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ABSTRACTS

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1. *In vitro* magnetic resonance microimaging permits improved visualization of structural organization of the rat olfactory bulb

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We have recently reported that it is possible to delineate and visualize, by using the method of noninvasive magnetic resonance imaging (MRI), the structural details of the rat olfactory system (Agahi *et al.*, 1994). This approach, which utilized a SISCO 4.7T/33 cm bore imaging spectrometer, allowed us to visualize the laminar organization of the olfactory bulb as well as the intricate conchal convolutions of the olfactory cavities. Using the same tools and approaches, we also succeeded in demonstrating that the differential atrophic effects of olfactory nerve degeneration (induced by neonatal destruction of the olfactory epithelium by zinc sulfate) on the various laminae of the olfactory bulb can be visualized and quantified (Agahi *et al.*, 1995).

To improve the resolution and visibility of the laminar structure of the rat olfactory bulb in magnetic resonance images, we used, in the present study, the Varian NMR spectrometer system (11.7T/50 mm vertical bore) with a Doty microimaging probe. This approach allows microimaging of a single detached rat olfactory bulb (<5 mm in each dimension) with a high resolution of 35–50 μm, compared with 300 μm possible in the *in vivo* images. This microimaging approach permitted a high degree of differentiation and visualization of the olfactory bulb's internal organization and its various layers, i.e. olfactory nerve layer, glomerular layer, external plexiform layer, internal granular layer, and the ependymal (ventricular) layer. In *in vitro* microimaging, the higher magnetic field and smaller, more efficient, radiofrequency coil, give a higher signal-to-noise ratio than in the *in vivo* studies, permitting smaller volume elements (voxel) and higher resolution for better visualization of the laminar structure of the olfactory bulb.

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2. Effect of amiloride on suprathreshold NaCl, LiCl and KCl in humans

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The purpose of this study was to investigate normal variances in the response magnitudes following delivery of suprathreshold concentrations of NaCl, LiCl and KCl salts and amiloride on the anterior human tongue. Randomly selected equimolar concentrations (0.12, 0.32, 0.72 1.8, 4.5 M) of salt solutions were delivered to spatially matched flow chambers attached to the anterior tongue of 20 volunteers at three different sessions. A cross modal magnitude matching procedure was used to scale salt taste intensity judgments. Each estimate of taste intensity was adjusted by the subject's mean estimate of the brightness of five visual stimuli. After each salt test, amiloride (100 μM) was delivered for 5 min and the test was repeated. A least squares regression analysis of each subject's function between log molar concentration and log response demonstrated that every subject was able to scale the dynamic range of each salt. There were no statistical differences in either the mean regression or intercept among the three salts. Repeated measures analysis demonstrated a statistical effect of amiloride on the before/after difference in the regression ($P = 0.02$) and intercept ($P < 0.0001$) of NaCl and LiCl power functions. There were no statistical differences in the regression ($P = 0.7$) and intercept ($P = 0.33$) between NaCl and LiCl amiloride/salt power functions. Amiloride had no effect on the before/after difference in regression ($P = 0.1$) and intercept ($P = 0.45$) of KCl power functions. There were statistical differences between KCl/amiloride and NaCl–LiCl/amiloride power functions. In summary, our

findings support the growing evidence of two different transduction systems which mediate salt taste in humans which are differentially affected by amiloride.

This study was supported in part by NIH grant DC01473 and OMSF Health Professions Student Award.

3. Characterization of transduction enzymes and G proteins in microvillar membrane preparations from mammalian vomeronasal organ

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To study the molecular mechanisms by which pheromones activate chemosensory neurons in the vomeronasal organ (VNO), we partially purified microvillar dendritic membranes from porcine VNOs. Western blotting with antibodies specific to G protein subunits reveals that these membranes contain G_s, G_{i2}, G_{i3}, G_o and a 40 kDa polypeptide related to G_q. In contrast to G_{i2} and G_o, previously localized to the cell bodies, axons and dendrites of subpopulations of vomeronasal neurons (Halpern *et al.*, 1995), the G_q-related protein is found mainly at the microvillar surface of the vomeronasal neuroepithelium. The large number of G protein subunits associated with vomeronasal neurons implies multiple transduction pathways. The concentration of the G_q-related protein at the dendritic microvilli suggests a central role for this G protein in pheromone signaling.

The microvillar membrane preparation is enriched in adenylate cyclase and phospholipase C. Stimulation of microvillar membranes from female VNOs with boar urine results in the production of inositol-(1,4,5)-triphosphate. Preliminary studies indicate that prolonged incubation with boar urine results in stimulus-dependent phosphorylation of a 44 kDa protein, about the size predicted for a putative pheromone receptor (Dulac and Axel, 1995). A monoclonal antibody against GRK-2 and GRK-3 detects both of these G protein-coupled receptor kinases in the microvillar membrane preparation. Moreover, antiserum against GRK-2, like the anti-Gαq antiserum, stains the microvillar surface of the VNO. Finally, antibodies against β-arrestin-1 and 2 reveal 4 immunoreactive species in the 48–52 kDa range. Thus, the microvillar membrane preparation we developed houses the transduction machinery for activation and desensitization of vomeronasal neurons in response to pheromones.

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4. Immunohistochemical changes in the anterior olfactory nucleus of the developing rat

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The anterior olfactory nucleus (AON) is located just caudal to the olfactory bulb in the olfactory peduncle. It receives input from both the ipsilateral and contralateral bulb (via the contralateral AON) and is involved in bilateral coordination of sensory information. The AON is commonly divided into six regions based on their location relative to the anterior limb of the anterior commissure, which forms the center of the structure. Relatively little is known about the function or development of the AON. We used ABC immunocytochemistry to visualize neuronal (Calbindin D28-k, NPY and MAP II) and glial (GFAP and RIP for astrocytes and oligodendrocytes respectively) development in postnatal (P) day 10, 20 and 30 rats. Calbindin immunoreactivity (ir) was found primarily in a subpopulation of neurons in the inner half of most divisions of the AON, except for par medialis, where a lower density of labeled cells with a more superficial distribution was observed. Visual inspection suggested that the number of labeled cells changes little from P10 to P30. NPY-ir cells were much less numerous, with a slight increase in numbers noted between P10 and P20. While these cells were also found widely scattered in deep portions of the AON, pars medialis appeared to be more densely populated than other areas. MAP II staining was quite dense in the AON by P20, indeed, much darker than that seen in the bulb. Substantial maturation occurred between P20 and P30, undoubtedly reflecting the maturation of dendritic processes. Both GFAP- and RIP-ir were localized to plexiform regions. GFAP staining was lighter than that seen in the bulb and restricted to the central core of the AON and a ring surrounding its peripheral surface. RIP-ir was limited to the region of the anterior commissure and lateral olfactory tract. Considerable maturation was observed for both between P10 and P20. Further work is in progress to complete the examination of all antibodies at all three ages.

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5. High-frequency olfactory nerve stimulation induces NMDA receptor-independent long-term potentiation in the glomerular layer *in vitro*

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We recently showed that 'theta-burst' stimulation of the olfactory nerve (ON) in olfactory bulb slices from 24- to 30-day-old rats induces long-term potentiation(LTP) of a long-latency, NMDA receptor (R)-mediated mitral spiking activity. We are currently investigating conditions (e.g. age, tetanus parameters) that support LTP of fast glutamatergic transmission, using field potential (FP) recordings from the glomerular layer (GL), the location of ON synapses.

In slices from younger (12–18 days old) rats, ON stimulation evoked a FP in the GL consisting of one major negative

component (peak latency 11–14 ms). APV (NMDA R antagonist) slightly reduced the FP, while CNQX (kainate/AMPA R antagonist) invariably blocked it, suggesting that this FP is largely mediated by kainate/AMPA Rs. A 50 Hz ON tetanus (5 s), but not theta-burst stimulation, induced LTP of the FP (185 ± 12%, n = 10). Potentiated FPs were blocked by CNQX, but not affected by APV, suggesting that LTP was expressed in the kainate/AMPA R-mediated component. APV and MK-801 did not prevent the induction of LTP (165 ± 11%, n = 6), suggesting that the induction does not require NMDA Rs. LTP was specific to the ON input since a control FP evoked by stimulation in the external plexiform layer was unchanged during LTP of the response to the ON input. Simultaneous recordings in the GL and mitral cell layer (MCL) showed that LTP of the GL FP was accompanied by LTP of a negative component in the MCL. This negativity may reflect mitral/tufted to granule cell synaptic activity and/or mitral spiking activity. Thus, LTP in the GL may result in LTP of the mitral cell output.

Together with our previous findings, these results suggest that the primary sensory synapses in the rat olfactory system are capable of expressing LTP in either the kainate/AMPA or the NMDA R-mediated transmission. NMDA R activation is not necessary for LTP of the ON-evoked FP in the GL of juvenile rats. Animal age and stimulation parameters appear to influence the induction and expression of LTP. We are currently examining the: (i) cell type(s) contributing to LTP in the GL and (ii) factors that determine which response components will express LTP.

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6. Explorations of pure chemotaxis with robo-lobster

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To explore the limits of pure chemotaxis without the aid of visual, mechanical, or electromagnetic cues, we built a lobster-sized, freely moving, underwater robot with bilateral chemical sensors. It is a battery-powered Autonomous Underwater Vehicle with two independently controlled wheels, a caster, gyros for course control, and a 'Tattletale' 486-based onboard computer. The essence of the lobster's chemosensory input and behavioral output are preserved: the robot moves and turns with lobster speed, and its sensors—scaled to lobster antennules—can be programmed to extract different temporal signal parameters. This allows us to explore different search strategies in turbulent odor plumes and it allows us to evaluate the importance of some of the known temporal filter characteristics of lobster olfactory receptor cells.

Initial experiments in turbulent jet plumes show that the most simplistic paradigm (turn toward the higher concentration; go straight if the signal is absent or equal across bilateral sensors) is successful only in the jet-dominated section of the plume. In an odor patch field these instructions fail to produce homing. A slightly modified paradigm (as above, but move backwards if no odor is sensed) produced different behavior, but fared no better in target acquisition. We are building more complex paradigms gradually so that we may understand the failures of simpler ones and relate them to odor dispersal properties and sensory signal processing. A rheotactic and a rheo-chemotactic robot are under consideration.

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7. Genetics of sucrose intake in the mouse

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Consumption of 0.12 M sucrose solution in 48 h two-bottle preference tests and electrical response of the chorda tympani gustatory nerve to sweet stimuli were studied in C57BL/6ByJ (B6) and 129/J (129) inbred mouse strains and hybrids of the F_2 generation derived from these strains. The B6 mice drank more sucrose solution and had lower thresholds of neural responses to sucrose and saccharin compared with the 129 mice. The F_2 hybrids (n = 171) were phenotyped for sucrose consumption, and genotyped using selected microsatellite markers. The F_2 mice that had high intakes of sucrose were significantly more likely to be homozygous for B6 alleles at loci on chromosome 4 (*D4 Mit4*, *D4 Mit7* and *D4 Mit42*) and chromosome 3 (*D3 Mit10*). Other biometric analyses confirmed that the strain difference in sucrose intake depended on a small number of genes. In selected F_2 mice with high and low sucrose consumption, chorda tympani electrical activity was recorded. The F_2 mice with high sucrose consumption had lower thresholds to sucrose and saccharin than were the mice with low sucrose consumption, which supports the presence of a genetically determined link between avidity for sucrose and peripheral gustatory sensitivity to sweet stimuli. The gustatory neural sensitivity to sweet stimuli was most closely linked to the *D4 Mit4* and *D4 Mit7* microsatellite markers located on a proximal part of chromosome 4, near the *dpa* locus (Ninomiya *et al.*, 1987). These results suggest that at least one gene on the proximal portion of chromosome 4, possibly the *dpa* locus, influences sucrose intake through alterations in peripheral taste sensitivity.

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8. Sensitivity and specificity for an odor fluency test in Huntington's disease

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Huntington's disease (HD) is an inherited, autosomal dominant

neurodegenerative disorder characterized by involuntary movements and cognitive disturbance. Onset is insidious and generally occurs in the 30s and 40s. Neuropathological changes due to HD include neuronal loss and degeneration in the caudate nucleus and putamen, with changes in the cerebral cortex and prefrontal cortical layers as well. Additionally, neuropathological changes have been documented in areas of the brain associated with olfactory functioning. Previous studies have found impaired olfactory functioning in people with HD on tests of absolute detection, short-term recognition memory, and odor identification Neuropsychological tests have shown that people with HD have difficulty with concentration and attention and memory deficits In particular, tests of verbal fluency including letter and category fluency have proven instrumental in understanding changes in memory and thinking in dementia. People with HD show severe to moderate deficits on tests of verbal fluency. Therefore, the purpose of this study was to design an olfactory analog to the verbal fluency tests. People with HD and normal controls were asked to provide as many exemplars as they could generate in 60 s that belong to the category, odors. They were then asked to provide as many odors as they could think of that begin with the letters P, C and S in 60 s. Finally, subjects were presented with odors contained in opaque glass jars and asked to name all of the odors that the stimulus brought to mind. Logistic regression analysis was carried out to examine differences in performance of HD and normal control participants. The results indicate that people with HD generate fewer responses for the category odors, fewer exemplars of odors beginning with specified letters, and fewer responses after being presented with an odor stimulus than normal controls. The odor fluency test correctly classified 83% of the HD patients and 90% of the normal controls.

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9. The relationship between the human taste threshold to NaCl and the duration of stimulus presentation

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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. Our recently developed Regional Automated Taste Testing System (RATTS) has made it possible to explore the relationship between detection threshold values and the amount of time the tongue is stimulated. This system was used to present spatially discrete stimuli to a region on the tongue tip for four different stimulus durations: 0.2, 0.4, 0.75 and 1.5 s. Detection thresholds for NaCl were determined in 12 college-age subjects using a single staircase procedure. Subjects were tested on all four stimulus durations in orders counterbalanced by 4 × 4 Latin Squares. The size of the tongue area stimulated was 25 mm^2. The mean threshold values decreased monotonically as the stimulus duration increased, ranging in concentration from -1.97 $\log_{10}$ M at 0.2 s to -2.84 $\log_{10}$ M at 1.5 s. This study is the first to demonstrate that taste sensitivity on the anterior region of the tongue is influenced by the duration of stimulus presentation.

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10. Depolarization- and c-AMP-dependent induction of tyrosine hydroxylase expression in primary cultures of mouse, neonatal olfactory bulb

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Tyrosine hydroxylase (TH), the first enzyme in the dopamine biosynthetic pathway, is localized to juxtaglomerular neurons in the mammalian main olfactory bulb. The initial expression of the phenotype occurs relatively late in the mouse olfactory bulb as compared with other dopamine systems such as the substantia nigra, gestational day (G) 17 and G12 respectively. However, in both regions, the regulatory mechanisms underlying induction of the phenotype have yet to be established. During development, onset of synaptic activity between receptor afferents and olfactory bulb neurons occurs contemporaneously with TH expression in juxtaglomerular cells, suggesting a depolarization-linked modulation of TH gene induction. Evidence also exists for cAMP-dependent mechanisms in TH gene regulation that act through a CRE-element in the near upstream region of the TH promoter. To investigate a role for both depolarization and cAMP-dependent processes in induction of the dopamine phenotype, TH expression was monitored immunocytochemically in primary cell cultures of olfactory bulbs prepared from postnatal day 2 mouse pups. At this age, a significant number of TH-immunostained neurons occur *in vivo* in the mouse olfactory bulb. Forty-eight hours after depolarization, produced by high potassium concentration (50 mM KCl) in defined medium, the number of TH containing neurons increased ~3-fold as compared with control cultures treated with equimolar sodium chloride (560± 10.5 neurons/culture versus 191 ± 5.8 respectively). Forskolin treatment (10 M in DMSO), which increases cAMP concentrations, produced a 1.5-fold induction in the number of TH-stained neurons as compared with vehicle (253 ± 9.8 neurons/culture versus 169 ± 12.4 respectively). Forskolin also altered dendritic morphology, increasing the number of primary dendrites. These data indicate that synaptic activity-dependent mechanisms, likely acting through the TH-CRE, are involved in the induction and maintenance of TH expression in the olfactory bulb.

