to participate in deactivation of pheromones or other odorants.

613. Lectin-binding identification of glycoconjugates on channel catfish (*Ictalurus punctatus*) olfactory mucosa: potential cell–cell interactions during *Edwardsiella ictaluri* infection of the olfactory epithelium

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The external location of the vertebrate olfactory organ places the organism at particular risk. For example, pathogens gaining entry into olfactory receptor neurons can potentially infect the central nervous system. Our previous studies have shown that the channel catfish olfactory organ is vulnerable to infection by *Edwardsiella ictaluri*, a Gram-negative bacteria, however, the full pathogenesis remains unclear. In the present study a panel of lectins were used to define patterns of glycoconjugates of the channel catfish olfactory mucosa. Glycoconjugates were examined because of their use by bacteria as potential receptor sites between host and pathogen during the critical initial adhesion stage of infection. Also identified in this study were lectin-like proteins of *E. ictaluri* by immunogold electron microscopy. The pattern of lectin binding showed D-mannose was ubiquitous with L-fucose and *N*-acetylneuraminic acid more specifically located in apical cell regions in sensory and nonsensory mucosa. Galactose, *N*-acetylgalactosamine and *N*-acetylglucosamine expression was restricted to subsets of olfactory receptor neurons. *Edwardsiella ictaluri* expressed lectin-like proteins WGA and UEA intensely with moderate expression of ConA, SBA and PNA. These results indicate that a differential spatial expression of carbohydrates in channel catfish olfactory mucosa exists. These glycoconjugates may serve as receptors for adhesion proteins expressed by *E. ictaluri* employed during initial host cell infection.

Supported by ALAV-283.

614. Gustducin's role in sweet and bitter taste transduction: insights from biochemical and transgenic studies

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Taste receptor cells express the G-proteins gustducin and transducin and a phosphodiesterase (PDE) that can be activated by these two G-proteins. In the presence of the bitter compound denatonium benzoate, bovine taste cell membranes activate transducin and gustducin, but not G_i. Peptides that competitively inhibit rhodopsin activation of transducin also block taste membrane activation of transducin or gustducin, arguing for involvement of seven transmembrane-helix G protein-coupled receptors. We have recently solubilized a taste receptor activity that responds to denatonium, quinine and several other bitter compounds to activate transducin and gustducin. The presence in taste cells of gustducin, transducin, a G-protein-regulated PDE and a bitter receptor that activates transducin and gustducin suggests that transducin and gustducin may play similar roles in taste transduction. One possibility is that bitter tastants activate specific receptors which couple to gustducin and/or transducin to regulate PDE, and thereby, taste cell cyclic nucleotide levels. Another possibility, suggested by the work of Spielman and Brand and others, is that bitter tastants may act via phospholipase C, IP_3 and Ca^{2+}.

To gain further insight into these issues we used gene targeting to generate gustducin deficient mice. We used positive/negative selection in embryonic stem cells to generate a gustducin allele with a *neo* insertion and deletion of the ATG^{met} initiation codon and the first protein-coding exon. Homozygous gustducin null mice were viable, healthy and fertile. Immunohistochemistry showed that the gustducin null mice fail to express gustducin. The homozygous null mice were indistinguishable from wild-type in their behavioral and electrophysiological responses to salty and sour stimuli. However, the null mice had reduced aversion to two bitter compounds and reduced preference for two sweet

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compounds. Furthermore, the null mice had diminished electrophysiological responses to these same compounds. These data suggest that gustducin is a principal mediator of both bitter and sweet signal transduction. Recent studies with transgenic mice expressing mutant forms of gustducin suggest that gustducin directly interacts with sweet and bitter receptors.

615. Circadian variations in blood pressure and heart rate in rats raised on low, mid, and high dietary salt levels

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We are investigating the long-term influence of perinatal salt exposure on the blood pressure and heart rate of adult rats. Most studies have restricted measurement of blood pressure and heart rate to short intermittent time periods during one or more days. Such brief measurement periods do not take into account the variations that occur throughout the circadian cycle. Using Data Sciences' pressure transmitters and receivers in conjunction with our own data collection system, we were able to measure mean arterial pressure (MAP) and heart rate (HR) continuously for a period of several months. Three adult male Sprague–Dawley rats were implanted with an aortic electronic sensor for transmitting blood pressure and heart rate signals by telemetry. Each rat was exposed to a different dietary NaCl level in the solid chow from conception to postnatal day 30, one basal (0.10%), one mid (1%) and one high (3%). During testing, the rats are maintained on ad lib food (Purina 5001 rodent pellet) and water (deionized) while on a 12:12 light/dark cycle. Based on data collected and averaged for each animal over several consecutive days, MAP and HR were relatively low and stable during the light phase, compared with higher and more variable levels during the dark phase. The MAP of the high salt rat (108 mm Hg) tended to be greater than those of the mid (103 mm Hg) and basal (93 mm Hg) salt rats throughout the 24 h period. The mean HR difference between light and dark phases was greatest for the basal salt rat, averaging 293 bpm during the light phase and 363 during the light phase. The average heart rate changed by 70 bpm for the basal rat, 60 bpm for the mid salt rat and only 34 bpm for the high salt rat. Variations in the MAP and HR during a 24 h period indicate that circadian rhythms play a part in the physiological condition of the rat. Additionally, differences in salt exposure during the perinatal period seem to have long-term affects on adult Sprague–Dawley rats in terms of their comparative blood pressures and heart rates.

This research was supported by NIH Grant DC 02641.

616. Integration of chemosensory and hormonal input in the male Syrian hamster brain

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Mating behavior in the male Syrian hamster requires chemosensory cues from the olfactory mucosa and vomeronasal organ and gonadal steroid hormones from the testes. If either signal is interrupted, mating is abolished. Sexually relevant odors are transmitted through brain regions rich in steroid receptors, including the medial amygdaloid nucleus (Me), bed nucleus of the stria terminalis and medial preoptic area (MPOA). The males' attraction to female hamster vaginal secretion (FHVS) is influenced by the hormonal milieu, and steroid concentrations in circulation increase after exposure to FHVS. The foregoing observations support the concept that there is communication between odors and hormones in hamster brain. The aim of our studies was to determine if such communication is required for male sexual behavior.

The approach was to place a single intracerebral implant of testosterone in MPOA or Me of castrated males and remove a single olfactory bulb (UBx), either ipsilateral or contralateral to the hormone implant. Unilateral testosterone stimulation of Me or MPOA restores sexual activity in long-term castrates, and UBx does not impair sexual activity in normal males. However, UBx ipsilateral to an intracerebral hormone implant segregates odor and steroid cues in opposite hemispheres. If chemosensory and hormonal communication is required for sexual behavior, such males will fail to mate. Twelve weeks after castration, males expressed only sporadic mounts (1.1 ± 0.4 mounts/10 min) during two tests with a receptive female. After hormone implantation in MPOA with contralateral UBx, mounting increased to 14.7 ± 4.5 mounts/10 min ($P < 0.05$). However, males with UBx ipsilateral to a hormone implant in MPOA averaged only 0.7 ± 0.6 mounts/10 min. By contrast, males with testosterone in Me failed to mate after either ipsilateral or contralateral UBx. The absence of sexual activity in males with ipsilateral UBx suggests that communication between odor and steroid cues is required for male sexual behavior in the Syrian hamster. Such communication probably takes place at multiple sites in the hamster mating behavior pathway. However, as demonstrated by the contrasting effects of contralateral UBx in males with testosterone in Me or MPOA, not all regions that are sufficient for hormonal stimulation of mating are equally capable of doing so. In this regard, MPOA appears to play a central role in chemosensory and hormonal integration.

617. Suppression of antennnular grooming behavior in the Florida spiny lobster, *Panulirus argus*, by binary mixtures

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Antennular grooming behavior (AGB) is a stereospecific grooming behavior in which the first pair of antennules, the major olfactory appendages in lobsters, are clasped and wiped repetitively by the third pair of maxillipeds. AGB, which is exhibited in many crustaceans, apparently functions to clear debris from the antennules. We have shown previously that L-glutamate (Glu) was the primary chemical stimulus of antennular grooming (Barbato *et al.*, 1996). Other chemicals also commonly found in their food were either nonstimulatory or weakly stimulatory. Furthermore, complex mixtures mimicking food extracts also evoked AGB but at much lower magnitudes than L-glutamate alone. It is possible that the observed suppression of AGB was the result of inhibition by 'command centers' for behaviors such as search which were also

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activated by complex mixtures. Thus solutions that activate search behavior may suppress AGB. Conversely, search response toward a complex mixture may be inhibited by simultaneous activation of AGB. We predict that suppression of both behaviors will occur toward mixtures containing compounds that elicit both behaviors while no suppression should be observed toward mixtures containing compounds that elicit only one behavior. To test this hypothesis we presented six lobsters with solutions of Glu, glycine (Gly), adenosine-5′-monophosphate (AMP), taurine (Tau) and binary mixtures containing Glu and one each of the other three compounds. Glu, AMP and Tau but not Gly are known to elicit significant search responses. AMP and Gly elicit weak but significant AGB while Tau is nonstimulatory. Observed search and AGB responses to binary mixtures are compared with responses predicted based on a noncompetitive model incorporating responses to single compounds. In addition we examine whether these two behaviors can co-occur or are mutually exclusive.

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618. Experienced-induced changes in the mature olfactory system

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Exposure of the mature olfactory system to an odorant can both increase and decrease sensitivity to odorants depending upon the circumstances. Increased sensitivity, or sensitization, has been shown most clearly among individuals who express a specific anosmia to androstenone. Brief, repeated periods of exposure of these individuals induces an ability to detect the odorant that was previously undetectable. The effects of exposure apparently occur both peripherally, perhaps via the up-regulation of receptors, and centrally, perhaps as a result of exposure-induced neuronal reorganization. This induced sensitivity persists for several months or even years. Exposure to an odorant also can decrease sensitivity through a process labeled adaptation. While there have been many published accounts of short-term adaptation, i.e. that occurring within a few s or min of perceiving an odor, less is known about the mechanisms underlying long-term adaptation of the type experienced by individuals who work in or live by a persistently odorous site, e.g. a tannery or rendering plant. Anecdotal reports from these individuals suggest that they do not experience the perceptual 'richness' of their environment, even after returning from a long weekend holiday. It is as if they have a specific anosmia or hyposmia to the environmental odorants. After a month-long vacation, however, the normal ability to appreciate fully the environmental odors has returned. Recent results from laboratory-based experiments suggest that a long-term shift in olfactory threshold can account, in part, for these anecdotal reports. The mechanisms underlying both sensitization and long-term adaptation, as well as the relationship(s) between the two phenomena, should be the focus of future research endeavors.

Supported by NIH TW00495, DC00298 and DC00214, and the Russian Foundation for Basic Research # 960451060a.

619. Nasal-trigeminal sensitivity in normal aging and clinical populations

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During monorhinal stimulation with odorants that are also irritants, it is not possible to determine which nostril is being stimulated (i.e. to lateralize) until the concentration of the odorant exceeds the irritation threshold. This lateralization threshold can be quantified by providing the subject, simultaneously, with an odorant/irritant.sample in one nostril and a blank/diluent sample in the other nostril. Subsequent to inhalation, the task of the subject is to choose which side of the nose was stimulated by the odorant/irritant. Using this approach in a modified staircase procedure we obtained test/retest, lateralization thresholds for 1-butanol from 142 normosmics (≥10 males and 10 females in each decade of age from 20 to 89). For comparison, we also obtained test/retest, olfactory-detection thresholds for butanol. Patients presenting at the Monell-Jefferson Chemosensory Clinical Research Center with chemosensory complaints also underwent similar testing. Results from normosmics indicate significant test/retest reliability for both olfactory detection (r = 0.93) and lateralization (r = 0.76); a significant decline of olfactory sensitivity with advancing age; and a significant, but much less pronounced, age-related decline in nasal-chemesthetic sesitivity. Clinic patients were age- and sex-matched with normosmics from the previously-tested 142 individuals. Results indicate that both hyposmics (n = 32) and anosmics (n = 27) had significantly elevated lateralization thresholds relative to normosmics, whereas patients with no olfactory deficit (n = 29) did not. Combined, these results indicate a normal decline of sensitivity to intranasal irritants with advancing age; however, individuals can alsoevidence reduced sensitivity irrespective of age if they experience olfactory loss. The later also implicates a functional interaction between olfaction and chemesthesis that necessitates further research.

Supported by NIDCD P50 DC000214.

620. Odor detection and discrimination in methimazole-treated rats

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A number of agents have selective toxic effects on olfactory epithelium ('olfactotoxins'). Methimazole, a thioamide, anti-thyroid drug has been reported to cause extensive degeneration of the rat olfactory mucosa and, in behavior tests, severe hyposmia (Genter *et al.*, 1996). In our study, rats, trained in an olfactometer on odor detection and discrimination tasks of increasing difficulty, were given 300 mg/kg i.p. methimazole (n = 6) or DMSO vehicle (n = 6) and retested 1, 2 and 3 days later. In other rats olfactory sacs were injected with horseradish peroxidase (HRP) 1–5 days after methimazole treatment and sacrificed 24 h later. Experimental rats were able to detect and discriminate odors; they made somewhat but not significantly more errors than controls on most tests. Methimazole produced a disruption of HRP transport to glomeruli on medial and parts of the lateral wall of the olfactory bulb but the density of reaction product in most glomeruli was similar to that of controls. The differences between these results and those of Genter *et al.* are probably due to our use of sensitive

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behavioral tests and histological methods which assess connections between the entire epithelium and the olfactory bulb.

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621. Molecular cloning of a phospholipase C-β and a G-protein β-subunit from the American lobster olfactory organ

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In lobster olfactory signal transduction, both cAMP and IP_3 act as second messengers. We have isolated cDNA clones for a guanine nucleotide binding protein (G-protein) β-subunit and a phospholipase C (PLC) from a cDNA library made from the olfactory organ of the American lobster. The putative Gβ of lobster has high percentage of amino acid identity with known G-protein and β-subunits: 90% with *Caenorhabditis elegans*, 87% with *Drosophila* β1, 85% with human β1 and β2, 83% with mouse β4. The deduced protein sequence of the lobster PLC clone indicates that it belongs to the PLC-β subfamily. It 57% amino acid identity with a *Drosophila* PLC (*NORPA*), which plays a role in both vision and olfaction, and 35% identity with rat PLC- β1, and 33% with human PLC- β2 and PLC- β3. These PLC-β and Gβ-subunit clones, as well as previously isolated Gα-subunit clones, are putative components of the olfactory ransduction pathways.

The research was supported by NIDCD award DC02366 to T.S.McC.

622. Determinations of human olfactory active center on the MRI brain map from the olfactory magnetic fields evoked by amylacetate

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We detected the olfactory evoked magnetic fields and somatosensory fields evoked by air from six healthy subjects using a 122-channel whole head type SQUID neuromagnetometer. The amylacetate (banana like odor) was used as odorant stimuli was a concentration of 1%. This odorant gas was made by the air bubbling method outside the magnetically shielded room(MSR) and which was guided non magnetic air operational valve near the subject in MSR. Odorant pulses were given duration time of 300 ms under the synchronization with the subject's respiration. The stimuli were delivered into the right or left nostril at the one session of the experiments at random every 3–12 respiration. Approximately 40 evoked magnetic responses were averaged. Auditory responses to the outer sound noize were avoided by using ear plugs and trials containing eye blink artifacts were rejected from the averaging process.

The generators of olfactory magnetic fields were estimated on the MRI brain mapping at two regions located fairly non-symmetrically near bilateral frontal areas.