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11. Chemoreceptor cell responses to different stimulus onsets

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In nature, odors are often distributed in discrete patches which form spatial gradients. In aquatic environments, these gradients contain information which may be used by an animal, such as the lobster, to locate an odor source. Experiments with high-resolution odor measuring devices carried by lobsters during chemotactic orientation have shown reliable distributions of odor concentration patches in aquatic odor plumes. These patches are seen as chaotic series of stimulus pulses by receptor cells. In a previous study using square pulses, cells responded best to pulse onset and integrated over 200 ms, after which they adapted completely within 1 s. In this study we determined cell responses to concentration slopes and amplitudes. These gradual increases in concentration resulted in min-long responses to intermediate slopes, gradually increasing adaptation and response shortening to steeper slopes, and no response to shallower slopes. We interpret these results as the outcome of a competition between excitation and adaptation processes. These cellular feature extraction capabilities may be the basis of gradient search in turbulent plumes.

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12. Olfactory activation of an antennular grooming behavior in the spiny lobster, *Panulirus argus*, is tuned narrowly to L-glutamate

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Antennular wipe behavior (AWB) 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. AWB, which is exhibited in many crustaceans, apparently functions to clear debris from the antennules. We wished to determine whether chemical odorants commonly found in food might activate AWB. Time-lapse videography showed that spiny lobsters evoke AWB sporadically throughout the day at relatively low frequencies. Lobsters were presented via pipette with 27 chemicals found in their food as single odorants. One chemical, L-glutamate, evoked very high frequencies of wiping. Most chemicals tested were not stimulatory while only a few were weakly stimulatory (adenosine-5'-monophosphate, glycine, D-glutamate). This is surprising because previous studies have shown that feeding behaviors (antennular flick, search) in spiny lobsters can be evoked by a much broader array of chemicals. Complex mixtures mimicking food extracts also evoked AWB but at much lower magnitudes than L-glutamate alone. Responses to complex mixtures appeared to be dependent on concentrations of L-glutamate present in the mixture. Distilled water ablation techniques were used to determine whether antennules or maxillipeds act as sensory organs for evoking AWB. Ablation of at least one of the antennules resulted in no response toward L-glutamate while ablation of the maxillipeds reduced preferentially auto-groom, a component of AWB in which the maxillipeds are rubbed back and forth. Thus, the antennules shown previously to be important sources of chemosensory input for feeding behaviors also appear to be the major sensory organs for evoking AWB. These results suggest that sensory input from the same organ can be filtered in different ways in order to drive different behaviors. Sensory input from many olfactory receptor types, each sensitive to different chemicals, may elicit feeding behaviors. In contrast, AWB may be activated by only a few receptor types.

13. Embryonic taste receptors will develop in the absence of neural crest

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Vertebrate taste buds arise late in embryonic development, and have been thought to be induced by ingrowing sensory nerves. Recently, however, a number of workers have shown that development of taste buds or their progenitors is independent of nerve contact. Since nerves do not control taste bud formation, what tissue might be responsible for taste cell induction? One likely candidate is the cephalic neural crest. This embryonic population of migratory cells differentiates into a variety of cell types within the head, including chondrocytes of the facial skeleton and branchial arches, and odontoblasts that, with epithelial ameloblasts, give rise to teeth. Given the location of neural crest derivatives (in the oropharynx where taste buds are found) and the timing of their migration during embryogenesis (migration is complete many days before taste buds form), perhaps distributed subsets of neural crest cells induce specific cells within the adjacent epithelium to become taste cell progenitors.

To test this hypothesis we performed parallel *in vitro* and *in vivo* studies using embryos of an aquatic salamander, the axolotl, in which the oropharyngeal region was isolated prior to migration of cephalic neural crest. Specifically, the ventromedial portion of the first two branchial pouches was removed from pigmented embryos at stage 18–19. (Neural crest migration begins at stage 22 in this species.) The explanted tissue was (i) placed in culture or (ii) grafted ectopically to the trunk of an albino host embryo. Isolated tissue and experimental embryos developed for 10–12 days until control embryos possessed taste buds.

When the experimental tissue was examined for the presence oftaste buds, we found well differentiated taste receptor cells within 80% of explants and 84% of grafts. These buds were indistinguishable from normal taste buds in that they were of comparable size, and possessed calretinin-immunoreactive fusiform cells and serotonergic Merkel-like basal cells. Typical neural crest derivatives, such as chondrocytes, teeth, melanocytes and sensory neurons, were not present, indicating that we succeeded in isolating the taste epithelium prior to the arrival of neural crest. Thus, vertebrate taste buds are not induced by neural crest. We are currently testing the potential inductive role of other embryonic tissues, such as cephalic ectoderm and paraxial mesoderm.

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14. Do taste–trigeminal interactions play a role in oral pain?

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The ability to taste PROP (6-*n*-propylthiouracil) shows genetic variation: supertasters (STs) perceive the most, medium tasters less, and nontasters the least bitterness. STs have the most fungiform papillae tastebuds and perceive the most intense oral burn from capsaicin (chili peppers) possibly because these tastebuds are innervated by two nerves: the chorda tympani (VII: taste) and the trigeminal (V: pain). Females are more likely than males to be STs. PROP paper (1 inch square pieces of filter paper impregnated with 1.2 mg PROP) and capsaicin candy (taffy containing 5–9 p.p.m. capsaicin) were distributed to lecture attendees and they were asked to rate PROP bitterness and the candy burn on the LMS scale. Comparing subjects 20–40 ($F = 248$, mean = 118) to those ≥53 years ($F = 68$, mean = 90) with ANOVA, capsaicin burned most to females [$F(1,512) = 4.324$, $P < 0.05$], those ≥53 [$F(1,512) = 10.132$, $P < 0.01$], and to STs [$F(2,512) = 13.047$, $P = 0.0001$]. The interaction between the three variables was significant [$F(2,512) = 3.396$, $P < 0.05$] and *t*-tests showed that female STs ≥ 53 years felt the greatest burn from capsaicin. We suggest that this may be the result of inhibition between VII and V. PROP receptors may be estrogen-sensitive and thus decline after menopause. If VII and V inhibit one another, loss in VII might intensify pain via V. Female STs might experience the greatest intensification since they have the greatest number of tastebuds. Inhibitory interactions between VII and IX result in intensified taste via one nerve when the other is damaged or anesthetized. Some subjects experience taste phantoms from the area which produces the intensified taste. If similar interactions occur between VII and V, some female STs might experience pain phantoms. This may be one mechanism for burning mouth syndrome in post-menopausal women.

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15. Biotin-induced chemokinesis in paramecium

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Paramecium tetraurelia respond with positive or negative kinesis to various chemical and environmental stimuli. Several bacterial metabolites have been identified as attractants such as folate, glutamate, cAMP, acetate and ammonia. At least three different transduction pathways induce hyperpolarization, which mediates attraction behavior. Some attractants activate the plasma membrane calcium pump while others activate adenylyl cyclase as well as the calcium pump or alter intracellular pH. Identification and characterization of novel attractants may yield additional information on current transduction models or may reveal another pathway leading to hyperpolarization of the cell.

The bacterial metabolite biotin was tested for its ability to induce attraction in *P. tetraurelia*. Biotin had previously been identified as an attractant of the bacteriaL-feeding nematode *Caenorhabditis elegans*. T-maze assays of chemoresponse behavior in response to various concentrations of biotin in Na^+ or K^+ solutions with Cl^- as the control ion indicate strong attraction, peaking at biotin concentrations of 2.5 mM with only a small response at 0.1 mM. Electrophysiology of *P. tetraurelia* whole cells showed 6.1 ± 3.4 mV hyperpolarization at biotin concentrations of 2.5 mM. Experiments using mutants defective in Ca^{2+} regulation (provided by Evans and Nelson) and quantification of second messenger molecules in wild type cells are being conducted to ascertain the transduction pathway responsible for biotin-induced cell hyperpolarization and attraction.

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16. The effects of exposure to exogenous amino acids on the cellular physiology of cultured zooxanthellae (*Symbiodinium microadriaticum*)

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Free amino acids (FAAs) may be the host factor that is involved in the symbiotic associations of zooxanthellae and anthozoans. The effect of exogenous FAAs on amino acid transport in zooxanthellae were examined in this study. Cultured zooxanthellae isolated from the sea anemone *Aiptasia pallida* were incubated in amino acid cocktail based on concentrations found in host tissue. The zooxanthellae were then rinsed and resuspended in amino acid-free medium. Internalization of the amino acids glycine and arginine was then assayed. Results demonstrated that incubation in amino acid cocktail resulted in the dramatic response by the zooxanthellae in the up-regulation of glycine and arginine transport. Values of the kinetic parameter V_{max} for arginine and glycine transport were, respectively, 5.9-fold and 7.4-fold greater than control values after 2 days of amino acid incubation.

Amino acid uptake may play an integral role in the symbiotic relationship that zooxanthellae has with various anthozoan hosts. Implications of such amino acid transport may include uptake of amino acids by this dinoflagellate as an alternative nitrogen source to ammonium, playing a significant role in the signaling of photosynthate release back to the host. However, the way in which

the signal emitted by the host is detected and transduced by the symbiont remains to be determined.

17. Effects of insulin-like growth factor-I and insulin on numbers of OMP+ olfactory receptor neurons in culture

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Both insulin and insulin-like growth factor-I (IGF-I) have multiple effects on neural cell division and differentiation. Therefore, we investigated their actions in primary cultures that support generation of olfactory marker protein-positive (OMP+) olfactory receptor neurons (ORNs). Newborn rat olfactory cells plated on newborn rat cortical astrocytes were grown *in vitro* for 0–1 days, switched into experimental media, fed every 3 days, fixed after 15 days and immunostained for OMP. Normal serum-free growth medium contains insulin at 5 μg/ml, which should activate both the insulin and IGF-I receptors. Removal of insulin reduced but did not eliminate all OMP+ ORNs. This suggests trophic support of OMP+ neurons by IGF-I or insulin, and also that these or other trophic factors may be endogenously produced. There are six known IGF-I binding proteins (IGFBPs) that bind IGF-I but not insulin. Ligand blotting of conditioned culture medium showed the cultures produced IGFBP-2, IGFBP-3 and possibly others of the six. Western immunoblotting of conditioned medium revealed a protease to IGFBP-4. Thus, IGF-I actions in the cultures are probably modulated by IGFBPs. Addition of IGFBP-3 to culture medium with insulin altered numbers of OMP+ neurons in a dose-dependent manner. Thus, insulin and/or IGF-I and the IGFBPs appear to play important roles in ORN differentiation (or survival) in culture.

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18. Gene-mapping of sweet and bitter tastants in *Mus musculus*

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We attempted to confirm that mouse chromosomes 4 and 6 contain polymorphisms which affect respectively, intake of sweet and bitter solutions. A 6 h access period was employed to measure intake of sucrose (10^{-1} M), saccharin (10 mM sodium saccharin) and quinine (1.1 mM quinine HCl) solutions by 133 F_2 mice derived from a cross of the C57BL/6J (B) and DBA/2J (D) inbred strains. Microsatellite markers encompassing the regions of interest on chromosomes 4 and 6 were used to type DNA from each animal. The marker D4 Mit42 at 80 cMs on distal chromosome 4 was the best predictor of intake of the sweet solutions. Relative to their water baseline, B-type homozygotes drank 324 ± 33% of sucrose and 356 ± 37% of saccharin compared with D-type homozygotes (sucrose, 199 ± 25%; saccharin, 213 ± 26%). The B allele was dominant (relative intake of BD heterozygotes: sucrose, 338 ± 22%; saccharin, 379 ± 23%). The best predictor of relative quinine intake was D6 Mit338 at 62.5 cMs on chromosome 6 (B-allele, 42 ± 7%; D-allele, 79 ± 8%). Dominance was in the direction of the D-allele (BD heterozygotes, 67 ± 4%).

A distal location on mouse chromosome 4 is confirmed for a gene which has a major effect on saccharin intake. The gene also strongly influences sucrose intake. In addition, we confirm that the Prp region (63 cMs) on mouse chromosome 6 contains a polymorphism which affects quinine aversion. Differences have been demonstrated recently between the chorda tympani response of the B and D strains to prototypic sweet and bitter tastants (Frank and Blizard, AChems 1996). The genes on chromosomes 4and 6 may mediate their behavioral effects by producing alterations in relevant peripheral processes.

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19. Evaluation of the reliability of sweet ratings by a trained panel for five sweeteners at four concentrations

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The purpose of the present study was to determine the reliability of sweet ratings by a trained panel. The panel's primary function is to evaluate accurately the sweetness of a variety of compounds. In this study, each panelist's sweet ratings for five representative sweeteners, acesulfame-K, aspartame, sodium saccharin, stevioside and sucrose, were evaluated over five repetitions. Each of the five sweeteners was tested at four concentrations. All sweeteners were rated at two concentrations isointense with 3 and 6% sucrose as determined by formulae developed by DuBois *et al.* (1991). The two higher concentrations >6% sucrose tested for each of the sweeteners depended upon the maximum sweetness levels that are achieved by each sweetener. The panel as a group was most accurate and reliable for sucrose and aspartame. The most variability occurred for acesulfame-K and stevioside. Sweet ratings for three of the panelists accounted for the majority of ratings that were significantly different from the group mean; however, there were no panelists who clearly stood out as consistently giving aberrant ratings. This panel, with a relatively small number of judges, gives responses over a number of taste sessions equivalent to those of a large panel in one taste session.

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20. Ultrastructure and morphology of putative chemosensory hairs on the chelae of the crayfish, *Orconectes virilus* and *Orconectes propinqus*

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Many crustaceans use chemical signals in mating and courting behaviors. Preliminary behavioral observations during mating in crayfish indicate the movements of the chelae are similar to typical chemosensory sampling behaviors of other appendages, such as flicking in antennules and sweeping in walking legs. In addition, it is necessary for many crayfish to molt from a stage I to stage II morphology before courting and reproduction can begin. From these preliminary observations, we hypothesize that there might be a change in the distribution or morphology of chemosensory hairs on the chelae of the crayfish which coincides with the onset of the observed chemosensory sampling behavior. To begin a series of ultrastructural studies designed to examine hair morphology and distribution on the chelae of stage I and stage II morphologies of male crayfish and a similar study of hair morphology on the chelae of female crayfish, we chose two species, *Orconectes virilus* and *Orconectes propinqus*. Samples were collected from males in both stages and from females, fixed with gluteraldehyde and dried in an alcohol series. Samples were prepared for SEM by mounting them on a specimen support stub and sputter coating them with gold palladium. SEM photographs revealed two distinct hair types, filiform and branched, along the chelae located in distinct pockets. Results show the filiform hair has a small pore at the distal tip. From the ultrastructural results and the behavioral observations, there are indications that these hairs may be chemosensory. In addition, there appear to be differences in the amount of filiform hairs present within a pocket between stage I and stage II males and differences between stage 1 males and females. This study will help elucidate both the role of chelae hairs and olfaction in chemically-mediated mating behavior of crayfish.

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21. Differential localization of carbonic anhydrase isozymes in taste buds

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Histochemical techniques previously have shown the presence of carbonic anhydrase enzyme activity in taste buds and some nerves associated with them. To determine which isozyme of carbonic anhydrase is associated with taste papillae, and to more accurately detail the distribution of the enzyme in taste cells, antibodies to various carbonic anhydrase isozymes, CA-I, CA-II, CA-III, CA-IV and CA-VI were used immunocytochemically on lingual tissue. Sections of circumvallate, foliate and fungiform papillae as well as kidney and liver were obtained from rats fixed with 4% paraformaldehyde. Some sections were also exposed to anti-serotonin or anti-gustducin for double label comparisons. Antisera directed against CA-I and CA-II yielded similar patterns of reactivity perhaps due to cross reactivity of these sera. Accordingly such activity will be referred to as CA-I/II. No specific reactivity was present to antisera directed against CA-III or CA-VI. Heavy CA-I/II immunoreactivity was present in most taste cells of the circumvallate and foliate papillae; only a few cells in the fungiform papillae were reactive and these to a lesser degree than in other taste papillae. All gustducin-immunoreactive taste cells also were reactive for CA-I/II although a few CA-I/II cells were not reactive for gustducin. CA-II, but not CA-I reactivity was present in coarse nerve fibers associated especially with fungiform papillae. CA-IV reactivity was prominent in a few cells of fungiform taste buds. These strongly reactive taste cells were spindle-shaped but often had irregular short extensions from the basal region. Circumvallate and foliate papillae contained scattered taste cells that were weakly immunoreactive for CA-IV. These results indicate that two different carbonic anhydrase isozymes are present in taste cells and that one form, CA-I or CA-II, is specifically associated with taste cells utilizing gustducin.

22. Chorda tympani responses to bitters in inbred and congenic mice

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Two inbred strains of mice, C3HeB/FeJ (C3) and SWR/J (SW), differ in their level of behavioral aversion to various bitter substances. C3 mice are relatively indifferent to bitters at concentrations where SW mice avoid in 48 h, two-bottle preference tests. We compared whole-nerve chorda tympani (CT) responses to the bitter tastants quinine hydrochloride (QHCl) and strychnine hydrochloride (SHCL) as well as salt (NaCl) in these inbred strains plus C3.SW-Soa^a congenic taster and C3.SW demitaster mice. These congenic mice contain the SW-type SOA taster allele, which influences behavioral responsivity to QHCL and SHCl but not NaCl, transposed on an ~99% C3-type genomic background. Millimolar (1–30) concentrations of strychnine and quinine elicited moderate to robust responses (10 s stimulus presentation) in all strains of mice. These responses, along with those to 10–300 mM NaCl, were determined relative to a standard stimulus, 500 mM NH_4Cl. The strains did not differ significantly in CT response magnitude for quinine, strychnine, or NaCl. In conclusion, bitter taste responses occurred in the CT nerve of mice but did not appear to reflect allelic variation at the *Soa* locus.

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23. A comparison of sucrose and saccharin consumption in mice

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Mice were behaviorally tested in a system designed for studying the microstructure of ingestive behavior. This system involved two-bottle tests (water versus sucrose or sodium saccharin) with the capacity to detect individual bouts of drinking and eating. Eight mice were tested for eight consecutive 23 h periods with 0.33 M (12%) sucrose, and then for eight periods with 0.01 M (0.2%) saccharin. These two sweet-tasting stimuli produced identical 24 h preference ratios (~99% preference for solution versus water) across 8 days. However, mice consumed twice as much sucrose as saccharin per 24 h period. During the sucrose test, the mice also consumed less food (mouse chow), such that the total caloric intake in the two conditions was similar. On average, mice had more bouts of drinking of sucrose (52) than for saccharin (42) per 23 h period, but there was a much higher lick rate for sucrose (146.4 ± 29.5 lix/min) than for saccharin (44.9 ± 13.1 lix/min). Saccharin bouts were longer, while the mice exhibited intense drinking of sucrose during relatively short bouts. Furthermore, there was evidence of a difference in the way animals switched from food to drink. Mice switched rapidly and often back and forth between saccharin and food. With sucrose, mice switched far fewer times, usually from food to sucrose, and the switches were spaced further apart. These findings, along with the lick rate data suggest there may be fundamental differences between sucrose and saccharin consumption in mice, similar to those found for the rat. This differential behavior suggests possible limitations to genetic interpretations of sweet taste in mice based solely on preference ratios.

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24. Labeling and isolation of mouse olfactory receptor neurons projecting to discrete regions of the olfactory bulb

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Previous electrophysiological and optical recording studies have suggested that there may be odorant-related projection patterns from the olfactory epithelium to the olfactory bulb. Recent molecular investigations have shown that individual glomeruli receive inputs from distributed olfactory receptor neurons that express the same or similar putative olfactory receptor mRNAs. However, no data are available comparing odor responses from different olfactory receptor neurons projecting to one or a few neighboring glomeruli. We have undertaken a project, the goal of which is to address (i) whether olfactory receptor neurons that project to neighboring glomeruli have similar odor-response profiles and (ii) whether receptor neurons that project to the same glomerulus exhibit the same odor-response profiles. As a first step, we are attempting to record from olfactory receptor neurons that project to small clusters of glomeruli. To do this, we are injecting retrograde tracers into small groups of visualized glomeruli in the mouse olfactory bulb, and isolating labeled cells for physiological analysis. Small injections of fluorescent latex microspheres into clusters of 2–5 glomeruli in the dorsal olfactory bulb labeled neurons found predominantly in the anterior dorsal recess and dorsal surface of endoturbinate IId of the olfactory epithelium. Labeled neurons have been identified in acutely dissociated preparations and efforts to obtain odor-responses from these neurons using fura-2 calcium imaging techniques are currently underway. These methods will allow us to describe the functional convergence of olfactory receptor neurons and to test the idea that the molecular convergence described by others correlates with a convergence of afferents with the same responsivity to odorants.

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25. Regulation of second messenger signaling in olfactory neurons

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The olfactory system responds precisely to iterative stimulation due to the phasic responses of the sensory neurons. The transient response is due to a rapid termination of the primary reaction mediated by specific kinases leading to phosphorylation of odorant receptors and thereby uncoupling of the reaction cascade. The desensitized system is reactivated by dephosphorylation of the receptors by specific phosphatases. Experiments employing highly selective inhibitors as well as specific antibodies indicate that phosphatase subtype 2A plays an important role in reactivation of desensitized transduction cascades. Thus, the coordinated interplay between phosphorylation and dephosphorylation of odorant receptors governed by specific kinases and phosphatases may control the responsiveness of olfactory neurons. Recent observations indicate that elevated levels of intracellular calcium and cGMP, which both may be the response to strong stimuli, significantly attenuate the responsiveness of olfactory cilia. Pharmacological experiments have demonstrated that the inhibitory effect of cGMP is mediated by a cGMP-dependent protein kinase. Using specific antibodies the cGMP kinase in olfactory neurons was identified as type I isoform. Studies in progress explore the notion if the cGMP kinase activity may interfere with the phosphorylation/dephosphorylation of receptors and thereby fine tuning the responsiveness of olfactory sensory neurons.

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26. Laminar segregation of receptor expressing sensory neurons in the rat olfactory epithelium

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In the pseudostratified nasal neuroepithelium the somata of mature olfactory sensory neurons are located in a laminar arrangement in the lower two-thirds of the epithelium; in addition they are organized in columns that are perpendicular to the surface and are assembled into columnar units. In situ hybridization studies using specific molecular probes have allowed to localize chemosensory cells that express distinct receptor types in one of several rostro-caudal zones in the nasal olfactory epithelium. Within their zones receptor-expressing cells are restricted to the layer of mature neurons in the lower two-thirds of the epithelium, however it remained unclear whether neurons display any patterning in the horizontal layers or vertical columns. Detailed analyses revealed that neurons expressing a distinct receptor type were preferentially located in a particular laminar zone of the epithelium. In addition, sets of several reactive neurons within the same laminar zone were found to be arranged in an orderly fashion; they were positioned at well-defined horizontal distances. It is not clear whether such a subset of cells has a common origin; however, it is conceivable that such a regular alignment of cells is based on the columnar organization previously described by Graziadei (1979). These results suggest that the topographic and laminar localization of sensory neurons is under stringent control leading to characteristic expression patterns.

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27. Sodium as a flavor potentiator: selective suppression of tastes

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To investigate the mechanisms by which salts may 'enhance' food flavor, taste interactions between a sodium salt (Na acetate), a bitter-tasting compound (urea), and a sweetener (sucrose) were studied. All possible combinations of three concentrations of urea (0, 0.5 and 1.0 M), four concentrations of sucrose (0, 0.1, 0.3 and 0.5) and three concentrations of Na acetate (0, 0.1 and 0.3 M) were evaluated with the method of magnitude estimation. Experienced subjects judged the bitterness, sweetness and otherness of each solution. Subjects rated all 36 solutions twice in blocks of 12, grouped by solution sodium level. In general, bitterness and sweetness suppressed each other equally. However, Na acetate suppressed bitterness but did not suppress sweetness. As a consequence, when Na acetate was added to urea-sucrose mixtures it increased the perception of the sweetness of the three-component mixture while simultaneously decreasing the bitterness. This occurred despite the fact that Na acetate in a two-component mixture with sucrose had no enhancing effects on sweetness. This increase in the perception of sweetness of the three component mixture is likely due to the release of the sucrose sweetness from suppression by the urea bitterness. Such selective suppression effects of sodium may account, in part, for its reputed flavor-enhancing effects.

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28. Nitric oxide gates subunit 2 of cyclic nucleotide-gated channels cloned from rat olfactory receptor neurons

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We have previously reported that NO can directly activate the cyclic nucleotide-gated (CNG) channel from salamander olfactory neurons independently from the cAMP second messenger cascade. Its action could be via the chemical modification of SH groups.

The native CNG channel is thought to be an hetero oligomer of rOCNC1 and rOCNC2 subunits. In cloned reconstituted CNG channels, the rOCNC1 subunits form a homomeric channel that can be activated by cAMP. The second subunit, rOCNC2 is either not expressed at the membrane or cannot be activated as a homomeric channel by cAMP.

Nevertheless, we have transfected human embryonic kidney (HEK) 293 cells with the second subunit rOCNC2 of the rat olfactory CNG channel. Patch-clamp recordings under excised inside-out configuration were made 1 and 2 days after transfection. No CNG activated conductances could be recorded when cAMP (50–500 μM) was perfused. However, after cAMP had been washed out, the nitric oxide donor, S-nitrosocysteine (SNC, 100 μM) was added to the cytosolic solution, resulting in single channel openings and long bursts of openings. The single channel conductance of the NO-gated rOCNC2 is smaller than that of the native CNG channel or the rOCNC1 subunit homomer, or of the heteromeric reconstituted rOCNC1 and rOCNC2 channel. To gain insight into the molecular mechanism by which this NO donor activated this conductance, we tested the effects of a sulfhydryl binding reagent, DTNB (50 μM) to verify whether the NO effects resulted from a chemical modification of sulfhydryl groups. This substance also induced an immediate activation of the rOCNC2 channel which was reversible by DTT (1 mM).

These results suggest that rOCNC2 subunits do form a channel expressed at the membrane of the 293 cells. This channel can be gated by NO, which might be its physiological ligand. The possibility of using NO to activate the second subunit of the CNG channel should aid in understanding the contribution of this subunit to the wild type channel.

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29. G-protein βγ-subunits expressed in isolated olfactory receptor neurons

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In channel catfish, phospholipase C is coupled to the odorant receptors by heterotrimeric GTP-binding proteins (G-proteins) composed of α, β and γ subunits. However, the nature of the G-protein subunit(s) that mediate odorant stimulation of phospholipase C remains unknown. Previous immunohistochemical studies showed that at least five G-protein α-subunits were expressed in olfactory cilia. These studies also showed that olfactory cilia displayed intense immunoreactivity to a G-protein β-subunit antibody (Abogadie *et al.*, 1995). This observation, coupled with the role of G-protein βγ-subunits in activation of downstream effectors (Iniguez-Lluhi *et al.*, 1993), prompted us to investigate the expression of these subunits in olfactory neurons. Isolated olfactory receptor neurons were obtained by dissociating olfactory rosettes with papain and harvesting morphologically identified cells with a microcapillary. Poly $(A)^+$ RNA was isolated from cells and used in RT-PCR with degenerate primers to amplify β- and γ-subunit sequences. Sequencing of 15 cloned β-subunit PCR products showed that 13 clones corresponded to the β2-subunit while two clones were similar to the β1-subunit. Screening of a cDNA library constructed from isolated cells yielded a 1.5 kbp clone encoding the β2-subunit. Sequencing of nine cloned γ-subunit PCR products showed that three clones were homologous to the γ2-subunit and six clones were homologous to the γ3-subunit. These results demonstrate the feasibility of studying gene expression in isolated olfactory neurons and suggest that these cells express a limited repertoire of G-protein βγ-subunits.

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30. Cell proliferation in the olfactory system of the adult zebrafish

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In the present study we examined the generation of new cells in the brain of adult zebrafish. Due to their small size, easy breeding, and genetic constitution, zebrafish have become the '*Drosophila* of vertebrate developmental biology' and have been intensively studied during the past few years. Adult fish were placed in aquaria containing a 1% solution of 5-bromo-2′-deoxyuridine (BrdU) for 1 h. Exposing the fish to the treated water allowed uptake of the thymidine analog, presumably via the gills. Fish were returned to small aquaria that were changed every hour for 4 h (to prevent reuptake of any excreted BrdU). After a short (4 h) or long (3 week) period, animals were killed, embedded in paraffin, and the sections reacted via ABC immunocytochemistry. In the forebrain of the short survival group, labeled cells were found almost exclusively in the subependymal zones around the telencephalic ventricle. In the olfactory bulb, labeled cells (presumably glia) were found only in the olfactory nerve layer. In the olfactory epithelium, labeled cells were found only in the basal layer. After long survivals, numerous cells were still observed in the ventricular region with very few cells anywhere else in the telencephalon. In the bulb, however, cells were found in both the olfactory nerve and internal cell layers. In the epithelium, labeled cells were distributed throughout all layers. This work suggests that, just as in mammals, cells continue to be added to the olfactory bulb of the adult zebrafish. Furthermore, as in mammals, these cells appear to originate from the proliferative zones around the telencephalic ventricle and migrate into the bulb. We are currently characterizing these newly generated cells in greater detail.

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31. Human reactions and pharmacokinetic response to low levels of the gasoline oxygenate methyl tertiary-butyl ether (MTBE)

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The EPA has mandated use of gasoline oxygenates during winter fueling in various regions. Such oxygenates can reduce emissions of some pollutants, but can also alter the sensory character of emissions from the fuel. Oxygenated fuel has led to some reports of irritation, headache, etc., that might arise from exposure to one or another volatile organic chemical (VOC). This investigation concerned the effects of 1 h exposures to MTBE at 1.7 p.p.m. on healthy adults. Exposures simulated the high end of those of commuters. In the first part, four subjects participated in a pharmacokinetic study of blood levels. Concentration of MTBE in blood rose twentyfold by the end of exposure and then declined to half within 40 min. Blood levels became high enough to simulate exposures of persons who had expressed symptoms. In the second part, 43 subjects participated in a study of acute reactions to exposure to MTBE, to a mixture of 17 VOCs (7.1 p.p.m.), and to air. Subjects rated symptoms (e.g. irritation), their mood, and various environmental attributes (e.g. odor, air quality), and also took performance tests. Measures of eye irritation (e.g. tear-film breakup) and nasal inflammation [i.e. measurements of polymorphonuclear neutrophilic leucocytes (PMNs)] were taken before and after exposure as correlates of symptoms. Subjects could perceive differences among the exposures via odor and air

quality, but showed no differences in symptoms. The only significant objective effect comprised a time-by-agent interaction in PMNs, with a higher level was 18–24 h after exposure to VOCs than just after. Because subjects' ratings replicated effects found previously (e.g. time-dependence of irritation, effect of gender on odor intensity), the absence of differential effects of MTBE on symptoms can apparently be accepted at face value.

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32. Toward development of a discrimination-based, multiple-choice test of odor identification with real-world items

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For various reasons, including clinical testing and screening subjects for participation in sensory testing, it would seem useful to have available real-world items for odor identification/recognition via multiple-choice. We have used a three-step process to develop items that range from easy to difficult for Americans. In the first step, 80 subjects simply sought to name 81 items, such as coffee, molasses, bean sprouts, etc. The subjects gave as many as 48 different responses for an item (sherry wine) and as few as 18 (cinnamon). After culling the responses for duplicates, such as wine, white wine and cooking wine for sherry, and coalescing very similar answers, whether correct or incorrect, we graded responses for probability of application to an item. The three most probable answers then served as foils to construct a four-alternative multiple-choice set per item. For the item ginger powder, the names nutmeg, pumpkin, and mint served as foils, for example. In the process of choosing appropriate responses the set was pared to 54. These were then presented to 100 Ss who ranged in age from 18 to 83. Median performance on the set equaled 74%, safely away from a ceiling. Performance on individual items ranged from 22% for butterscotch to 97% for banana extract. As a third step, we reduced the set further, to 40 items, and divided that into two sets that we expected would yield equivalent performance based on results of 70 and 71% average correct from the 100 subjects. The 20 items of each set were then arranged in order from best to worst and given to another group of Ss in that sequence, one set to one nostril and the other set to the other nostril. As expected, errors increased from the easier to the more difficult items and indicated thereby that screening for functioning could be shortened in various ways. For example, if a patient with a complaint of anosmia misses just three or so easy items, the test could stop after just three trials. The more trials a person takes to make a criterion number of errors, the better his/her sense of smell. Number of trials to a criterion number of errors could thereby serve as the index of performance.