In this olfactory MEG experiments event-related magnetic fields were often dominant same as the olfactory evoked potentials on the ipsilateral side than the contralateral side at the lateral orbital sulcus in the deep frontal area which was clearly different from the contra-laterality of the trigeminal area obtained by the three times stronger fresh air stimulation more than usual odorant stimulation condition.

623. Neural processing of hedonics and quality of taste stimuli in the rat brain

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Chemical senses are processed in terms of hedonic evaluation and quality discrimination. In our experiments using c-*fos* immunoreactivity as an anatomical marker for activation of central neurons, we have shown that the subdivisions of the parabrachial nucleus (PBN) of the rat represent different aspects of hedonics and qualities of taste stimuli.

The dorsal lateral subnucleus (dls) is a possible site for representation of hedonically positive feeling, or for expression of ingestive behavior. This suggestion is derived from quantitative evaluation of c-*fos* labeling in the dls and other areas in different experimental paradigms: (i) c-*fos* expression was observed in the dls when water-deprived rats ingested palatable liquids such as water, saccharin and NaCl; (ii) c-*fos* expression was not observed in the dls to stimulations with unpalatable liquids and aversively conditioned taste solutions; (iii) familiar 0.15 M NaCl induced more c-*fos* labeling in the dls than novel one; (iv) when Na-deficient rats drank 0.3 M NaCl, those rats showed strong c-*fos* expression in the dls, while little c-*fos* expression was found in naive rats; and (v) when lysine-deficient rats ingested 0.4 M lysine–HCl, c-*fos* was expressed in the dls, while no c-*fos* was expressed in the dls when normal control rats ingested the same stimulus.

Decerebrate rats showed essentially the same c-*fos* expression pattern in the PBN when saccharin or quinine were intraorally infused as normal control rats did. c-*fos* expression in the dls following ingestion of water was diminished to some extent after bilateral section of the superior laryngeal nerve.

Neuroanatomical study using the WGA–HRP method showed that neurons in the dls mainly project to the medial preoptic area, paraventricular nucleus and the lateral area of the hypothalamus.

These results suggest that the dls receives both peripheral gustatory inputs and central inputs relating to ingestion behavior, and send information to higher brain centers including the hypothalamus.

624. Discrimination of odor differences in MHC-deficient mice

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We have shown that mice exhibit body odors of exquisite individuality (odortypes) representing polymorphism of major histocompatibility complex (MHC) genes and, less potently, of

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other chromosomal sites. Perception of odortype likely underlies mate selection, pregnancy success and parental behavior, serving to maintain normal social interactions and to promote genetic diversity through avoidance of inbreeding. Although several lines of evidence suggest MHC genes themselves code for odortypes, incontrovertible evidences of this would focus the search for mechanism directly on these known genes. Thus the purpose of this study was to determine whether mice with targeted gene disruptions (knock out mice) that do not express one class of MHC genes (termed class I) differ in body odor from otherwise identical animals expressing these genes. Mice trained in a Y maze served as the bioassay. We tested the ability of five trained mice to distinguish the urine odor of C57BL/6J β2m-deficient (–/–) male mice from the urine of C57BL/6J (+/+) male mice. The results demonstrated that mice lacking class I expression have different odortypes than otherwise identical mice expressing class I molecules. This result provides definitive evidence that MHC molecules themselves are central to determining MHC odortypes.

625. Odor-induced activation in rat olfactory bulb observed by functional magnetic resonance imaging

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In the mammalian olfactory pathway, axons from 10^7–10^8 olfactory receptor cells (ORC) in the epithelium converge onto ~2000 glomeruli in the olfactory bulb (OB). Functional analyses with 2-deoxyglucose and more recent molecular analyses of odor receptor expression suggest that the convergence of subsets of ORC axons onto specific glomeruli may underlie the establishment of an odor map in the olfactory bulb. Functional magnetic resonance imaging (fMRI) provides a completely non-invasive method for mapping brain activation, based on activation-induced changes in blood-oxygenation level. Using fMRI, we had previously mapped the activation of single whisker barrel in rat brain. In the present study, we apply fMRI to map activation in the rat olfactory bulb in urethane-anesthetized rats stimulated with amyl acetate, using multi-slice gradient-echo imaging method (in-plane resolution = 150 mm × 150 mm, slice number = 4, slice thickness = 1 mm, imaging time = 30 s and experiment protocol = 16 images per experiment, 1–8 as control and 9–16 with stimulation) at 7 Tesla. We observed spatially localized and statistically significant activation ($P > 0.001$) in the medial and lateral regions of the olfactory bulb, apparently correlating with the location of the glomerular layer. The results were reproducible intra- and inter- animal. Prior analyses of functional activity within the OB were either spatially limited, as occurs with electrophysiological analyses, or temporally limited, as occurs with 2DG. fMRI overcomes both of these limitations in that the entire OB can be repeatedly assessed with high temporal resolution (30 s per image), thus enabling intra-animal comparisons over extended periods of time.

Supported by NIH DK27121 and by NIDCD, NASA and NIMH (Human Brain Project).

626. MR evaluation of olfactory bulb in normal and anosmic patients

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MR imaging has been used as a noninvasive diagnostic tool for the detection of olfactory apparatus in patients with congenital anosmia like Kallmann's syndrome. For the evaluation of change in olfactory bulb size in patients with reduced or no sense of smell, 22 normals and 11 hyposmics or anosmics were examined by nasal endoscopy, olfactory tests and magnetic resonance imaging (MRI) of olfactory bulb. Causes of 11 hyposmics or anosmics were head trauma (3), post-URI (5), post-surgery of the nose (1) and unknown (2) without congenital origin. The durations of olfactory disorder in patients were between 1 and 10 months. Olfactory tests consisted of threshold test by bounded three-alternative forced-choice modified ascending method of limits (CCCRC test) and step method using threefold dilutions of 1-butanol and olfactory identification test using 32 natural odors familiar to Koreans. MR evaluation included the estimation of olfactory bulb size by using a 13 cm diameter general purpose surface coil with 3 mm T1-weighted MR coronal sections without interval. Cut area of olfactory bulb in MR coronal sections were measured by Hope Graph Planimeter model No. 9-003 after 5-fold enlargement.

All subjects showed well-preserved olfactory fissure in endoscopic examination without obstructive or inflammatory disease in the nose. The olfactory bulb were observed in all subject in three cuts of MR imaging and measured area of the olfactory bulb showed no significant difference between the right and left in normals and anosmics, however, area of normal subjects was larger than anosmics statistically. Relation between olfactory tests and area of olfactory bulb showed negative correlation with low correlation coefficient. MR imaging can be a useful diagnostic tool for patients with hyposmia or anosmia.

Supported by Hallym University Medical Center.

627. Role for calmodulin and the plasma membrane calcium pump in glutamate chemoresponse

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There appear to be three different signal transduction pathways in *Paramecium* for chemoresponse to acetate, NH_4Cl and glutamate, respectively. There is evidence that a plasma membrane calcium pump rather than a channel is responsible for the hyperpolarization of the cells upon stimulation with acetate and glutamate, and evidence against such a pump's involvement in chemoresponse to NH_4Cl. The pump is calmodulin regulated and we attempted to demonstrate the involvement of the pump indirectly by down regulating calmodulin with antisense oligonucleotides (ODNs). Electroporation of the antisense ODNs resulted in loss of chemoresponse to Na-acetate and normal response to NH_4Cl. However, electroporation disrupted response to glutamate, with both sense and antisense ODNs. To test for the involvement of calmodulin and the pump in glutamate chemoresponse, we developed expression plasmids for antisense RNA forcalmodulin. ELISAs show a decrease up to 50% in calcium protein in some transformed lines. RT–PCR confirms that

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there is a decrease in calmodulin mRNA in these lines. T-maze assays of chemoresponse show a consistent decrease or loss of response to glutamate and variable loss of response to acetate, as a function of cell growth condition. These data complement those of the earlier electroporated ODNs and implicate calmodulin and the plasma membrane calcium pump in two of the three pathways.