33. Effect of gender and experience on multidimensional sorting of corn and potato chips

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Multidimensional sorting (MDS) allows the study of perceived relationships between objects. Panelists sort a set of samples into groups based on similarities and differences. MDS is relatively simple for panelists to understand and can be completed quickly.

The objective was to determine if there were differences due to gender or experience in performing MDS on 15 varieties of corn and potato chips. Experienced males (EM), experienced females (EF), inexperienced males (IM), and inexperienced females (IF) comprised four groups of panelists (n = 10 for each group). Experienced panelists had previously participated in descriptive sensory analysis testing. Inexperienced panelists had no prior experience in sensory testing.

Each panelist was presented with the 15 samples in a completely randomized design and asked to sort them into no less than two and no more than 14 groups. No criteria for sorting was given. Panelists identified key words to describe each group. Two methods were used: a visual sort (VIS), in which panelists sorted only by looking, and a taste/touch sort (TTS), in which panelists were allowed to taste, touch and manipulate the samples as desired.

EM formed 3–13 product categories, EF 5–14, IM 2–11 and IF 5–12. Samples were separated along dimension 1 (D1) on the basis of corn versus potato, with corn products split into two distinct groups (light- and dark-colored). Along dimension 2 (D2), product separations were based on appearance and taste characteristics such as smoothness or roughness, presence or absence of ridges, and saltiness and oiliness. Use of D1 and D2 varied between panelist groups. EM and IF placed nearly equal weight on D1 and D2, while EF and IM weighted D2 more. With TTS, IM and IF created tighter product groups than EM or EF. Experienced panelists used the dimensions differently for VIS and for TTS, while inexperienced panelists used the dimensions similarly for both methods.

34. A quantitative study of rat petrosal ganglion neurons innervating the posterior tongue

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Neurons of the petrosal ganglion are primary afferent sensory neurons whose axons run in the glossopharyngeal nerve and project centrally to terminate in the rostral nucleus of the solitary tract (rNST). Petrosal ganglion neurons with axons in the lingual-tonsillar branch of the glossopharyngeal nerve relay sensory information derived from oral gustatory, thermal and tactile receptors and are therefore the first link in the sensory pathway between the posterior oral cavity and the central nervous system. Little is known about the biophysical and morphological characteristics of these neurons. Therefore, we have initiated

studies to characterize the properties of petrosal ganglion neurons that innervate the posterior tongue.

Either Fluorogold or Lucifer Yellow crystals were used as retrograde tracers to fill the cell bodies of neurons of the lingual-tonsillar branch of the glossopharyngeal nerve. When using Fluorogold the nerve was cut in anesthetized rats and the proximal end isolated and covered with the tracer. The Fluorogold was left in place for 30 min, the animals allowed to recover and then killed 72 h later. In the Lucifer Yellow experiments the anesthetized rat was first perfused with saline and the tracer placed on the cut nerve. It was left in place for 6 h and the animals were then immersion fixed. Either horizontal or transverse 15 μm serial frozen sections were cut and examined under epifluorescent illumination. The cell bodies of the lingual-tonsillar neurons are 20–35 μm in diameter and are located immediately proximal to the junction of the vagus and glossopharyngeal nerves. In addition there are a small number of 10–20 μm diameter neurons. The number of somata was determined either by counting the number of sectioned cell bodies and dividing by the average number of sections occupied by a soma, or by only counting soma with visible nuclei. The mean number of lingual-tonsillar cell bodies was 1748 ± 63 (SE). Now that we have information on the location and morphology of the petrosal ganglion neurons innervating the posterior tongue, we plan to study the membrane properties of these primary afferent sensory neurons.

Supported by NIDCD, NIH Grant DC 00059.

35. Influence of the Sac and dpa loci upon preference phenotypes for sodium saccharin, D-phenylalanine and the low-weight L-amino acids among inbred strains of mice

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Two-bottle preference testing of five inbred strains (129/J, BALB/cByJ, C3HeB/FeJ, C57BL/6J, and DBA/2J), a replicated half-diallele of Intercross (F_1) progeny from these strains, (C57×129)×129 backcross (B_1) progeny, and (C57×129)×(C57×129) intercross (F_2) progeny was used to analyze phenotypes for sodium saccharin (10^{-3} M) and D-phenylalanine (10^{-1} M). The sodium saccharin phenotype was found to be monogenic in both segregating populations. Lack of significant linkage for this phenotype with the Mup-1 locus on chromosome four has been taken as further evidence that Sac and dpa are independent loci. Variation for the D-phenylalanine phenotype (in the 129×C57 lineage), is apparently determined by two genes, one of greater influence (almost certainly the Sac locus) and a second of lesser influence (presumably the dpa locus). Two-bottle preference testing (using the same five inbred strains across an ascending series of molar concentrations) of some low-weight amino acids (glycine, L-alanine, L-serine, L-threonine) and an amino acid (L-proline) indicated variation for preference among the strains consistent with a two-gene model. An assignment of alleles to the inbred strains for the two genes is suggested:

dpa^{TT}/Sac^{dd}, DBA/2J;
dpa^{NN}/Sac^{dd}, 129/J.

This research was supported by NIH grant DC00150.

36. OMP immunoreactivity enhanced in individual ORNs following olfactory bulbectomy in the rat

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Olfactory marker protein (OMP) is a 19 kDa acidic protein distributed throughout the cytoplasm of mature olfactory receptor neurons (ORNs) in the olfactory epithelium (OE). Its function remains unknown. Following olfactory bulbectomy (OB-X), the proportion of ORNs mature enough to express OMP declines greatly. However, we have noticed over the course of several immunohistochemical studies that the intensity of OMP immunoreactivity (IR) in these few remaining mature ORNs appears to be higher than that shown by mature ORNs in nonbulbectomized OE. We have now undertaken a systematic immunohistochemical examination of this phenomenon.

Rats were subjected to unilateral OB-X and perfused with Bouin's Fixative at various post-operative times (3 days–6 months), as described elsewhere. Paraffin sections (10 μm) were reacted with various dilutions (1:500–1:50 000) of goat α-OMP (generously provided by Dr F. Margolis). Presence of OMP IR was visualized using a standard ABC kit (Vector). OE on the unoperated, contralateral side served as control tissue in each animal. Completeness of bulbectomy was verified histologically.

Results confirmed our earlier observations: at all post-operative periods and all α-OMP titers examined the intensity of OMP IR in individual ORNs was higher in operated than unoperated OE. Moreover, with increasing α-OMP dilution, IR intensity in ORNs in unoperated OE declined faster than in operated OE. At 1:40 000 OMP(+) ORNs were no longer distinguishable in control OE while even at 1:50 000 a few still remained ipsilaterally. Consistent with OMP expression being limited to mature ORNs, the scattered OMP(+) ORNs in ipsilateral OE were confined to just 1–2 apical cell layers. These observations suggest that ORN OMP levels may rise in response to direct axonal injury and the absence of OB trophic signals.

This research was supported in part by NIH grants DC02774, DC01593 and DC00347.

37. Stimulants of fish feeding behavior in tissues of marine organisms

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Techniques of ion exchange chromatography, HPLC, and thin

layer chromatography were used to analyze low molecular weight substances serving as feeding stimulants in extracts of 10 species of marine teleosts and 20 species of invertebrates. Multi-dimensional scaling techniques show that distinct taxon-specific groups are formed by comparing the relative concentrations of the free amino acids, quaternary amines, guanido compounds, and nucleotides in fishes, mollusks, and crustaceans. The greatest differences in relative concentrations are between the fishes and the invertebrates. Similarities are evident between the mollusks and crustaceans where eight of the nine most abundant substances are identical: i.e. betaine, taurine, trimethylamine oxide, glycine, alanine, proline, homarine and arginine.

A literature review is used to correlate the major tissue components in the fishes and invertebrates with the compounds previously shown to stimulate feeding behavior in 35 species of fish. Glycine and alanine are major tissue components that are the two most frequently cited feeding stimulants in the 35 species. Mollusks and crustaceans contain high concentrations of five of the most frequently cited stimulants (glycine, alanine, proline, arginine and betaine); these substances all occur in much lower concentrations in fish. Prior studies also show that some minor tissue components, such as tryptophan, phenylalanine, aspartic acid, valine and uridine 5′-monophosphate are important feeding stimulants for some fish species. Several major feeding stimulants (free amino acids and quaternary amines) also serve as 'compensatory solutes' which stabilize enzymes and structural proteins.

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38. An investigation into the effect of odour on product evaluation in 'virtual' environments

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In a study investigating consumer behaviour, an experiment was undertaken, examining subjects' evaluation of a hand towel, in the presence or absence of odours.

In an independent groups design, the experiment consisted of three conditions (no odour control; 'clean laundry' odour; and 'mildew/mould laundry' odour). Subjects (n = 60) were required to evaluate the product on a number of different dimensions, on a computer-generated questionnaire.

Preliminary results, using analysis of variance showed that there was a significant difference among the means of the three groups, on: 'softness-to-touch': $F(2,56) = 8.278$, $P = 0.001$; 'stitching': $F(2,57) = 3.972$, $P = 0.024$; and 'thickness/fluffiness': $F(2,56) = 7.285$, $P = 0.002$. All other evaluative questions were not significant. The results obtained indicate that the role of odour is not quite as straightforward as was suggested in a similar laboratory-based study (Hirsch, 1993). It is possible that other factors, especially context, combine with olfactory and auditory stimuli to provide the information necessary to judge a product holistically.

While previous studies involving consumer behaviour (Teerling *et al.*, 1992; Knasko, 1993) have been successfully conducted in 'real-life' retail settings, the use of 'real-world' environments is impractical and difficult to control. We propose that virtual reality will enable researchers to investigate the contribution of odour to product evaluation whilst providing complete control over the environment.

A series of experiments are being designed to address the issue of product evaluation in a virtual world. In order to develop the theoretical and commercial aspects of odour in environments, we must advance our understanding of the underlying psychological mechanisms of the olfactory commercial aspects of odour in environments, we must advance our understanding of the underlying psychological mechanisms of the olfactory system; VR may be a key strategic tool in advancing that understanding.

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39. Chocolate cravings: a cross-cultural investigation

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Chocolate is one of the most highly craved foods among Americans, especially females. The cause of this particular craving is not known. A number of possibilities have been proposed including physiological need (especially since females report their chocolate cravings as being perimenstrual) and the reinforcing sensory properties of the chocolate.

A questionnaire study was conducted investigating chocolate cravings of American and Spanish males and females to determine if there is any cultural influence on chocolate cravings and in particular the relationship of such cravings to the menstrual cycle.

While the proportion of males craving chocolate was lower than the proportion of females in both Americans and Spaniards, the difference was smaller for the Spaniards. This was due to the higher proportion of Spanish than American males who craved chocolate. The two groups of females craved chocolate to an equal extent. This result suggests some cultural influence on the rate of chocolate craving in men.

The relationship of chocolate cravings to the menstrual cycle in the two groups of females was also significantly different. Sixty percent of American females who craved chocolate reported that it was related to their menstrual cycle while only 24% of the Spanish females reported such a relationship. This result also suggests some cultural difference. It could be due to a difference in rates of dieting between the two populations. Since a high incidence of dieting occurs among American women it is possible that they use their menses as an excuse to eat chocolate (a forbidden food).

40. Human cortical taste areas studied with fMRI

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Although relatively well known in the monkey, gustatory projections (deep anterior insula, frontal operculum and orbitofrontal cortex) are poorly documented in humans: a few reports of lesions involve the parietal lobe, surrounding the sylvian fissure. Recent studies using PET or fMRI revealed taste evoked activity in left insula and temporal lobe.

We performed an fMRI study using a 3 Tesla whole-body MR scanner equipped with a head gradient coil allowing echo-planar imaging. Twelve transverse slices 6 mm thick centered on the sylvian fissure were sampled (64 × 64 pixel, FOV 200 mm, TE = 40 ms, TR = 500 ms) during a 5 min experiment. Six healthy male volunteers, aged 21–25, participated in this study. Stimuli were sodium chloride, aspartame, quinine hydrochloride, D-threonine, glycyrrhizic acid and 5′-guanosine monophosphate; low level concentrations were used to avoid trigeminal stimulation. Each subject tested three stimuli and water as a control, i.e. four experiments in the same session. Liquids were delivered directly to the subject's mouth through micro syringes and polyethylene tubings as a bolus of 50 µl manually pushed every 3 s. The stimulation paradigm consisted in two ON periods of 30 s and three OFF periods of 90 s during which water served as a reference and rinsing solution. Subjects used a linear potentiometer finger-span connected to an AD converter to continuously match the perceived intensity. The resulting time-intensity profile was correlated to the MR signal time course of each pixel of functional images.

We found activations for stimuli that were not elicited by water control experiments in both left and right insula, left and right frontal operculum and temporal gyri. We also found some activations in parts of visual areas (lingual gyrus) and limbic system (cingulate gyrus) related to stimulus evoked mental activity and emotion.

41. Membrane properties and excitatory synaptic transmission by mitral cells in slices of rat olfactory bulb

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Recent studies have provided evidence for the roles of excitatory and inhibitory synaptic circuits acting through specific types of receptors in the processing of odor information by the mammalian olfactory bulb. Building on a previous study of excitatory transmission in the isolated turtle olfactory bulb, we have developed a slice preparation of the rat olfactory bulb for analyzing these circuits.

Olfactory bulb slices were obtained from 20–40 day old rats by horizontal sectioning with a vibratome. Both whole-cell and sharp electrode intracellular recordings were made from cells in the mitral cell body layer. Some of these cells were identified as mitral cells by biocytin intracellular staining (n = 8). In response to depolarizing current injection, most mitral cells showed several distinct membrane properties: (i) delayed onset of firing (suggesting the presence of a type of potassium A current); (ii) subthreshold oscillations of the membrane potential; and (iii) repetitive firing of clustered action potentials during prolonged injection of threshold current.

Olfactory nerve (ON) stimulation evoked a long-lasting EPSP in most of the mitral cells. This long EPSP was completely blocked by combined application of antagonists of NMDA and non-NMDA receptors (20 µM CNQX and 100 µM APV), suggesting that, as previously shown in the turtle, glutamate is the neurotransmitter of synapses from the ON to mitral cells. The ON stimulation-evoked EPSP was often preceded by a prespike, which was resistant to membrane potential hyperpolarization. This prespike may be indicative of an active response in the dendritic tufts of mitral cells, similar to that seen in the rabbit and turtle. Stimulation of the lateral olfactory tract evoked an antidromic impulse followed by an EPSP-like depolarization which could also be elicited independently of an antidromic impulse in that cell. Since the only excitatory synapses on a mitral cell are from the ON, this antidromically induced EPSP may reflect self-excitation of a mitral cell by glutamate released from its dendrites by antidromic impulse invasion, or lateral excitation from neighboring invaded dendrites.

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42. Ca^{2+} transients in salamander mitral/tufted (M/T) cells: characteristic and properties

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Complex responses have been reported in salamander M/T cells either following odor or electric stimulation. An initial brief depolarization includes not only a compound EPSP from the olfactory nerve action potentials, but also active dendritic depolarizations, fast prepotentials (FPP), which persist even when hyperpolarizing current injections in the M/T somata block regular action potentials. In addition, although olfactory nerve stimulation typically evokes a single action potential followed by a hyperpolarization, low stimulation intensities (400–550 µA) can evoke in some M/T cells a second period of late depolarization. In this study, Ca^{2+} sensitive probes were used to determine the relationship of these membrane depolarizations with Ca^{2+} transients. Most experiments were conducted in regular slices. Horizontal bulb slices attached to the epithelium by intact olfactory nerve fibers and put in a split-bath tissue chamber were used to evaluated odor responses. Ca^{2+} transients evoked either by electric or odor stimuli were distributed non homogeneously in M/T dendrites suggesting multifocal electrogenic zones. Blockade of inhibitory feedbacks increased the magnitude, duration and spatial distribution of these Ca^{2+} transients. These conditions also facilitated the propagation of some transients to the soma. Ca^{2+} channel blockers (Cd^{2+} or Co^{2+}) suppressed all Ca^{2+} signals, even

those arising from stimuli focally applied at the glomeruli. Replacement in the bath solution of Ca^{2+} by Ba^{2+} or Mg^{2+} also suppressed Ca^{2+} responses. Following olfactory nerve stimulation, Ca^{2+} transients associated to FPPs and late depolarizations had different properties. Ca^{2+} changes associated to FPPs had fast onsets, were evoked predominately at high stimulation intensities (900–1500 μA; 500 μs) in distal dendritic regions. Ca^{2+} changes associated to late depolarizations had longer latencies and were evoked instead at low stimulation intensities (400–550 μA) in dendritic regions closer to the soma. These transients also differed in their pharmacological profile. These data suggest that different Ca^{2+} transients participate in the regulation of the spatio-temporal distribution of activity in M/T dendrites and have different implications in the generation of membrane potential changes.