Supported by NIH and the VCC.

628. Calcium pump in chemoresponse: role of the calmodulin binding domain

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Two of at least three signal transduction pathways in *Paramecium* appear to include the plasma membrane calcium pump in the stimulus pathways. Plasma membrane calcium pumps are calmodulin regulated through a C terminus calmodulin binding domain. This domain distinguishes the plasma membrane pump from intracellular pumps. One approach to verify the participation of the plasma membrane pump is to down regulate calmodulin, which regulates the pump and correlate this down regulation with reduced chemoresponse. However, in an attempt to affect pump activity in a more direct way, we have subcloned the C terminus region of the *P. tetraurelia* plasma membrane calcium pump and expressed it both in bacteria and paramecia. The calmodulin binding domain is expressed in bacteria as a GST fusion protein. Both the fusion protein and the cleaved calmodulin binding domain have calmodulin binding activity on electroblots. The peptide produced in bacteria is phosphorylated on serine and not threonine. The C terminal peptide sequence has been subcloned into a newly developed expression plasmid and used to stably transform paramecia. The expression of the peptide is demonstrated with Western blots using a polyclonal antibody developed against the 17 C terminal amino acids of the pump. We are now poised to overexpress this domain in *P. tetraurelia* to determine its effects on the three chemoresponse pathways.

Supported by NIH and the VCC.

629. Effects of functional blockers injected into the amygdala on conditioned taste aversion in the rat

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To study possible involvement of the amygdala (AMY) in conditioned taste aversions (CTAs), the effects of microinjections of reversible blockades of glutamate receptors or protein kinase C (PKC) into the AMY on CTA formation were investigated. Soon after Wistar rats ingested 0.005 M sodium saccharin (conditioned stimulus, CS), they received bilateral intra-amygdala infusions of glutamate receptor antagonists or PKC inhibitors: 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) as an amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptor antagonist, D-2-amino-5-phosphonovalerate (D-APV) as an *N*-methyl-D-aspartate receptor antagonist, α-methyl-4-carboxyphenylglycine (MCPG) as a metabotropic glutamate receptor antagonist, polymyxin B sulfate (PMXB) or 1–5-(isoquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H-7) as PKC inhibitors. With a 30 min interval, they administered an i.p. injection of LiCl (unconditioned stimulus, US; 0.15 M, 2% of body wt). The injections of CNQX or MCPG significantly disrupted the acquisition of CTAs in comparison with the injections of the vehicle in control rats. In contrast, CNQX infusions into the vicinity of AMY were ineffective. CTA acquisition was attenuated by infusion of D-APV. PMXB or -7 infusions into the AMY between the CS–US interval significantly disrupted CTA formation. These results suggest that glutaminergic neuronal transmission and intracellular signal transduction cascades including PKC in the AMY participates in the brain mechanism of CTA acquisition.

The effects of high dose of D-APV, calcium/calmodulin-dependent protein kinase (CaM K) inhibitor and voltage-dependent calcium channel blocker are now under investigation.

Supported in part by grants from the Japanese Ministry of Education (No. 08780767, 07457438).

630. The effects of iso-alpha-acid, organic acids concentration and pH on sourness and bitterness

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Iso-alpha-acids are the principal bitter compounds in beer. Beer also contains several kinds of organic acids from processed fermentation. We have examined the gustatory effects and interaction between the bitterness and sourness of these acids. We used mixtures of tetra-hydro-iso-alpha acid (T: 10, 15 and 20 mg/l) and three kinds of organic acids, citric acid (CA: 0.00032–0.0032 M), lactic acid (LA: 0.001–0.01 M) and acetic acid (AA: 0.001–0.01 M). Five concentrations of each organic acid were prepared in quarter log dilution steps. The sourness intensities of each organic acids were roughly equal at the five concentration levels. The taste of the mixture was evaluated before and after pH adjustment to 4.3, which is the usual pH level for beer. A sample of 32 young (20–27 years of age) subjects rated the bitterness and sourness intensity of 30 mixtures of each organic acid and T using a 13 points category scale. Before pH adjustment, the bitterness of T (10 mg/l) was suppressed by AA but not LA and CA. However, in the case of 15 mg/l T, there was a point of bitterness enhancement by the organic acids. Following pH adjustment, the bitterness of the T (10mg/l) was suppressed by AA but not LA and CA. At pH 4.3 the intensity of sourness was greater for AA than CA and LA. No enhancement of bitterness was observed at this pH. To further characterize the sensitivity to T, we will compare responses in 5% ethanol with those in aqueous solutions.

631. Morphological study of the receptor cell early regeneration in the vomeronasal epithelium

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The receptor cells are capable of replacing in the dual olfactory system of adult mammals. Previous study revealed that transection of the vomeronasal nerves caused the degeneration of the receptor cells. In addition, the number of the receptor cells reached the lowest level by recovery day 6 and exceeded control levels by day

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15. To analyze the structural changes of the degenerating and regenerating cells, an electron microscopic study was done in the vomeronasal sensory epithelium following a nerve transection. Male rats were examined for each postoperative recovery time of 4, 6, 10, 15 and 21 days. The microvilli of the receptor cells decreased in number at day 4 and could not be recognized at days 6 and 10. Degenerating receptor cells were observed in the day 6. Some dendritic endings of the receptor cells filled with many centrioles at days 10 and 15 similarly in the developing vomeronasal sensory epithelium. The microvilli of the receptor cells were observed again at day 15 and increased in number by day 21. The number of these microvilli, however, did not reach that of control side by day 21. These results suggested that the receptor cells degenerated by day 6 and gradually differentiate by day 15. But these cells showed an immature state even at day 21. To elucidate the recovery process of the vomeronasal sensory epithelium, it will be necessary to examine longer recovery time.

632. The evaluation of the anxiety-reduction effect by odor using an EEG measure

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We tried to reduce the anxiety accompanied with mental stress by odor stimuli. Anxiety has two aspects, one is trait anxiety and the other is state anxiety. We assumed that odors could alter state anxiety. However, as the degree of trait anxiety might affect state anxiety, we used two groups of subjects that had high or low scores of the T-STAI. Lavender oil and tuberose absolute were used as stimuli. State anxiety scale (S-STAI), psychological evaluation (awareness, mood) and electroencephalogram (EEG) were used to investigate the effect of odor on anxiety-reduction. The EEG was measured from right and left forehead (Fp1, Fp2) and slope coefficients of frequency-fluctuation in the alpha wave band were calculated. The result indicated that the state anxiety was highly correlated with mood and decreased when subject felt a good mood. The effect of odor on awareness was not conspicuous. Tuberose improved mood state in both trait anxiety groups and decreased the score of S-STAI whereas Lavender increased the score of S-STAI. As a result, it was suggested that tuberose should decrease the state anxiety whereas lavender should not. Regarding to the EEG, the slope coefficient increased in a better mood state and was dominant in the left forehead. In tuberose that reduced state anxiety, the slope coefficient highly increased in the low score group of T-STAI and the laterality between Fp1 and Fp2 was also significant. The state anxiety was estimated using the multiple regression analysis: S-STAI score as a dependence variable (y) and both right and left slope coefficients as the independence variables ($X1$, $X2$). The estimation formula of $y = -36.2 \times X1 - 28.1 \times X2$ ($n = 56$, $P < 0.001$) was provided. $R^2 > 0.85$, suggesting that the slope coefficient of frequency-fluctuation in the alpha wave band may be a useful index to estimate state anxiety.