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43. Self-metered oral doses of trigeminal irritants do not promote conditioned odor avoidance in starlings

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Birds, and in particular starlings (*Sturnus vulgaris*), avoid consumption of fluid or food treated with the natural plant products, methyl anthranilate (MA) and *o*-amino acetophenone (OAP). Avoidance is an unlearned response mediated via chemically sensitive fibers of the trigeminal nerve. Involuntary exposure to high concentrations of these irritants can lead to conditioned odor aversions. However, if starlings are allowed to self-meter their exposure to the irritants they fail to form conditioned odor aversions. The odor of these compounds is not aversive to starlings as judged by olfactory nerve cut experiments. Similarly, self-metered oral exposure to these compounds fails to lead to conditioned avoidance of colors that have been paired with the irritant. The failure to form conditioned avoidance responses when exposed to trigeminal irritants may have implications for the evolution of fruit chemical defenses. Trigeminal irritants may prevent untimely frugivory without causing seed dispersers to form strong generalized avoidance of sensory cues associated with fruit. Birds typically sample items that have been protected with trigeminal irritants. In contrast, birds that have been made ill with a toxicant form strong avoidance responses to flavor, odor or color. I hypothesize that the biochemistry of bird-dispersed fruits changes with respect to avian trigeminal irritants during the course of maturation. Specifically, unripe fruits should have high concentration of aromatic avian irritants and the maturation of the seed should coincide with deactivation or withdrawal of these compounds.

44. The role of 'death receptors' Fas and tumor necrosis factor receptor in apoptotic cell death in rat olfactory epithelium

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Adult olfactory epithelium is characterized by the continuous production of new neurons and continued death of existing neurons. In this study we asked whether the Fas antigen–Fas ligand (Fas–FasL) or the tumor necrosis factor receptor (TNFR)–TNFα systems were involved in cell death in olfactory epithelium. Both Fas and TNFR are found on many cell types and, when activated, a cascade of events is initiated that results in apoptotic cell death.

Olfactory mucosa was removed from adult rat nasal septum and turbinates and total RNA was extracted. RNA was reverse transcribed and, using appropriate primers, a polymerase chain reaction was carried out to determine whether the transcripts of Fas, FasL, TNFα and TNFR were present. In unperturbed rats all four transcripts were found. The experiment was repeated on unilaterally bulbectomized rats, 1, 2, 5 and 21 days after surgery. Massive death of neurons occurs within the first week (acute stage) and the rate of cell death is upregulated by two- to fourfold in the chronic stage, after 1 week. Of the four transcripts only that of TNFα255C5,5,0,0,0,0 was noticeably upregulated on the operated side at all times following surgery; TNFα was also up-regulated on the unoperated side 1 and 2 days following surgery. The presence of both cell death-related systems in olfactory epithelium suggests that both may participate in regulating apoptotic cell death in olfactory epithelium in the unperturbed state. The up-regulation of TNFα transcripts after surgery suggests the TNF system may be prominent under some physiological conditions.

Finally, the presence of FasL and TNFα in olfactory epithelium might explain why lymphocytes (most of which carry both the Fas antigen and TNFR on their membrane) are rarely seen in the epithelium; this suggests that olfactory epithelium, like other tissues including brain and testis, might be, to some degree, immunologically privileged.

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45. Nasal localization thresholds in normosmics mirror nasal pungency thresholds in anosmics, for homologous *n*-alcohols

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In the past, we have sought to discriminate between the level at which an airborne chemical begins to produce and odor from that at which it begins to produce nasal pungency (via trigeminal nerve stimulation) by testing normosmic and anosmic subjects respectively. Anosmics, lacking olfaction, can only detect vapors in the nose through the trigeminus. In recent studies, it has been indicated that localization of an airborne compound to the right or left nostril is only possible through trigeminal activation. If this

is so, measuring nasal localization thresholds in normosmics might represent an alternative way of obtaining 'olfactory unbiased' nasal pungency thresholds, as previously suggested. Using homologous *n*-alcohols (1-propanol, 1-butanol, 1-hexanol and 1-octanol) as stimuli we are measuring nasal localization, odor, and eye irritation thresholds in normosmics. Substances are presented from 'squeeze bottles', employing a two-alternative, forced-choice procedure and an ascending concentration method of limits. We are also measuring nasal pungency and eye irritation thresholds in anosmics, using identical stimuli and methodology. Ongoing measurements show that nasal localization and nasal pungency thresholds—measured in normosmics and anosmics respectively—fall very well into register. Furthermore, 1-octanol, the only compound that failed to elicit nasal pungency, also failed to be localized, even at vapor saturation. The results support the notion that nasal localization thresholds in normosmics and nasal pungency thresholds in anosmics represent equivalent ways of obtaining olfactory unbiased nasal trigeminal thresholds.

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46. Cardiovascular responses during taste-mediated licking in rats

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We developed a method for measuring licking responses to brief presentations of taste solutions simultaneously with blood pressure and heart rate. Three male Sprague–Dawley rats were used. One rat was raised, from conception to postnatal day 30, on a maternal diet containing basal 0.1% NaCl and two males rats were raised on a maternal diet containing high 3% NaCl. After postnatal day 30, all three were switched to a mid 1% NaCl diet and they remained on this diet throughout training and testing. The rats were trained to drink immediately to brief 30 s presentations of 0.2 M sucrose. After training, the rats were implanted with an aortic electronic sensor for transmitting blood pressure signals by telemetry. The animals were adapted to a water deprivation schedule such that they received water for 60 min 1 h before testing and again for 60 min 1 h after testing. For each test session, the water-deprived animals received two random 10 s presentations each of water, 0.2 M sucrose, 0.1 M NaCl and 0.008 mM quinine hydrochloride. The animals received nine sessions with this stimulus protocol. A microcomputer controlled stimulus presentations and recorded licks, mean arterial pressure and heart rate during each stimulus presentation. Preliminary results show that solution drinking increased blood pressure 10–15 mm Hg. This pressor response was similar for water, sucrose, NaCl and quinine. The baseline blood pressure and heart levels of the two high salt rats were significantly higher than those of the basal salt rat. The pressor response to licking steadily increased throughout the 10 s stimulus presentation for the two high salt rats, but increased then decreased for the basal salt rat. Thirst may obviate differential cardiovascular responses to taste solutions. Maternal salt feeding influences long-term blood pressure and heart rate levels and may influence blood pressure reactivity to solution drinking in rats.

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47. Pheromone behavioral responses in unusual male European corn borer hybrid progeny not correlated to electrophysiological phenotypes of their pheromone-specific antennal neurons

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In genetic studies on the sex pheromone communication system of two races of European corn borer, which use opposite pheromone blends of the E and Z compounds, it was found that antennal olfactory cell response amplitudes to the two compounds were controlled by an autosomal factor, whereas behavioral responses to the blends were controlled by a sex-linked locus. Because of the difference in genetic controls, it was postulated that some unusual males would be produced in F_2 crosses between these two races. These unusual males would have antennal olfactory cells that respond as the Z-race males, but would respond behaviorally to the E blend. The present studies combined behavioral studies in a flight tunnel and single cell electrophysiological studies to show that these unusual males do indeed exist. These findings show that the spike amplitude of peripheral olfactory cells is not important in regulating species- or race-specific pheromone responses, as compared with some central nervous system factor assesses the spike frequencies from different pheromone-component-specific cells on the antenna. This factor seems to be essential in governing the pheromone-blend specific behavioral responses of male moths.

48. Effect of odor concentration on the olfactory event-related potential in introverts and extroverts

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Compared to auditory and visual event-related potentials (ERP), relatively few studies have been performed on olfactory ERPs (OERP) due to the difficulties involved in their recording. Problems with recording include lack of stimulus control and concurrent stimulation of the somatosensory, auditory, or trigeminal system. Although recent improvements in recording technique have overcome these difficulties, recording parameters which produce an optimal OERP waveform continue to be explored. In this study, participants tested for introversion and

extroversion with the Myers–Briggs type indicator were presented low, medium, and high concentrations of the odorant isoamyl acetate. Previous ERP studies of stimulus intensity in the auditory modality have found differences in the amplitude of the P300 (P3) between introverted and extroverted individuals. Furthermore, ERP research in the olfactory modality has shown a change in P3 latency as the odor concentration, or stimulus intensity, changes. In the present experiment P3 amplitude was influenced by the odor concentration. Specifically, P3 amplitude was smaller for the low concentration compared with the medium concentration across both personality types. An interaction between personality type and odor concentration at the Cz electrode site produced a larger N2-P3 interpeak amplitude for the introverts at the low concentration and the extroverts at the high concentration. Furthermore, personality type interacted with electrode site and odor concentration. Introverts demonstrated a shorter P3 latency at Cz and longer P3 latencies at Fz and Pz for both the low and medium concentrations compared with extroverts. These findings suggest that the OERP is sensitive to changes in odor concentration. However, further studies are needed to elucidate the effects of odor concentration as a function of personality type.

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49. Phantom tastes/phantom smells: comparisons and contrasts

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Taste–smell confusions may lead to simultaneous reports of taste and smell phantoms and blur distinctions between these syndromes. However, most patients who present with a complaint of a chemosensory phantom do label it as either a taste or a smell. To better delineate these two forms of chemosensory dysfunction, we compared data obtained from 84 patients who complained of a phantom smell (PS) and 107 who reported a phantom taste (PT); both of these groups of patients were also compared with patients who complained of chemosensory loss or distortions (LOSS) in the absence of phantom sensations (n = 532). The PS and LOSS patient groups were comparable in age, but PT patients were significantly older than either ($P < 0.005$), with over half being at least 60 years of age. Women predominated in all three patient groups; this was most pronounced in PS patients (68% female). Over 70% of PS patients evidenced a clinically significant loss of smell on standard olfactory testing. This rate of smell diagnosis is identical to that seen in LOSS patients, and significantly higher than that observed in PT patients (19.6%; $P < 0.001$). In contrast, only 18.7% of PT patients evidenced whole-mouth taste loss; however, this is still significantly higher than the rate of taste diagnosis in either PS or LOSS patients (6–7%; $P < 0.001$). PS complaints were significantly more likely to be associated with a recognized etiologic factor (83.3%) than were PT complaints (40.2%; $P < 0.001$). Follow-up interviews with subsets of patients, however, indicate that PS (n = 28) and PT (n = 44) patients were equally likely to experience improvement in or resolution of their phantom symptoms (reported by ~43% of each group).

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50. Olfactory bulb development in the postnatal mouse is altered by postnatal exposure to retinoic acid

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The transcriptional activator retinoic acid (RA) is an endogenous morphogen for the developing CNS. Systemic exposure of early embryos, especially during organogenesis, to high levels of exogenous RA is highly teratogenic. However, the effects of postnatal exposure to RA on the still-developing nervous system are less well known. We have given retinoic acid to neonatal mice and studied the gross morphologic and anatomic sequelae in the olfactory bulb and nasal epithelium at various postnatal ages. At 14 days of age (P14), olfactory bulbs and noses appear shorter in length but broader in width. The number of glomeruli in RA-exposed animals is substantially reduced (by as much as 40%) compared with littermate controls. The regions of the olfactory bulb that appear most affected are the dorsolateral and the ventrolateral quadrants. At this age the nasal epithelia are also thinned in width, both throughout the turbinates as well as the nasal septum. Even by P43 there are still apparent differences in the organization and structure of olfactory bulb glomeruli and nasal epithelia. Thus, whatever the multiple molecular mechanism(s) that are developmentally regulated by retinoic acid, the continual turnover of the postnatal mouse olfactory system does not appear to completely compensate.

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51. Use of similar experimental protocols to evaluate differences in coding of mixtures by spiny lobsters and catfish

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Two animal models used extensively to study coding of odorant mixtures are the spiny lobster *Panulirus argus* and the channel catfish *Ictalurus punctatus*. The incidence of mixture suppression and enhancement reported in the literature is different for these species. However, it is uncertain if these differences are due to dissimilar experimental protocols and definitions or interspecific differences in the mechanisms of processing of mixtures. To resolve this issue, we have used a protocol on spiny lobsters similar to that used on catfish. We examined responses of individual taurine-sensitive olfactory neurons to mixtures of two excitatory compounds that either do (taurine, β-alanine, hypotaurine) or do not (taurine, L-glutamate, ammonium, adenosine-5′-monophosphate) compete for the same receptor sites, as indicated by

previous binding and electrophysiological data. Two indices were calculated: mixture discrimination index (MDI) and independent component index (ICI). Our results for spiny lobsters were generally similar to those reported for catfish. For both species, binary mixtures of competitors had MDI values close to 1.0, as expected, and ICI values <.0, indicating a lack of independence in their activity. Mixtures of non-competitors had MDI values >1.0 and ICI values <1.0. The major interspecific difference was that the ICI values were slightly lower for spiny lobster. We conclude the following. (i) Individual cells of spiny lobsters are excited by more than one food odorant because they express more than one type of receptor, and that one receptor type is typically of much higher density or affinity. (ii) Generally similar results for spiny lobster and catfish suggest many similarities in processing of odorant mixtures. (iii) The lack of independence between non-competitors could be due to a number of interactions between them at transduction sites within a cell, and is an example of previously reported mixture suppression. (iv) It will be important to analyze a greater diversity of olfactory cells from both species, including those excited by one component and suppressed by another.

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52. Localization of cholecystokinin mRNA to lingual epithelium in rat tongue

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Cholecystokinin (CCK) is a peptide hormone with multifaceted functions that is localized in the GI tract and in central neurons. Previous work in this laboratory using immunocytochemical techniques has localized CCK-like immunoreactivity to sub-populations of taste cells in foliate and circumvallate papillae and in the nasoincisor ducts (NID). We sought to confirm and further characterize these findings by localizing messenger RNA encoding the CCK peptide using techniques of non-radiographic *in situ* hybridization. A 35 base pair antisense probe complimentary to a region of mRNA that encodes the carboxy terminal amino acids of the peptide was tailed with digoxigenin (DIG) labeled nucleotide and visualized with an phosphatase labeled anti-DIG antibody using *x*-phosphate and nitroblue tetrazolium (BCIP/NBT) as color substrates. Hybridization was performed on paraformaldehyde-fixed cryostat sections of rat posterior tongue and, as a positive control, brain at room temperature and 37°C. Cytoplasmic labeling with clear nuclei was present in cortical neurons. Negative controls included tissue sections incubated with sense probe, without probe or without antibody. Reaction product from *in situ* hybridization was more widely distributed than immunocytochemical product. Positive labeling was confined to lingual epithelium of the foliate and circumvallate papillae and the NID. It was absent under conditions of sense probe, no probe or no antibody. Under the most stringent wash conditions (0.1× SSC) reaction product was observed more specifically in taste buds of circumvallate and foliate papillae. In general, these studies support the notion that the peptide cholecystokinin is localized in some taste cells, and perhaps more generally within the lingual epithelium.

B.C. and T.L. contributed equally to this work. Supported by NIH grant DC00401.