633. CO_2 recovered taste nerve responses of bullfrogs from NH_3 induced suppression

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We investigated the suppressive effect of CO_2 and NH_3 on the taste nerve responses of bullfrogs to 0.1 mM quinine. $NaHCO_3$ and NH_4Cl were used to yeild CO_2 and NH_3, respectively. The responses were decreased with decreasing pH of stimulating solutions in the presence of 15 mM $NaHCO_3$. Also the responses were decreased with increasing concentration of $NaHCO_3$ at pH 6.5. The data thus obtained fell on a single curve as a function of CO_2 concentration calculated with Henderson-Hasselbalch equation. The similar results were obtained with NH_4Cl; 5 mM NH_4Cl decreased the taste responses with increasing pH and NH_4Cl fixed to pH 9.0 decreased the taste responses with increasing NH_4Cl concentration. These suppressive effect was reversible. These results indicate that CO_2 and NH_3 are responsible for the suppression whereas other formes such as HCO_3^- or NH_4^+ are ineffective. It appears that the suppressive effect results from CO_2-induced acidosis and NH_3-induced alkalosis of taste cells. The irrigation of the lingual artery with NH_3 also suppressed the responses reversibly. The suppressed responses were recovered with the surface application of CO_2 for 3 s prior to 0.1 mM quinine. The recovering effect was decreased with the longer application of CO_2. Therefore, it is likely that the diffusion of CO_2 neutralizes the intracellular solutions of taste cells to recover the responses and the longer application acidifies inside taste cells to suppress the responses. The time dependent recovery suggests that the neutralization of taste cells at a point or layer is critical in taste transduction.

634. Changes in odorant quality perception following methyl bromide induced lesions of the olfactory epithelium

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Previously, we described the injury to the mucosa caused by methyl bromide (MeBr) exposure and established the time course of anatomical recovery. Further, we also demonstrated a capacity for the recovered system to support function, as measured by performance on a five odorant identification task. Although the recovered system could support function, the questions as to whether the perception of odorant quality had been changed as a result of damage and recovery still remained to be resolved.

To examine the question, we again trained rats on a five odorant identification confusion matrix task (AOCM). Following criterion training, animals received forty overtraining/testing sessions using a standard 5 × 5 confusion matrix design. After the last session, animals in the lesioned group ($n = 7$) were exposed to 330 ppm MeBr gas for 6 h and then allowed to rest for two months (the time required by anatomical recovery as defined by measures that assess epithelial reconstitution, neuronal maturation and reconnection with the bulb). Similarly, control animals ($n = 7$) were also rested following overtraining. At the conclusion of the rest period control and lesioned animals were tested on a daily basis until recovery of

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criterion performance was achieved. Subsequently, an additional forty testing sessions were collected in order to acquire data for the comparison of odorant quality perception between these two groups.

Following the rest period, control animals retained the identification task at 76 ± 4.48% correct (mean ± SEM). In contrast, lesioned animals performed at 30 ± 4.06%. With additional testing, control animals required 3.57 ± 0.65 testing sessions to achieve criterion performance versus 13.57 ± 5.88 for the lesioned group. the composite matrix for each animal was compared with every other animal yielding a dissimilarity matrix of animal AOCM responses. An MDS analysis of the dissimilarity data yielded a four dimensional solution with each animal (both control and lesioned) occupying a point in MDS space. Formal statistical analysis (MANOVA) demonstrated significant effects of treatment ($P < 0.05$) in determining the location of an animal in the MDS space. these data, taken together with the anatomical observation of incomplete epithelial recovery and aberrant reconnection with the bulb, suggest that although the capacity to support function was restored by 2 months after lesion, odorant quality perception had been altered.

Supported by NIDCD Grant # DC00220-13.

635. Magnetic resonance imaging of patients with post-traumatic olfactory deficits

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We sought to define the primary sites of injury in patients with post-traumatic anosmia and hyposmia using high resolution magnetic resonance imaging (MR). Thirty-five patients (ages 16–51, mean 36) had surface coil examinations of the olfactory bulbs and tracts (OBT) and head coil images of the brain with volumetric analyses performed for the OBTs and temporal lobes (TL). The MR studies were compared with results of the University of Pennsylvania Smell Identification Test (UPSIT) and other specific tests of olfaction Anosmia was defined as UPSIT scores ≤ 18. Inter- and intraobserver tests of reliability yielded intraclass correlation coefficients of over 0.90 for all volumetric measures. OBT (88% of patients), subfrontal (60%) and TL (32%) injuries were found. Bilaterality to the OBT and frontal lobes injury was present in 90% of the cases. There was a statistically significant difference between the OBT volumes of anosmic (mean 52.4 mm^3 on the left and 62.5 mm^3 on the right) and non-anosmic patients (means 95.8 mm^3 and 105.8 mm^3) ($P = 0.032$). The TL volumes of the anosmic patients (mean left and right TL volumes of 68285 mm^3 and 69746 mm^3) were not significantly different than those of patients who had residual smell function (mean left and right TLs 68892 and 68273 mm^3 respectively). The OBT volumes of age matched controls were significantly greater than those of all post-traumatic patients ($P = 0.029$) and post-traumatic anosmic patients ($P = 0.008$), but not post-traumatic hyposmic individuals ($P = 0.307$). There was no statistically significant difference in TL volumes between control patients and patients with post-traumatic olfactory deficits.

Supported by NIDCD research grant PO1 DC 00161, NIH.

636. Functional magnetic resonance imaging using olfactory stimulants

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We sought to assess areas of activation in the brain using functional magnetic resonance imaging under conditions of variable olfactory stimulation. Four individuals with normal senses of smell assessed by the University of Pennsylvania Smell Identification Test (UPSIT) were repetitively evaluated with a paradigm using odorants that stimulate the olfactory and trigeminal nerves. A 1.5 T GE Signa MR system fitted with Advanced NMR EPI hardware and software was used for imaging. The task paradigm consisted of alternating rest-stimulus cycles (30 s rest, 30 s stimulus) over 5 min. The odors were presented through a custom built MR-compatible olfactometer. Generic activation for the group of volunteers was determined by computing the median value of the fundamental power quotient at each voxel of the observed parametric maps, and comparing it to a null distribution of median FPQ values computed from the randomized median FPQ. The group averaged activated voxels were overlaid on a high resolution MRI template. These displays allowed the identification of the standard Talairach stereotactic coordinates and labeled Brodmann areas for each regional focus of generic activation. The stimulation maps using olfactory nerve mediated odors predominantly activated the frontal lobes of the brain and the anterior temporal poles. On repetitive stimulation, the activation appeared to decrease. The stimulation maps using odors that also activated the trigeminal nerve showed much more brain activity than those with selective olfactory nerve stimulation. The frontal, occipital, and temporal lobes and the cerebellum showed significant activity. This activity was amplified on retesting.

Supported by NIDCD research grant PO1 DC 00161, NIH. and The Burroughs Wellcome Travel Grant Program.

637. Cellular expression of $G_{\alpha i\text{-}3}$ and α-gustducin in rat vallate taste buds

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In addition to α-gustducin, there is evidence that other G proteins may be involved in gustatory transduction. In the present study, the cellular location of the G proteins, $G_{\alpha i\text{-}3}$ and α-gustducin were investigated using immunocytochemistry combined with semi-thin transverse sections through the vallate taste buds of the rat. Tissue containing the vallate papilla was cut into 300 μm sections with Vibratome, dehydrated in alcohol and embedded in polyester wax. After careful orientation, 3 μm sections were cut transverse to the long axis of the taste buds. Sections were reacted with a polyclonal antibody against $G_{\alpha i\text{-}3}$ [Santa Cruz Biotechnology: anti-$G_{\alpha i\text{-}3}$ (C-10), which cross reacts with $G_{\alpha i\text{-}1}$ and $G_{\alpha i\text{-}2}$] and a monoclonal antibody against α-transducin (Transduction Laboratories; anti-G_{α}), which we demonstrate to stain all the cells that contain α-gustducin, using anti-αt and Santa Cruz anti-Gαgust (I-20).

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Sections were processed for $G_{\alpha i\text{-}3}$ immunoreactivity using the avidin-biotin (ABC) method or for double-labeling for α-gustducin/α-transducin (anti-α_t) using fluroescein- and rhodamine-conjugated secondary antibodies. Sections were viewed and digitally photographed on either a conventional compound microscope or a Zeiss confocal laser-scanning microscope. Results show that cells with morphologies consistent with dark (Type I) cells were labeled with anti-$\alpha_{i\text{-}3}$; labeling was considerably more concentrated in the apical portion of the taste buds, where there is an accumulation of dark cell cytoplasm. Double-labeling with antibodies against α-transducin/α-gustducin (anti-$G_{\alpha t}$) and $G_{\alpha i\text{-}3}$ demonstrated that those cells that were immunoreactive for α-gustducin were light (Type II) cells that were also immunopositive for $G_{\alpha i\text{-}3}$. Since C-terminal amino acid sequence that was used to create anti-$G_{\alpha i\text{-}3}$ is similar to that of α-gustducin, it is possible that the light cell staining by anti-$G_{\alpha i\text{-}3}$ is the result of cross reactivity.

Supported in part by NIDCD DC00347 (D.V.S.).

638. Involvement of amyloid precursor protein isoforms in the proliferation and differentiation of olfactory receptor neurons

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Amyloid precursor protein (APP) is the source of β-amyloid of senile plaques in Alzheimer's disease (AD). Although the abnormal expression and processing of APP have been implicated in AD, its normal role is not yet understood. Olfactory receptor neurons in nasal epithelium are replaced at a slow rate throughout life. Accelerated degeneration and repopulation of these neurons was achieved by destroying their target olfactory bulb and this model was used for studying the role of APP in neuronal proliferation and differentiation. We have previously reported the expression of all three major APP mRNA and protein isoforms in the olfactory epithelium (OE). In situ hybridization data indicate that APP mRNA isoforms are present in the neuronal layer of OE. Immunocytochemical analysis using APP antibodies confirms that APP protein isoforms are present in neuronal layer of OE as well as in the nerve bundles. In this study, quantitative RT–PCR was used to measure APP 695, 751 and 770, at 3, 5, 7 and 14 days following unilateral bulbectomy. Changes in GAP-43 and olfactory marker protein mRNA in the epithelium were monitored as indicators of neurite outgrowth and mature differentiated neurons, respectively. At day 3, a 75% decrease was observed in the levels of all three APP mRNA isoforms, comparable to the loss in olfactory marker protein mRNA. While APP 695 lagged behind, recovery of APP 751 and 770 was evident at 5 days during early stages of neuronal proliferation. By 7 days, APP 695 had increased to pre-bulbectomy levels, while APP 751 and 770 isoforms already surpassed the control levels. During the phase of active neurite outgrowth at 14 days, amounts of all three APP isoforms were almost double those of the control steady-state epithelium, comparable to the increase and continued elevation of GAP-43 mRNA. These results suggest a role for APP 751 and 770 in initial phases of neuronal proliferation and differentiation, while APP 695 may be involved in subsequent stages of neuronal differentiation and neurite outgrowth.

639. Evidence that tenascin (TN) signals axonal outgrowth by negative repulsion during regeneration of the olfactory nerve in larval sea lampreys

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Continual neurogenesis within the vertebrate olfactory system provides an appropriate model for examining molecular cues that guide the outgrowth of axonal processes. Tenascin (TN), a glycoprotein of the extracellular matrix localized along developing axonal pathways, may function as a guidance molecule by repulsion, restricting axons within the boundaries of nerve fascicles. We have previously observed that intense TN immunostaining of olfactory nerve fascicles (ONF) in larval sea lampreys was lost during retrograde degeneration of the olfactory nerve. In the present study, we investigated the involvement of TN during axonal growth of reconstituted ONF following retrograde degeneration. Two proteins associated with developing neurons, growth-associated protein 43 (GAP-43) and acetylated tubulin (AT) were present in unlesioned ONF. Three days post-lesion, ONF were devoid of AT, GAP-43 and TN-immunoreactivity. However, AT and GAP-43 immunoreactivity were present in diffuse unfasciculated axonal processes subjacent to the olfactory epithelium. At 14 days post-lesion, when olfactory knobs repopulated the olfactory epithelium, ONF exhibited GAP-43, AT and TN immunoreactivity. These results support the view that TN in larval lamprey ONF is involved with fasciculation of olfactory neuronal axons.

Supported in part by NSERC, NIH NS10174 and DC00210.

640. Differentiation in an olfactory neuronal cell line: analysis by differential display

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The olfactory epithelium is a unique system, in which new neurons are continually generated throughout adult life. Olfactory neurons are derived from stem cells that lie adjacent to the basal lamina of the olfactory epithelium; these stem cells divide several times and their progeny differentiate into mature sensory neurons. In our laboratory immortalized cell lines have been derived from these dividing cells. The morphology of these cell lines and their expression of neuronal markers varies with culture conditions. When grown in low serum medium one of these cells lines, OLF 442, differentiates by extending long neurites and increasing its expression of neurofilament and B50/GAP43 proteins at the same time reducing expression of GFAP.

Identification of differentially expressed mRNA in cell lines has previously relied on both screening for known markers, and the use of subtractive techniques for identification of unique mRNA species. The differential display techique allows simultaneous detection of differentially expressed mRNA at different time periods and growth conditions. A modified

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Liang and Pardee differential display technique was used to screen OLF 442 over a number of time intervals in serum-depleted media, and compared with OLF 442 grown in complete media. The differentially displayed fragments were cloned and sequenced, leading to the identification of a number of sequences, both known and unknown. The known sequences include SPARC, which is reported to function as a modulator of the cell matrix, and RHAMM, the receptor for hyaluronan mediated motility. Both the known and the unknown sequences are being studied further to provide insight into the differentiation of olfactory neurons.

Supported by the Australian Research Council and the Garnett Passe and Rodney Williams Foundation.

641. Early development of the olfactory organ in lower actinopterygian fish: *Acipenser* (sturgeon) and *Polypterus* (bichir)

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It has been shown that the olfactory placode of the zebrafish *Danio rerio* originates from a subepidermal monolayer which gives rise to all cell types of the sensory epithelium. In contrast, the olfactory epithelium of the clawed frog *Xenopus laevis* derives from two different cell layers, receptor neurons from cells of the sub-epidermal layer and supporting cells from epidermis cells. Since amphibians in some characters show a more primitive condition than teleosts, our light and electron microscopic study had to be extended to more basic groups of actinopterygian fishes (Acipenseridae and Polypteridae) in order to allow for an approach at the basic osteichthyan pattern.Embryos of sturgeons show cell differentiations in the epidermis on either side of the head: in the sterlet 56 h after fertilization (h AF) and 72 h AF in the Sibirian sturgeon. These regions invaginate to form the primary olfactory pits (64 and 90 h AF, respectively). About 10 h later, the bottom of the olfactory pit shows the apical endings of supporting cells and receptor cells. The latter seem to penetrate through the surface epithelium from below. These ontogenetic processes resemble those described for *Xenopus*. In contrast, the olfactory placode of the bichirs develops below the epidermis, starting from a monolayer, which is similar to zebrafish. Other than in zebrafish, however, the lumen inside the olfactory organ anlage appears before it opens to the exterior. Accordingly, the primary opening is formed in a different manner in sturgeons and bichirs, either by invagination (sturgeons), which is considered the primitive mode of formation, or by perforation (bichirs), considered as a derived state. In sturgeons as well as in bichirs the primary opening of the olfactory pit elongates and is then constricted by a bridge that separates incurrent and excurrent nares. The evolutionary aspects of these differences and similarities are discussed.

642. Odors activate a sodium-gated nonselective cation channel in lobster olfactory receptor neurons

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We have previously characterized a Na^+-gated nonselective cation channel in cultured lobster olfactory receptor neurons (Zhainazarov and Ache, 1995). We now identify a functional role for the channel in lobster olfactory receptor cells *in situ*. The role of Na^+-gated channels in normal cellular function is still elusive in any system. We show that the Na^+-gated channel occurs in the outer dendrites, the site of odor transduction. Ion substitution experiments show that a substantial part of the odor-evoked depolarization in the cells can be ascribed to a Na^+-gated conductance. Two pharmacological blockers of the channel, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfanamide (W7) (EC_{50} = 15.1 mM) and trifluoperazine (EC_{50} = 54.9 mM), could be identified. Both probes perturb odor-activation of the cells *in situ*, implicating a role for the channel in the control of odor excitability. The results of the ion substitution and pharmacological experiments taken together strongly imply that the channel secondarily amplifies the primary receptor current, presumably as a result of Na^+ influx through the primary odor transduction channel, with the interesting implication that Na^+ may serve as a second messenger in these cells.