53. The effects of varying duration of naris occlusion on olfactory bulb laminar volume

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Closing a single external naris soon after birth drastically alters the size of the ipsilateral olfactory bulb. For example, naris closure on the day after birth (P1) in rats results in a 25% reduction in the volume of the ipsilateral olfactory bulb by P30. We have recently developed a method for reversibly blocking nares which involves the insertion of removable nose plugs. The present study employs this technique to examine the effect of varying durations of naris occlusion on the size of the ipsilateral olfactory bulb. Rat pups underwent reversible unilateral naris closure on P1. Nose plugs were removed on either P10, P15 or P20, and animals were tested to ensure patency of the previously occluded naris. On P30 animals were killed and serial nissl sections of their olfactory bulbs produced. Volume measurements were made of the glomerular, external plexiform, and granule cell layers of both bulbs using a computer planimetry system. Results suggest that longer durations of naris occlusion result in greater reductions in the overall volume of the ipsilateral olfactory bulb: occlusion from P1 to P10, P1 to P15 and P1 to P20 resulted in an approximate 6, 12 and 15% reduction in the overall volume of the bulb respectively. Largest changes were seen in the GLM in animals plugged from P1 to P10, while the EPL was most affected in pups occluded from P1 to P20. Previous studies using permanent naris closure revealed that cells born on the day after the onset of naris occlusion underwent a 50% reduction in cell survival in the ipsilateral olfactory bulb (*J. Comp. Neurol.*, 289). In an on-going study, pups were reversibly occluded from P1 to P3, P1 to P6, P1 to P10 or P1 to P15, and cells born on P2 were labeled with BrdU. Quantification of BrdU-immunoreactive cells at P30 will allow us to define the length of naris occlusion necessary to affect survival in this population of bulb cells.

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54. Middle-aged females exhibit lower orthonasal and retronasal olfactory perception than young females

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Olfaction serves as a dual sense to perceive odors orthonasally (via the nostrils) and retronasally (via the mouth). We examined if middle-aged females (n = 29, mean age = 51 ± 5) show lower olfactory function than young females (n = 15, mean age = 22 ± 4). Middle-aged subjects completed a health and dental history and 11 of the sample were still menstruating and were dentate. Subjects used the labeled magnitude scale (Green *et al.*, 1993) to rate intensity of a five member concentration series of NaCl (quarter

log dilutions from 1 M), and chocolate and orange odorants sampled orthonasally and retronasally (log dilutions from 0.07 and 0.21% respectively). Orthonasal odorants were sniffed from squeezable polyethylene bottles. The retronasal odorants were delivered in a sweetened gelatin (0.15 M) which diminished volatile release until orally manipulated and masked any primary taste qualities of the odorant. Subjects were asked only to rate the intensity of the odorant in the sweetened gelatin. Stimuli were tested in a random order and replicated. Odorant perceived intensities were normalized to 1 M NaCl (Snyder *et al.*, 1996). In ANOVA, a significant main effect of group occurred for both orthonasal and retronasal odorants: orthonasal orange [$F(1, 42) = 9.32$, $P < 0.01$)] and chocolate [$F(1, 42) = 4.71$, $P < 0.05$)]; retronasal orange [$F(1, 42) = 4.03$, $P = 0.05$)] and chocolate [$F(1, 42) = 5.87$, $P < 0.05$)]. Middle-aged females rated significantly lower intensities ($P < 0.05$) for all but the top odorant concentrations. In the total sample, orthonasal and retronasal functions correlated significantly ($P < 0.01$) across all concentrations for both odorants. When comparing young females to dentate, menstruating middle-aged females, the significant main effect of group remained for the orthonasal odorants but disappeared for the retronasal odorants. In summary, these data reveal that middle-aged females, on average, exhibit lower orthonasal and retronasal olfactory perception than young females. Lower olfactory perception was apparent in middle-aged females even when attempting to control for menstrual and dental status.

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55. Neural responses to bitter compounds

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Single-unit responses to a variety of bitter tasting compounds were recorded from rat chorda tympani (CT) ($n = 28$) and glossopharyngeal (GP) ($n = 33$) neurons. Responses to several alkaloids were obtained: 10 mM quinine, 50 mM caffeine, 1 mM nicotine, 1 mM yohimbine and 1 mM strychnine, plus a number of non-alkaloid bitter compounds including, 0.1 M KCl, 0.01 M $MgCl_2$, 1 mM L-tyrosine, 1 mM PTC and 1 mM denatonium. Capsaicin (10 μM), NaCl (0.1 M) and HCl (pH 2.0) were also tested. One goal was to determine whether neurons that are activated by one alkaloid, quinine for example, are activated by other alkaloids. The neural activity was determined by counting the difference in the number of action potentials 5 s before and after a stimulus was applied to the tongue. The data were analyzed by calculating the neural mass differences between stimuli and plotting these differences using multidimensional scaling (MDS). In MDS space, the distance between stimuli are related to their similarity. As controls, responses to quinine (GP) and NaCl (CT) were obtained before and after the other stimuli were applied. GP neurons showed similarity between quinine, denatonium and nicotine. CT neurons showed similarity between quinine, $MgCl_2$ and KCl. These observations indicate that the class of alkaloids do not activate a common receptor.

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56. Coordination of chemosensory orientation in the starfish *Asterias forbesi*

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Starfish are known to orient to distant odor sources but previous research has not examined how the decentralized, aganglionic nervous system of the starfish can be coordinated to reach a distant goal. I observed orienting starfish in a y-maze and used local ablations of chemoreceptors by treatment with distilled water to approach these questions. Starfish orienting in an odor plume tend to lead with the ray facing the odor source, typically the upstream ray (48%, significantly greater than chance, $P < 0.01$). When the upstream ray is ablated by treatment with distilled water, the starfish leads with the upstream ray less frequently (7.6%, less than the control, $P < 0.01$), but success at reaching the source is not affected (80% of controls and 76% of ablated starfish reached the source, both significantly greater than chance, $P < 0.01$). When two or three adjacent upstream rays are ablated, success is rare (16.7%) and downstream movement is frequent (50% of trials). It seems likely, then, that the ray receiving the strongest chemosensory input becomes the leading ray, causing movement in the direction that typically would take it to the source. Downstream movement in ablated starfish makes the use of a rheotactic sense unlikely. A computer model based both on this conclusion and on earlier starfish research is successful at finding an odor source even in a patchy simulated odor plume.

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57. The effects of varied exposure schedule on the intensity and locus of sensory irritation from acetone

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Repeated exposure to a volatile chemical often diminishes its perceived odor and irritancy. To study the temporal parameters of adaptation to an irritant, we varied the interval between 20 min exposures to 800 p.p.m. acetone in a chamber and collected ratings of perceived odor and irritation during exposure. To better characterize the locus of sensory irritation from acetone, subjects in three studies were also asked to identify the location of the dominant nasal and throat irritation on a schematic diagram of the upper respiratory airways.

With inter-session intervals of 2 days or 1 week, subjects showed adaptation to odor and irritation both within and across the exposure sessions. In contrast, when the inter-session interval was

only 15 min, subjects showed adaptation within each session, but transient sensitization to odor and irritation across the sessions. Interestingly, rated irritation was significantly lower when subjects used the diagram to identify the locus of irritation than when they rated irritation alone. The diagrams revealed that the locus of strongest perceived irritation often shifted within and across exposures. Because the pharynx and throat were frequently identified as the dominant site of irritation (on 36 and 63% of the trials respectively), the results suggest that the irritancy of acetone is not entirely mediated by the trigeminal nerve.

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58. Squid olfactory receptor neurons respond to betaine in a dose dependent manner via a second messenger mediated chloride conductance

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We performed nystatin patch recordings on isolated olfactory receptor neurons (ORNs) of the squid *Lolliguncula brevis*. We report that betaine activates a Cl^- conductance in squid ORNs that can be reversibly abolished by inhibiting phospholipase C (PLC) with the inhibitor U-73122. Nystatin patch recordings reveal that the I_{Cl} is unaffected by changing internal or external $[Na^+]$or $[K^+]$ and that its reversal potential follows the Cl^- reversal potential as it shifts with changes in internal $[Cl^-]$. The betaine induced current also appears to be slightly inwardly rectifying in symmetrical Cl^- solutions. At –50 and +50 mV, the average peak currents were –80 ± 15 pA and 24 ± 5 pA respectively (n = 9). Finally, the betaine induced current shows slow desensitization, with τ = 2.89 s in response to a 1′ s application of 10 mM betaine. Evidence supporting an IP_3 mediated response includes the fact that the currents wash out in whole cell recording but can be maintained in nystatin patch recordings. The time to half peak is a slow 115.2 ± 11.7 ms (n = 11) as compared with voltage gated I_{Na} of 3 ms at –40 mV. Finally, the response can be reversibly inhibited by the PLC inhibitor U-71322. Previous studies indicate that IP_3 in the patch pipet activates a chloride dependent conductance in these neurons (Lucero and Piper, 1994). Based on these pieces of evidence, we conclude that betaine activates a dose dependent Cl^- conductance that could either depolarize or hyperpolarize the cell, depending on the internal Cl^- concentration, and that this response is mediated by an IP_3 second messenger system.

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59. Responses to alcohol in chorda tympani taste fibers of *Macaca mulatta*

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Responses of whole chorda tympani nerve as well as single taste fibers in rhesus monkey were recorded to taste stimulation of the anterior part of the tongue. Our main goal was to investigate the effect of ethanol on the taste fibers. The approach was first to characterize each fiber with an extended array of taste stimuli. We used three salts, two umami compounds, three acids, three bitter compounds and 12 sweeteners. Having sufficient background about the response profile of the fibers we stimulated them with 1 M and 3 M ethanol alone or mixed with four basic stimuli: 0.07 M NaCl, 0.04 M citric acid, 5 mM quinine and 0.3 M sucrose. The whole nerve was stimulated with series of 8 concentrations (from 0.3 to 8 M) of alcohol alone.

As in recordings from human chorda tympani nerve, but different from non-primates, the response to ethanol consisted of a phasic and a tonic part. 0.3 M ethanol was the lowest concentration tested and it did give response. The response–concentration relationship can be described by a sigmoidal function (correlation coefficient 0.99).

The response profiles of 41 single fibers to 25 stimuli were subjected to hierarchical cluster analysis. Four major clusters of fibers, characterized by predominant sensitivity to NaCl, sucrose, quinine and citric acid were separated. At 1 M, ethanol stimulated only sucrose-best fibers; at 3 M it elicited a strong response in sweet fibers and a small response in salt fibers. In contrast acid and bitter fibers did not respond to ethanol.

In sucrose-best fibers the responses to mixtures of NaCl, citric acid, quinine and sucrose with 1 or 3 M alcohol were greater than the responses to the basic four stimuli themselves. But in salt-, acid- and quinine-best fibers most responses to the four basic stimuli were suppressed when the tongue was stimulated with alcohol mixtures.

The fact that ethanol stimulated mainly the sweet chorda tympani fibers may explain the preference for low concentrations of alcohol in behavioral experiments with monkeys.

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60. Dopaminergic neurons in the gustatory zone of the nucleus of the solitary tract in the hamster receive direct synaptic inputs via the chorda tympani

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The gustatory zone of the nucleus of the solitary tract (NST) in the hamster contains a substantial population of relatively large tyrosine hydroxylase (TH) immunoreactive neurons that are probably dopaminergic. The function of these dopaminergic neurons is unknown. Data are presented that such neurons do not

project to the parabrachial complex and, consequently, do not contribute to the ascending gustatory pathway. These dopaminergic neurons could provide a descending projection that influences a variety of visceromotor reflexes that accompany ingestional behavior. We hypothesize that the initiation of such reflexes would require a direct and secure input from peripheral taste receptors. We explored this possibility by destroying the chorda tympani (CT) with the toxic lectin ricin and examining the synaptic relationships between the degenerating CT terminal boutons and TH immunoreactive processes. Normal axonal terminal boutons of the CT appear as large bulbous endings that are densely packed with medium-sized, clear spherical vesicles uniformly distributed with no accumulation near the active synaptic site. At short survival times (3–7 days), the degenerating terminals of the CT become relatively dark and the synaptic vesicles appear swollen, but the degenerating boutons are still morphologically intact and associated with identifiable synaptic complexes. Of 70 well-defined degenerating CT axon terminal boutons, 57% were in synaptic contact with non-immunoreactive dendrites and 43% were in synaptic contact with the shafts of TH immunoreactive dendrites. No axosomatic contacts were encountered. This is the first demonstration of an anatomical segregation between pathways mediating taste discrimination and the control of ingestional reflexes, and a direct synaptic relationship between the CT and a functionally distinct class of gustatory neurons.

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61. Citrate enhances glossopharyngeal taste responses to L-proline in the channel catfish

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Citric acid, an acidulant and preservative in food processing, is often used commercially as a flavor modifier, but knowledge of its specific physiological effects on the sense of taste, other than as a proton donor, is scarce. It has been indicated that citrate ($Na_3^+citrate^-$) enhanced (i) the glossopharyngeal taste response to L- and D-arginine in the largemouth bass and (ii) behavioral and cellular taste responses to sweet and amino acid stimuli in rodents. Unpublished data indicate that citrate releases both appetitive and consummatory feeding behavior in channel catfish. The present study demonstrates that citrate enhances the glossopharyngeal (IX) taste responses to L-proline in the channel catfish, *Ictalurus punctatus*; however, citrate does not enhance L-arginine or L-alanine taste activity in either VII or IX. Studies on the possible effects of citrate on facial taste responses to L-proline are currently ongoing. Taste enhancement occurs over a narrow range of citrate and proline concentrations (~1.0 mM), less than or equal to threshold for citrate and at mid-dynamic range concentration for L-proline. The taste enhancement to the mixture of L-proline + citrate was also pH-dependent and generally occurred between pH 8.5 and 9.0, a pH range commonly measured in local catfish ponds. In contrast, high concentrations (0.3–1.0 M) of citrate in the binary mixtures often resulted in taste responses less than the response to the component amino acid alone. The present report is also consistent with previous data indicating the independence of receptor sites for L-proline from those to L-alanine and L-arginine.

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62. A cyclic nucleotide-gated chloride conductance in olfactory receptor neurons

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Sensory responses are elicited in olfactory receptor neurons (ORNs) by odor-activated processes that alter the membrane conductance and change neuronal firing properties. Several different conductances are involved in odor responses including a cyclic nucleotide-gated cation conductance and a Ca^{2+}-activated Cl^- conductance. In ORNs from *Necturus maculosus*, odors elicit increases as well as decreases in the Cl^- conductance (Dubin and Dionne, 1993), suggesting the presence of two different types of chloride channels in these cells. We examined this possibility using whole-cell patch-clamp recordings, ion substitution, and flash photolysis of caged cyclic nucleotides. A Ca^{2+}-activated Cl^- current can be blocked in these cells, by substitution of Ba^{2+} for extracellular Ca^{2+}, or by blockade of Ca^{2+} influx with 100–200 mM Cd^{2+} ($n = 9$). When the Ca^{2+}-activated Cl^- current is blocked by Cd^{2+} or by Ba^{2+} substitution, a voltage-dependent Cl^- current can still be elicited. The sudden release intracellularly of cyclic nucleotides by flash photolysis of caged-cAMP or caged-cGMP activates a Cl^- current that is blocked by DIDS in a dose-dependent manner. The flash-activated Cl^- current persists in the absence of extracellular Ca^{2+} ($n = 4$), in the presence of high Ca^{2+} both inside and outside the cell ($n = 4$), and cannot be elicited by the photolytic release of IP_3 ($n = 7$). When the broad spectrum protein kinase inhibitor H-7 was loaded with the caged compounds into cells, flash-activated Cl^- current was still observed ($n = 5$). These data suggest that cyclic nucleotides can directly activate Cl^- current in *Necturus* ORNs and that it is the voltage-dependent Cl^- conductance which is affected.