Supported by grant # DC01655 from the National Institute on Deafness and Other Communication Disorders.

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643. Membrane properties of taste receptor cells in the soft palate

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The majority of the information on the physiology of taste receptor cells (TRCs) in the oral cavity has been obtained from recordings on cells from the fungiform, foliate and vallate papillae of the tongue. Even though there are roughly equal numbers of taste buds contained in the soft palate and in the fungiform papillae, virtually nothing is known about the electrophysiological characteristics and chemosensitivity of these palatine TRCs. We have developed methods to isolate the epithelium and taste buds from the soft palate and the geschmacksstreifen (GS) in rats to allow the use of electrophysiological techniques to address these basic questions. Since the greater superficial petrosal (GSP) nerve in mammals which innervates the soft palate is quite responsive to NaCl and sweet-tasting stimuli (Harada and Smith, 1992), we began by measuring Na^+ transport across the epithelium containing palantine taste buds. Similar to our prevoius results in lingual epithelia containing fungiform, foliate or vallate taste buds (Zhang *et al.*, 1996), Na^+ transport in palatine epithelia is saturable, ouabain-sensitive, partially sensitive to mucosal amiloride (0.5 mM) and to the tight junction blocker, $LaCl_3$ (6

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mM). Thus, amiloride-sensitive and -insensitive pathways and paracellular pathways contribute to Na^+ transport across the palate. In patch-clamp recordings, isolated palatine and GS TRCs exhibit voltage-activated Na^+ currents and a variety of K^+ currents similar to those in TRCs from the tongue. In preliminary experiments, saccharin (10 mM) and polyunsaturated fatty acids (0.1–10 μM) inhibited K^+ channel activity in TRCs. We are currently exploring the chemosensitivity of the TRCs in greater detail.

Supported in part by NIH grants DC02507 (T.A.G.) and DC00353 (D.V.S.).

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644. Functional characterization of a nematode (*C. elegans*) odorant receptor

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The nematode *C. elegans* exhibits chemotactic behavioral responses to both volatile and non volatile compounds. Responses to the volatile odorant diacetyl (2,3-butadione) require the function of a seven transmembrane domain receptor encoded by the *odr-10* gene. ODR-10 protein is localized to the ciliated ending of the AWA chemosensory neuron, suggesting that ODR-10 might directly interact with diacetyl. We expressed ODR-10 in human embryonic kidney (HEK293) cells and used a fura-2 based imaging technique to detect activation of ODR-10. When ODR-10 expressing human cells are exposed to diacetyl, a rapid and transient Ca^{2+} elevation response is observed. This response is produced by release of Ca^{2+} from intracellular stores and is likely to be mediated via coupling of ODR-10 to a Gq-a class G protein. The EC_{50} for the ODR-10 diacetyl response is ~2.25 mM. ODR-10 dose not respond to closely related volatile compounds such as 2-butanone, 2-hydroxy-3-butanone (acetoin) or 2,3-pentanedione. We found, however, that ODR-10 does interact with pyruvate and with citrate. Interestingly, these two compounds are direct intermediates in the diacetyl synthesis pathway used by some bacterial species. These suggest that ODR-10 activation could be a signature for the presence of specific bacteria that can be used as a food source by nematodes.

Supported by NIDCD and NIMH.

645. Functional expression of an olfactory receptor in olfactory epithelium by adenovirus-mediated gene transfer

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We have been using olfactory neurons as an *in vivo* expression system for the functional expression of odor receptors through adenovirus-mediated gene transfer. A recombinant adenovirus vector carrying a bicistronic expression cassette controlled under the strong CAG promoter for both the I7 odor receptor gene, and the green fluorescent protein (GFP, S65T mutant) driven by an internal ribosome entry site (IRES) following the I7 coding sequence, was used to infect olfactory epithelium of rat *in vivo*. GFP expression marked both the place and rate of infection in living tissue. Antibody staining for GFP confirmed that more than 80% of GFP positive cells in olfactory epithelium were olfactory neurons. The infection rate reached up to 25% of the olfactory neurons in an infected area.

Electro-olfactogram (EOG) recording was used to screen responses to multiple odors in infected epithelia. In a normal olfactory epithelium an olfactory receptor (1/1000 genes) is expressed on average in ~0.1% of the total olfactory neurons. However the cloned receptor could be expressed by the adenovirus vector in up to 25% of olfactory neurons in the infected area. Thus, the response, in infected epithelium, to the odor ligand that activates this receptor should be much larger than that in a normal epithelium, a difference that could be detected easily with an EOG recording. EOGs were recorded from I7-virus infected turbinates which showed green fluorescence 3–7 days after virus infection, and, as controls, in the same location from non-infected turbinates. Turbinates infected with a similar amount of an adenovirus carrying the LacZ gene were also used as a control. The lacZ virus infection was confirmed by X-gal staining after EOG recording. Virus-infected olfactory epithelium responded to all odorants tested. The spatial pattern of responses was unchanged in infected animals, though the amplitude of the EOG to all odorants decreased by an average 30%. Over 60 odors were tested. One odorant induced a significant increase in the response from I7-virus infected epithelia versus control epithelia, indicating the functional expression of the I7 receptor and identifying its odor ligand.

Supported by the Whitehall Foundation and McNight Foundations.

646. Modulation of cAMP levels in rat taste epithelia following exposure to monosodium glutamate

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A metabotropic glutamate receptor, mGluR4, is expressed in taste buds in the circumvallate (CV) and foliate papillae of rat. Behavioral studies have implied that mGluR4 or other type III mGluRs may be involved in the transduction of monosodium glutamate (MSG) or *umami* taste. mGluR4 activation in other systems has been shown to result in a decrease of cyclic adenosine monophosphate (cAMP). Thus, we examined whether cAMP levels in taste tissue also are modulated following stimulation with MSG.

Epithelial sheets rich in taste buds were isolated from CV or foliate papillae of the rat tongue. Because resting levels of cAMP were found to be low, the tissue was pretreated with 10 μM forskolin to stimulate adenylyl cyclase. Following pretreatment with forskolin, one strip of foliate epithelium or one half of each CV epithelium was incubated in Tyrode's solution containing a tastant, while the other foliate strip or other half of CV from the

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same rat was incubated in Tyrode's alone. After incubation for 30 s, the cells were permeabilized and cAMP concentration was determined by enzyme linked immunoassay. L-MSG at 10 and 20 mM elicited a significant decrease in cAMP concentration in epithelium enriched in taste buds. D-MSG, the optical isomer, does not produce a taste quality similar to L-MSG in rats or humans. We found that cAMP concentration in taste epithelium remained essentially unchanged following treatment of 20 mM D-MSG. Additionally, 0.5 mM IMP in combination of 20 mM L-MSG elicited a larger depression in cAMP concentration than did L-MSG alone. This result is consistent with the known synergistic effect of IMP on glutamate taste. IMP alone did not affect cAMP concentration in taste epithelium. Epithelial sheets free of taste buds did not respond to L-MSG, D-MSG or IMP. These results suggest that cAMP may be a prominent second messenger in the transduction of *umami* taste in rats and are consistent with previous interpretation that metabotropic glutamate receptors mediate the taste of MSG.

Supported in part by NIDCD 03013.