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63. An IP_3 receptor partial cDNA from rat olfactory organ

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Evidence from a number of laboratories implicate inositol 1,4,5-trisphosphate as a second messenger in olfactory responses to some odorants. Lischa and co-workers provided evidence of direct gating of plasma membrane channels by IP_3 in rat olfactory

neurons. Antibodies directed against the 19 C-terminal amino acids of the mouse cerebellar form of the receptor localize to ciliary regions of olfactory neurons and recognize membrane proteins of M_r 260, and 120 kDa in proteins extracted from rat olfactory cilia while photoaffinity labeling identified specifically labeled proteins of 120 kDa in the same membrane preparation, suggesting that multiple forms of the IP_3 receptor may be present in olfactory neurons. We have begun to further examine the molecular identity of the olfactory IP_3 receptor by amplifying a partial cDNA with homologies to known IP_3Rs from reverse transcribed olfactory ciliary RNA using primers from highly conserved regions of the IP_3R and PCR. Northern analysis with the cerebellar form of the IP_3 receptor resulted in no hybridization to mRNA from rat olfactory tissues under high stringency conditions. However, when Northern analyses were performed using a probe prepared from the partial cDNA product expression of a 10 kb band as well as an additional band of ~4 kb were identified in rat olfactory RNA. Further studies are being conducted to extend the olfactory cDNA clone and to determine whether multiple isoforms of the IP_3R are present in olfactory tissues.

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64. Implications of *Pax-6* gene for olfactory system development studied in small eye (*Sey*) mice

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Pax-6 is a member of a family of developmental control genes which encode proteins containing a unique DNA binding domain known as the paired-box. *Pax* gene products are transcription factors believed to be involved in inductive developmental events in the nervous system. During development *Pax-6* is expressed in the eye, olfactory system and pituitary. This gene is believed to be involved in the induction of both the eye and nose, since it is expressed in both the inducing and induced tissues. Small-eye (*Sey*) is a semidominant, homozygous lethal murine mutation affecting eye and nose development. A point mutation in the coding region of the *Pax-6* gene is responsible for the *Sey* phenotype. Homozygous *Sey* mice lack eyes and an olfactory system. These animals die soon after birth because they are not able to breath. Heterozygous (*Sey*/+) mice have reductions in lens size and a range of accompanying eye defects. In this study we are examining the implications of the *Pax-6* point mutation on olfactory system development in *Sey*/+ mice. We found that adult *Sey*/+ mice (>60 days of age) have a 30% reduction in total main olfactory bulb volume as compared with wild-type controls. Significant reductions in volume were found in all three laminae measured. The glomerular layer was reduced by 26% and the external plexiform and granule cell layers were both reduced by ~31%. Interestingly, total accessory olfactory bulb volume was not significantly different between *Sey*/+ mice and wild-type controls, indicating a surprising degree of specificity in our main finding. These data demonstrate the necessity of having two normal alleles of this developmental control gene for normal olfactory bulb development.

65. Anion size and simple taste reaction times in humans

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A recent model of taste bud function suggests that some gustatory stimuli can act both at ion-selective channels in the apical portion of taste bud receptor cells and by means of an extracellular field potential established when ionic taste stimuli diffuse through the tight junctions that connect the apical portions of taste receptor cells (DeSimone and Heck, 1993). Metallic salts such as NaCl and KCl would be examples of taste stimuli that could act through both mechanisms if relevant apical channels existed. This model also predicts that if anions such as gluconate (Glu) are substituted for chloride, diffusion through tight junctions will be diminished and slowed. A consequence of this would be that taste responses would not only be reduced in magnitude but also would have a markedly slower time course. Using neurophysiological measures, a change in time course has been observed in experimental animals (Ye *et al.*, 1994).

This study investigated the temporal pattern of NaCl, KCl, NaGlu, KGlu and sodium acetate in humans using psychophysics. Simple taste reaction times were recorded for 100 mM aqueous solutions of these compounds using a closed-system flow machine (as described in Kelling and Halpern, 1986) that both restricted stimulation to the tip of the tongue and precisely controlled stimulus onset times. Results from a panel of twelve subjects indicate that reaction times increase with increasing molecular weights, with the average reaction times for NaCl, KCl, sodium acetate, NaGlu and KGlu being 0.67, 0.83, 0.88, 1.29 and 2.06 s respectively. These findings are in accordance with the DeSimone and Heck (1993) model, supporting the appropriateness of extending this model (developed with experimental animals) to humans.

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66. Alternative mechanism for taste compensation following chorda tympani anesthetization

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Psychophysical observations demonstrate that lingual nerve damage or chorda tympani (CT) anesthesia minimally affects the

perceived intensity of taste stimuli applied to the whole mouth. Disinhibition of responses from taste receptors innervated by other nerves is a proposed compensatory mechanism. The goal of this study was to determine whether such disinhibition occurs in the nucleus of the solitary tract (NST) of the rat. Extracellular recordings of NST responses to taste stimulation with a mixture applied to the whole mouth (WM), anterior tongue (AT), nasoincisor ducts (NID) and foliate papillae (FOL) were made before, during and after CT anesthetization with 2% lidocaine. Responses were obtained from 13 single and 13 multiunit sites responding to stimulation of the AT ($n = 6$), AT + NID ($n = 14$) or taste buds not on the AT ($n = 6$; FOL = 4, NID = 1, soft palate = 1). The WM response for AT sites was virtually eliminated during CT anesthesia (= –99.5 ± 1.24% SD). The WM response for AT+NID sites also decreased (= –66.7 ± 34.9% SD, $P < 0.001$) compared with the pre- to post- anesthesia change. For NST sites not responsive to AT stimulation, there was no effect of anesthesia ($P > 0.05$). Thus, NST WM taste responses exhibited an overall decrease during CT anesthesia, despite a few ($n = 3$) instances in which an individual receptive field response increased. Therefore, disinhibition does not occur frequently in the NTS during CT anesthesia. This conclusion, however, is based on standard measures for taste responses (spikes/s-spontaneous rate). When responses were quantified as a percent change relative to spontaneous rate, the average WM response at AT+NID sites was stable during CT anesthesia, while many NID responses exhibited marked increases. These effects were due to the profound decrease in spontaneous activity at these sites during CT anesthesia. An alternate possibility for compensation may be a decrease in spontaneous activity rather than an increase in gustatory response rates.

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67. Chemosensory cues are required for attraction of predators to salmon eggs in Iliamna Lake, Alaska

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Iliamna Lake, Alaska, supports the largest run of sockeye salmon (*Oncorhynchus nerka*) in the world. One of the major factors that may affect the production and freshwater ecology of salmon in Iliamna Lake is egg predation by coastrange (*Cottus aleuticus*) and slimy (*C. cognatus*) sculpins. Just prior to and during the period of salmon spawning, sculpin densities on spawning beaches rise dramatically and sculpin predation on eggs may be significant. The mechanisms which guide sculpins to spawning beaches and, ultimately, to detect recently spawned eggs in redds are not known. In 1994, we conducted field and laboratory experiments to examine the potential roles of visual and chemosensory cues in facilitating egg detection by sculpins.

Field studies involved baiting minnow traps on spawning beaches with (i) free eggs, (ii) eggs enclosed in ziplock bags with perforations (visible/odors present), (iii) eggs enclosed in ziplock bags (visible/odors absent) and (iv) eggs enclosed in darkened ziplock bags with perforations (not visible/odors present). Eggs enclosed in perforated bags were less attractive than free eggs (160.45 ± 15.13 versus 105.55 ± 11.51 mean number of sculpins per trap ± SE). The number of sculpins attracted did not differ between visible/odors present and not visible/odors present traps (105.55 ± 11.51 versus 100.35 ± 12.74 mean number of sculpins/trap ± SE), suggesting that visual cues were not required for detecting eggs. However, attraction to sockeye eggs did require chemical cues emanating from the eggs. The number of sculpins attracted to visible/odors absent traps was dramatically lower than in visible/odors present traps (6.50 ± 1.20 versus 105.55 ± 11.51) and indistinguishable from the numbers attracted to empty traps.

As an initial step in characterizing the chemical attractants that emanate from salmon eggs, we also tested whether sculpins were attracted to test odors in a two-choice maze. Test odors were prepared by soaking unovulated or ovulated eggs in lake water. For both unovulated and ovulated eggs, sculpins preferred the arm of the maze scented with egg wash compared with lake water alone ($n = 54$; χ^2 test; $P = 0.0065$ and $P = 0.0295$ respectively). Sculpins demonstrated no tendency to enter the arm scented with ovarian fluid versus the lake water control arm ($P = 0.179$), suggesting that the attractive substances are derived directly from egg material. Future studies will attempt to further characterize the role of chemoreception in egg predation by sculpins and more clearly define the chemical nature of attractants emanating from sockeye salmon eggs.

68. Field potential oscillations in amphibian olfactory bulb and epithelium

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Odor-induced or enhanced oscillations in field potential (FP) have been recorded in the olfactory bulb of species from a number of classes of vertebrates, as well as in olfactory processing areas of several invertebrate species (Bressler and Freeman, 1980; Gelperin and Tank, 1990; Laurent and Davidowitz, 1994). These oscillations are thought to arise as a result of the excitatory and inhibitory connections within the olfactory bulb. The ubiquitous nature of the FP oscillations suggests that they may be important in the neural coding of olfactory information. We have recorded odor-induced oscillations in olfactory bulb FP and in the olfactory epithelium EOG in two species of amphibians, *Rana pipiens* and *Ambystoma tigrinum*. While FP and EOG oscillations are not always observed in the same preparation, the oscillations recorded in the two areas do have several features in common: (i) EOG and FP oscillations have similar time courses, with approximately the same onset (relative to the stimulus) and duration; (ii) oscillations that are recorded simultaneously in the olfactory epithelium and bulb of one animal are of about the same frequency, in the range of 10–20 Hz for different individuals of both species; (iii) oscillations in both areas are first observable in response to moderate to high odorant concentrations and increase in amplitude with increasing stimulus concentration; and (iv) odorant concentration does not affect the oscillation frequency in either area. Application of lidocaine to the olfactory epithelium abolishes oscillations in both the epithelium and olfactory bulb. In addition, preliminary data indicate that there are small amplitude olfactory bulb FP oscillations in the absence of odor stimulation at about the same frequencies as the evoked oscillations. This

background oscillation is also eliminated by lidocaine treatment of the epithelium. Taken together, these observations suggest an alternative hypothesis for the source of the olfactory bulb FP oscillations: in frogs and salamanders, oscillations in the olfactory bulb may in part be driven by oscillatory input from the epithelium rather than arising solely from the intrinsic circuitry of the bulb.

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69. Olfactory dysfunction in Usher syndrome

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Usher syndrome (USH), the most frequent cause of combined deafness and blindness in adults, is a heterogenous group of autosomal recessive disorders characterized by sensorineural hearing loss (HL), retinitis pigmentosa (RP) and, in some cases, vestibular dysfunction (VD). Two distinct clinical entities are commonly recognized: type 1 (USH1), characterized by profound HL, RP and VD; and type 2 (USH2), characterized by mild-to-severe HL, RP and no VD.

Since ciliary dysfunction is strongly implicated in the pathogenesis of USH, and since olfactory cells are ciliated, we determined whether USH patients experience olfactory loss. If this is true, then olfactory testing may be useful in identifying new forms of USH.

The UPSIT was administered to eight USH1 and 14 USH2 patients, as well as to age-, gender- and smoking habit-matched controls. In addition, a single staircase phenyl ethyl alcohol (PEA) detection threshold test was given to seven USH1 and 10 USH2 patients, and to matched controls. A trained tester administered both tests to each subject, taking particular care that the procedures were completely understood.

The UPSIT scores of the USH patients were significantly below those of the controls (Wilcoxon test, $P < 0.005$). The USH1 and USH2 scores did not differ significantly from one another (U test, $P = 0.70$). Five USH1 and six USH2 patients had test scores at or below the 25th percentile of a normal reference group, and six of these 11 patients scored at or below the 10th percentile. A trend towards higher PEA threshold values in the USH than in the control subjects was present (respective medians: –5.19 and –6.13 $\log_{10}$ v/v; $P = 0.09$).

These data demonstrate that some individuals with USH have decreased ability to smell. The presence of olfactory dysfunction, in combination with losses in vision, hearing, and, in some cases, vestibular function, extends the definition of Usher's syndrome. Given the evidence linking ciliary abnormalities to the USH-related pathology of the retina and inner ear, future research should determine whether the decreased olfactory function in USH is due to altered olfactory cilia.

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70. Genetic sensitivity to 6-*n*-propylthiouracil (PROP) predicts hedonic responses to bitter but not to sweet tastes

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Genetically-mediated sensitivity to the bitter taste of 6-*n*-propylthiouracil (PROP) has been associated with enhanced taste sensitivity to other bitter compounds. However, few studies have explored the impact of PROP taster status on preferences and dislikes for sweet and bitter tastes. This study separated 87 young women (mean age 25.6 years) into PROP nontasters ($n = 18$), regular tasters ($n = 49$) and supertasters ($n = 20$) on the basis of their PROP taste thresholds and responsiveness to suprathreshold solutions of both PROP and NaCl. Taste thresholds were established using 15 PROP solutions ranging in concentration from 1.0×10^{-6} to 3.2×10^{-3} M PROP and incrementing in quarter log steps. PROP/NaCl intensity ratios were based on the ratings of five suprathreshold solutions of PROP (range 3.2×10^{-5} to 3.2×10^{-3} M) relative to five solutions of NaCl (range 0.01 to 1.0 M). Nontasters had thresholds $>.8 \times 10^{-4}$ M PROP. Supertasters had thresholds $<3.2 \times 10^{-5}$ M PROP and mean PROP/NaCl intensity ratios >1.7. There was a significant link between PROP taste thresholds and both PROP intensity ratings ($r = -0.61$) and the dislike of bitter PROP solutions ($r = 0.59$). As expected, increasing perception of bitterness was tied to a greater dislike of PROP solutions ($r = -0.0.80$). Nontasters, tasters and supertasters differed strongly in their perception and in their dislike of PROP solutions. The subjects also tasted and rated five sucrose solutions (2–32% wt/vol). PROP taster status failed to predict hedonic ratings for sucrose.

Past studies on PROP have been concerned exclusively with the sensitivity to bitter taste. Since the rejection of bitter taste is closely tied to the measures of threshold and intensity scaling, hedonic profiles may provide an additional, and more rapid, index of taster status.

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71. Influence of GABA on acutely isolated neurons from the gustatory zone of the rat nucleus of the solitary tract

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We have recently developed an acutely isolated preparation of neurons from the rostral nucleus of the solitary tract (rNST). Compared to the properties of rNST neurons in brain slices, the acutely isolated neurons maintain their biophysical and morphological characteristics but provide a means for rapid application of pharmacological agents. The preparation can be combined with direct visualization of neuron morphology to make correlations between neuron structure and function. We now

report the effects of GABA on the biophysical properties of these isolated neurons.

Neurons were isolated as previously described (Bradley and Du, 1995) and observed under an inverted microscope. Drugs were applied by pressure injection from a multibarrel pipette placed close to the cell soma. Whole cell recordings were made under current clamp conditions and neurons were biophysically characterized and grouped (Bradley and Sweazey, 1992). Neurons were also grouped into elongate, multipolar and ovoid morphological types based on simple morphometric characteristics. All neuron types responded to GABA. Application of GABA (10^{-6}–10^{-3} M) resulted in a hyperpolarization of the cell resting membrane potential and an increase in cell conductance in a concentration dependent manner. The $GABA_A$ receptor agonist muscimol (10^{-7}–10^{-4} M) also resulted in membrane hyperpolarization and an increase in conductance but was effective at a lower concentration than GABA. The half maximum effective concentration (EC_{50}) of the GABA and muscimol induced effects were 5.7×10^{-5} and 1×10^{-5} respectively. These results indicate that the profound effect of GABA applied to the soma of rNST neurons is probably mediated by $GABA_A$ receptors. Since all rNST neuron types respond to GABA, inhibition must play a significant role in synaptic processing in rNST.

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72. Supertasters of PROP (6-*n*-propylthiouracil) rate the highest creaminess to high-fat milk products

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Perceived bitterness of PROP provides a marker for genetic taste status. Nontasters (NT) rate concentrated PROP as very weak, medium tasters (MT) as moderately bitter, and supertasters (ST) as very bitter. Significant associations exist between PROP status and rated intensity of other tastes (i.e. bitters, sweeteners) and oral irritants, and the density of fungiform papillae and taste buds. Recent data show associations between PROP status and both dietary fat behaviors and related nutritional risk. Our aim was to determine if oral fat perception varied with PROP status. We hypothesized that ST would rate greater creaminess than MT or NT. Scaling of PROP and NaCl in 52 females and 17 males ($x = 25 \pm 8$ years) produced 12 NT, 41 MT and 16 ST. On a separate day, subjects provided magnitude estimates of a NaCl series and were instructed to use the same scale to rate creaminess of milk products with varying percent fat: <0.5, 1, 2, 3.5, 11.5, 36 and 54%.