647. The ecological function of amino acids as feeding attractants

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Amino acids are feeding attractants and stimulants for a taxonomically diverse assemblage of terrestrial and aquatic animals. Although amino acids have been widely used in laboratory studies, they have not been tested in the field to clarify their ecological roles. In the current field study, we measured dissolved free amino acid (DFAA) fluxes from prey and coefficients of eddy diffusion, shear velocities, and roughness Reynolds numbers were determined to help describe the hydrodynamic transport of amino acids following release in seawater. DFAA fluxes significantly differed between live intact and injured prey. For injured clams and fiddler crabs, DFAA release rates were 88 and 6,804 nmol/min/g wet tissue mass, respectively. Whereas live intact fiddler crabs released DFAAs at extremely low rates (0.1 nmol/min/g), live intact clams actually removed DFAAs from seawater.

When presented with DFAA solutions mimicking those from injured crabs or clams, omnivorous mud snails moved towards the stimulus input source. Significantly more snails were attracted to amino acid release sites simulating an injured crab than an injured clam of equal tissue mass. Differences in attraction were not caused by changes in either DFAA composition (mixture blend), total DFAA concentration, or fluid input rate. DFAA flux, the product of concentration and fluid input rate, was the critical factor regulating mud snail attraction. Crab and clam DFAA mixtures were equally attractive when presented at the same flux, and the lowest flux having a significant effect was 180 nmol/min. Based on current and previous results, we hypothesize that amino acids are seldom released in sufficient quantities by live intact prey to act as feeding attractants. The principal effects of amino acids are in stimulating ingestion and consumptive phases of feeding and in attracting carnivores to injured prey and carrion.

Supported by NSF IBN 92-22225 and OCE 94-16749.

648. Mitral cells and ruffed cells: two physiologically different types of relay neurons in the olfactory bulb of *goldfish*

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Anatomical differences characterizing mitral cells (MC) and ruffed (RC) cells were published 10–15 years ago by Kosaka and Hama in three teleost species (goldfish, catfish and sea eel). Physiological responses from the two different types of relay neurons were recorded extracellularly and simultaneously in the plexiform layer using a single tungsten microelectrode electrode (A-M systems 5770; 10–12 MW). During interstimulus intervals MC responded with higher, frequently burst-like impulse rates (mean value 2.7 s^{-1}) triggered by the activity of epithelial receptor neurons. The MC activity could be totally suppressed during anesthesia of the epithelium. RC impulse rates were low (mean value.75 s^{-1}), and each potential triggered a long-lasting (3–5 ms), continuously variable, summed granule cells (GS) potential (~3 ms peak to peak). MC activity via GC laterally inhibited RC potentials. In contrast to MC, blockade of epithelial receptor cells increased the activity of RC. During olfactory stimulation (non-familiar stimuli: amyl acetate, β-ionone, 2-phenylethanol 10^{-6} M; relevant natural stimuli: four amino acids 10^{-6}–10^{-10} M; preovulatory pheromone 17,20-β-dihydroxy-4-pregnen-3-one 10^{-9}–10^{-13} M, bile acid taurolithocholic acid, 10^{-6}–10^{-10} M) contrasting interactions between MC and RC were present that even in the lowest concentration resulted in a drastic intensification of centrally transmitted information. More frequently an inhibition was recorded from MC, and decreasing lateral inhibition via GC resulted in an activation of RC. Activation of RC resulted in an activation of pools of GC via initial pedunculate protrusions from the long unmyelinated portion of their axons, laterally inhibiting pools of MC in their vicinity. This is the first physiological determination of different types of relay neurons in the olfactory bulb.

Supported by DFG 112/7-1.

649. Role Of cyclic GMP in olfactory transduction and adaptation

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Sensory adaptation is essential for the proper regulation of the gain of signal transduction and the setting of the sensitivity at different stimulus intensities, in order to prevent saturation of the transduction machinery. Recent biochemical and electrophysiological progress has made it possible to identify distinct forms of odor adaptation operating at the level of isolated vertebrate olfactory receptor neurons (ORNs), and to begin to characterize the underlying molecular mechanisms. Adaptation in ORNs is manifested at two different levels: (i) a rapid (seconds) change from phasic to tonic response during continuous stimulation; this effect recovers over several s and is triggered by Ca^{2+} entry through cAMP-gated cylic nucleotide-gated (CNG) channels; and (ii) a slow and long-lasting decrease in responsiveness that persists for several min in the absence of odor stimulation; this effect has been termed cumulative or long-lasting

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adaptation (LLA). Evidence indicates that LLA depends on the cGMP second messenger system. Pharmacological blockade of the generation of the diffusible messenger carbon monoxide (CO), which acts as an endogenous stimulator of cGMP formation in adult ORNs, disrupts LLA but has no effect on odor excitation and cAMP-mediated signal transduction. Blockers of the nitric oxide system are unable to eliminate LLA. During blockade, LLA can be fully restored by adding micromolar doses of exogenous CO to the bath. cGMP-mediated Ca^{2+} entry through CNG channels appears to play a critical role in LLA by triggering negative feedback regulation. Confocal microscopy demonstrates that low levels of 8-Br-cGMP cause substantial Ca^{2+} entry into olfactory cilia. LLA is abolished under conditions in which the Ca^{2+}-feedback is removed. Our results demonstrate that the cGMP second messenger system mediates a distinct form of odor adaptation that can be dissociated from excitation by pharmacological methods. The data offer a mechanism by which a single brief odor stimulus can be translated into long-lasting intracellular changes that could play an important role in the perceptual adaptation to odors, and explain the long-standing puzzle that the olfactory CNG channels can be gated by both cAMP and cGMP.

This research was supported in part by NIH grants DC02227 and DC00086.

650. The role of chemical signals in structuring crayfish hierarchies: recognition of dominance status by olfaction

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An integral part of any crustacean community are dominance hierarchies. Typically, these are arranged based upon physical aspects of individuals and the results of conflicts between individuals. In some crustaceans, individual position within the hierarchy can be recognized through sensory cues, primarily through chemoreception. Based on work in other crustacean systems and preliminary work in our lab, we hypothesize that chemical signals will play a role in status recognition within the hierarchy for the red swamp crayfish, *Procambarus clarkii*. In our experiment, dominance hierarchies were set up for groups of three individuals. Based on analysis of isolated conflicts between individuals, ranks were assigned. Subsequently, we tested the ability of crayfish to recognize hierarchical status through olfactory cues using a choice paradigm in a flow-through Y maze. Individuals used for the chemical stimulus presented only chemical cues to test individuals. Visual or mechanical cues were eliminated. We measured initial arm choice, time spent within each arm, and number of approaches towards an individual. Trials were run with both male and female populations, including addition naive individuals as test subjects. Our results show that crayfish can recognize status through olfactory stimuli. By using olfaction as a cue for both individual and status recognition, crayfish may reduce time spent in physical conflict or aggressive displays.

Supported by NSF grant # (IBN-9614492).

651. Studies towards structure determination of substituted pyrazines

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The problem of the exact structure of several commercially significant pyrazines arises from reading the product lists of some important pyrazine manufacturers. These pyrazines are mainly those which consists of a substituent at position 5 or 6 in addition to a substituent on position 2, e.g. 2-methyl-5- or 6-methoxypyrazine and 2-methyl-5- or 6-*n*-propoxypyrazine. The exact structure elucidation was performed using ^{15}N-NMR spectroscopy, especially polarization-transfer for sensitivity enhancement (INEPT) and 2D-correlation techniques. Nitrogen NMR spectroscopy has been well described in review literature, to show the importance of nitrogen chemistry and the potentialities and difficulties of NMR work with the two magnetic nuclei, ^{14}N and ^{15}N. Both have rather low magnetogyric ratios (γ), and so rather low NMR sensitivity-about one-tenth that of ^{13}C. ^{15}N has spin 1/2 but low natural abundance (0.365%) and negative NOE factors (γ being negative), and may be slow to relax. ^{14}N is nearly 100% abundant but is quadropolar ($I = 1$), though with a relatively small quadrupole moment ($Q = 0.017 \times 10^{-28}$ m^2) so that broadening may not be excessive.

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