The ANOVA (see above) showed a significant main effect of PROP status [$F(2,66) = 5.436$, $P < 0.01$] and interaction between PROP status and milk product [$F(12, 396) = 4.187$, $P = 0.0001$] on creaminess ratings. With *t*-tests as planned comparisons, ST > MT > NT at the 54% fat level ($P < 0.001$). At the 36 and 11.5% fat levels, ST > MT ($P \leq 0.005$) and ST>NT ($P \leq 0.01$). In summary, PROP ST provided highest creaminess ratings to high-fat milk products. These and previous data suggest that ST have the greatest propensity to experience trigeminal stimuli. These findings might explain why genetic taste status associates with dietary fat behaviors.

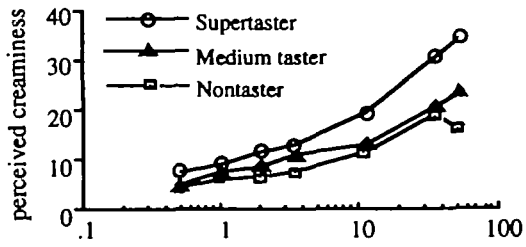

Figure 1 Creaminess ratings of milk products with varying % fat in PROP taster groups. Ratings were obtained with magnitude estimation and magnitude matching to ratings for 0.32 and 1 M NaCl.

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73. Gonadotropin releasing hormone modulates outward currents in olfactory receptor neurons from mudpuppies, *Necturus maculosus*

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The terminal nerve is an anterior cranial nerve that projects to the olfactory and vomeronasal epithelia in tetrapods as well as to the retina in teleosts. In most vertebrates, the terminal nerve contains one or more forms of the peptide gonadotropin releasing hormone (GnRH), and is suspected to play a role in modulating the activity of peripheral sensory neurons. GnRH from the terminal nerve alters the activity of retinal ganglion cells (Walker and Stell), but its effects on olfactory receptor neurons have not been described.

We are investigating the effects of GnRH on olfactory receptor neurons in the mudpuppy (*Necturus maculosus*) using whole-cell patch recordings in epithelial slices. Because the terminal nerve of amphibians has been suggested to contain both mammalian and salmon forms of GnRH (mGnRH and sGnRH respectively), we use a mixture of 10 μM mGnRH and 10 μM sGnRH. Within 5–10 min of bath application, GnRH produces a decrease in an N-shaped outward current. This current is voltage-activated and appears to be Ca^{2+}-dependent. The decrement in this current is reversible, and partial recovery is obtained within 30 min.

In pituitary gonadotrope cells, GnRH has been shown to influence Ca^{2+}-dependent K^+ currents as well as Ca^{2+} mobilization and Ca^{2+} influx (Merelli *et al.*; Tse and Hille). We are presently conducting experiments to determine whether GnRH acts directly on the Ca^{2+}-dependent outward current in olfactory

receptor neurons, or if the effect is the indirect result of a reduction in Ca^{2+} influx or mobilization of Ca^{2+} from intracellular stores.

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74. Effects of aging on olfactory event-related potentials in middle-aged adults

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There has been a great deal of research of event-related potentials in the human special senses of vision and audition. In the past, difficulties in producing reproducible, precise rise times in odor concentration had stymied progress in olfactory event-related potentials. Today, researchers are capable of recording reproducible non-trigeminal olfactory ERPs. The purpose of this study was to determine how the components of the OERP are affected differentially by age. OERPs were recorded monopolarly at the Fz, Cz and Pz electrode sited on eight middle-aged adults (41–56 years of age), 16 young adults (19–27 years of age) and 16 older adults (60–78 years of age). Based on results of a previous study a 60 s inter-stimulus interval with amyl acetate was selected as the odorant. Latency and amplitudes of N1, P2, N2 and N1/P2 peak-to-peak amplitudes were measured. Result demonstrated that the latency values of the major N1, P2 and N2 waveform complex tend to increase as a function age Significant differences in latency value in P2 were noted between the young and middle-aged groups. P2 latency was increased in middle-aged subjects when compared with young subjects. This study of OERPs in young and middle-aged and elderly subjects show age related changes in latency occur before age related in amplitudes.

75. Uneven distribution 0f PCBs in the olfactory bulb and brain

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The contents of the persistent Organochlorides Polychlorinated Biphenyls (PCBs) in the ferrets' (*Mustela putorius* f. furo) CNS was tested. Ferrets were reared indoors and fed on a PCB-contaminated diet (17 ng PCBs/g diet; daily consumption ~50 g diet/kg body wt; ADI 1000 ng/kg). Control animals were reared outdoors and fed on the same diet. Animals of different age classes were killed by a intracardial injection of T61 and the brains were prepared for further analysis immediately. The olfactory bulbs (~0.30 g) were separated from the brain and both tissues homogenized. PCB extraction and analysis followed the procedures as described in the literature except for olfactory bulbs and corresponding whole brain controls. Due to the small amount of these tissues soxhlet-extraction was replaced by extraction in glass vials at 60°C. Solvents used were of high purity. PCB-analysis was carried out with GC/MS.

In the brains of both groups average concentrations of PCB (ng PCBs/g extractable fat) slightly increased depending on age (1 year, 101; 3 years, 137; 5 years, 235). No age dependent increase in the concentrations in corresponding other tissues (liver, 112–205; subcutaneous fat, 207–275) could be observed. The comparison of the olfactory bulbs of both groups revealed that in indoor but not outdoor reared ferrets the PCB concentrations in the olfactory bulbs were five- to tenfold higher than in the rest of the brain. Since metabolic differences between brain and olfactory bulb are not known we think that the source of the high PCB concentrations in the olfactory bulb are airborne PCBs (~250 ng/m³ air; <1000 ng/m³ recommended) emitted from elastic sealings in the animal room. We therefore conclude that PCBs enter the CNS directly via the olfactory nerve. Ongoing research with labeled PCBs will help verifying this hypothesis.

76. Olfactory event-related potentials in Alzheimer's disease

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Twenty-four patients with probable dementia of the Alzheimer type (55–82 years old, 13 females) and 24 controls (56–87 years old, 14 females) were tested. Only patients with Mini-mental State Examination scores between 10 and 26 were included in the study. Other causes of dementia were excluded by MR imaging and blood testing. No subjects were taking psychoactive medications. Olfactory event-related potentials were recorded using previously described techniques. Odorant stimuli consisted of 50% amyl acetate and an air control presented monorhinally via nasal cannula (1.6 mm I.D.) at 4 l/min, 100 ms duration and 5–10 s interstimulus intervals. Psychophysical olfactory testing was also performed and included the Smell Identification Test (Sensonics Inc.), and odor detection thresholds for phenylethyl alcohol, isoamyl acetate and CA phenone (Olfacto-Labs Inc.).

Reproducible olfactory evoked potentials could not be obtained from seven severely demented patients. Analysis of variance revealed that olfactory psychophysical test scores and P1 latency, N1 latency and N1P2 interpeak amplitude differed between the patient and control groups. Linear regression analysis showed significant correlations between the olfactory measures and performance on neuropsychological tests (e.g. recall and recognition memory). These data confirm previous findings of progressive olfactory dysfunction in Alzheimer's disease and suggest that the olfactory evoked potential may be useful in following these changes.

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77. The role of growth factors in olfactory cell proliferation

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For the past few years we have studied the effect of several growth factors on olfactory epithelium in organotypic cultures. Data from organotypic cultures are more useful than FROM other *in vitro* methods because they mimic most closely *in vivo* conditions for studies of cell proliferation. We have tested 10 growth factors for their effect on proliferation of both the neuronal and supporting cell populations in cultures of olfactory mucosa from E19 rats: TGF-α, TGF-βΦ2551, TΓΦ–β2, EGF, NGF, PDGF, Amphiregulin (AR), bFGF, IGF-I and IGF-II. Of these, TGF-α had the greatest potency in increasing the rate of proliferation by threefold in both neuronal and supporting cell populations in doses of 50–100 pM medium, and the effect on supporting cells was somewhat higher than in controls. TGF-β1, TGF-β2, EGF, AR, IGF-1, IGF-2 and PDGF had moderate effects (at 5–20 nM) on both neurons and supporting cells. Basic FGF had a marginal enhancing effect on cell division specifically in the neuronal population. NGF had virtually no effect on cell division in either population. We also did RT-PCR studies on RNA preparations of olfactory mucosa from unilaterally bulbectomized adult rats to determine whether the mRNA for TGF-α was up-regulated in animals in which we knew from previous studies that cell proliferation was up- regulated. The transcript for TGF-α was indeed up-regulated on the bulbectomized side, compared with the unoperated side or with unoperated animals. Moreover, mRNA for another growth factor, neu differentiation factor (not tested in cultures) was concomitantly up-regulated. These data indicate there are multiple pathways for regulating proliferation of neurons and supporting cells in olfactory epithelium. Multiple pathways may have evolved to enable the organism to respond to a variety of environmental challenges.

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78. Development of peripheral olfactory innervation in hatchling lobsters

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In the adult lobster, the lateral flagellum of the antennule is the primary olfactory organ, with chemosensory aesthetasc sensilla present at the distal end. Pelagic lobster larvae possess lateral antennulae. Aesthetasc sensilla are present in larval stages II and III, and also the postlarval stage IV lobster. However, the stage I larva does not have aesthetasc sensilla. Instead, there is an apical giant sensillum, which may be chemosensory in function. Previous studies showed that fibers which innervate aesthetasc sensilla are present at the base of the giant sensillum of the stage I larva. Although there have been anatomical studies of the brain (deutocerebrum) of the embryonic and larval stages, the anatomy of the periphery during these stages and the projections has not been well characterized.

During late embryonic development, the preaesthetasc dendrites of the lateral antennule move distally to the apical end of the lateral antennule. In the stage I larva, when the giant sensillum is everted, the clusters of pre-aesthetasc dendrites protrude into the base of the giant sensillum. Sagittal serial sections show that upon hatching, the axons leading from the putative sensory somata of the lateral antennule project to the olfactory lobe. Thus, the periphery is 'wired for smell'. These studies suggest that the giant sensillum may be capable of processing olfactory information upon hatching.

79. Fos expression in olfactory cell cultures exposed to odorants

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Olfactory receptor neurons (ORNs) are the only mammalian neurons that are continually replaced in adults, consequently, this system is uniquely suited for studying regulation of neurogenesis and differentiation. Pixley has developed a dissociated culture system of neonatal rat olfactory cells grown on a CNS astrocyte feeder layer that supports both division and differentiation of olfactory progenitor cells *in vitro* (Pixley, 1992). To determine if the ORNs in these cultures could respond to odorants, the expression of the immediate early gene, c-fos, was used as a marker of neuronal activation. An odorant solution (isoamyl acetate, heptanone or a 10 odorant mixture at 10, 50 and 100 μM), diluted in culture medium or medium without odorant was added to cultures. Following stimulation, cultures were fixed and processed for Fos protein and olfactory marker protein (OMP) immunocytochemistry (ICC).

Our results showed increased Fos expression in cultures exposed to odorants relative to controls. Fos expression was scattered in the cultures with certain clusters of cells more intensely stained than others. Double label ICC for Fos and OMP showed OMP positive/Fos positive, OMP positive/Fos negative and OMP negative/Fos positive cells. Some feeder layer cells also showed Fos expression after odorant application. Addition of high KCl solution, to depolarize the cells, caused intense Fos expression in all neurons and astrocytes. This response was unlike the differential Fos expression seen in cultures exposed to odorants. Further studies will include manipulation of components of the sensory transduction cascade to determine the specificity of the Fos response to odorants.

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80. Odor perception and multiple-chemical sensitivity

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Individuals who report unusual sensitivity to low level chemical exposures (multiple chemical sensitivity—MCS) often report heightened sensitivity to odors. The purpose of the present study was to compare the odor perceptions of MCS subjects to those of normals, asthmatics and people with chronic fatigue and/or depression (CF/D). Odor detection thresholds for phenyl ethyl alcohol (PEA) and pyridine (PYR) were obtained, along with trigeminal-sensation ratings and aesthetic ratings for three suprathreshold levels of these odorants. Subjects also completed the U of PA smell identification test (UPSIT). MCS subjects did not differ from normals or asthmatics in detection thresholds for PEA or PYR; CF/D subjects detected PEA at a lower concentration than the other groups. MCS subjects did not differ from any other group in the UPSIT test while the CF/D group made fewer errors than the asthmatics. Relative to normals and CF/D subjects, MCS subjects rated sensations of burning and stinging as greater for 0.01% PEA. MCS subjects also reported more numbness than normals for 1% PEA. For PEA at 100%, MCS subjects differed only from asthmatics in their perception that PEA was more unpleasant. However, at the lowest concentration (0.01%) the MCS group perceived PEA to be more unpleasant than all the other groups. Relative to the other groups, MCS subjects perceived all suprathreshold concentrations of PEA to be more unsafe. Few differences were observed among the groups at any of the PYR suprathreshold concentrations. The findings indicate that MCS subjects do not detect odors at lower concentrations or identify odors more accurately than controls. However, consistent with clinical reports, they do report more symptoms and are concerned more about safety at low concentrations of odorants normally regarded as pleasant.

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81. Gustatory reflex systems in the brainstem: from primary sensory nucleus to pharyngeal motor complex

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Taste information from the vagus nerve regulates ingestive behaviors such as swallowing or regurgitation. In catfish, vagal taste fibers project to the vagal lobe, a structure equivalent to the portion of the mammalian nucleus of the solitary tract that processes vagal taste information. In catfish, the vagal lobe, but not other primary gustatory nuclei project to the vicinity of the dendrites of the nucleus ambiguus motoneurons that innervate pharyngeal muscles presumably involved in swallowing. The present anatomical investigation was undertaken to determine: (i) whether vagal lobe outputs directly contact nucleus ambiguus motoneuron dendrites; and (ii) what cell types in the vagal lobe give rise to these reflex connections.

Double label studies were performed in which biotinylated dextran amine was applied to the vagal lobe while HRP was applied to the vagal motor root. Thus in these preparations, biotinylated dextran was transported anterogradely to label the vagal lobe outputs while HRP was transported retrogradely to fill the motor neuron dendrites. The two labels could then be reacted differentially for electron microscopy. Labeled axon terminals were found to directly contact the dendrites of the ambigual motoneurons.

To determine which vagal lobe cell types gave rise to the ambigual projection system, dil was applied post mortem to the lateral reticular formation area in which the ambigual motoneuron dendrites are situated. Fluorescence microscopy revealed that small neurons situated medially and ventrally in the lobe provided the bulk of the reflex system.

In summary, the vagal portion of the primary gustatory nucleus sends output directly to the motoneurons involved in swallowing and regurgitation. The output from the primary gustatory nucleus arises from small or medium-sized somata.

82. Unilateral naris closure and RNA expression in the rat olfactory bulb

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Early unilateral naris occlusion in the rat results in a 25% volume reduction in the ipsilateral olfactory bulb by postnatal day 30 (P30). Although the long term consequences of naris closure are well characterized, immediate cellular changes remain unclear. Previous studies indicate reduced [^{3}H]leucine incorporation 24 h after naris occlusion, suggesting decreased protein synthesis in the ipsilateral bulb. To characterize potential factors coincident with this change, we have used several techniques to assess levels of both ribosomal and messenger RNA. Alterations in RNA (and thus protein) levels in response to unilateral naris occlusion may reveal active genetic mechanisms involved in the permanent effects seen after longer intervals. Rat pups were unilaterally occluded on P1 and olfactory bulbs removed 24 h later. First, regional RNA production was assessed by examining [^{3}H]uridine incorporation patterns via autoradiography and image analysis to quantify silver grain densities. Second, rRNA levels were visualized with ABC immunocytochemistry using a monoclonal antibody (Y10B, provided by Dr E. Rubel), and optical densities measured. The antibody labels rRNA within ribosomes (the nucleolus does not stain) and thus provides a marker for ribosomal density. Finally, overall levels of mRNA were determined via spectrophotometry. Olfactory bulbs were pooled into ipsilateral and contralateral tissue homogenates and mRNA and DNA extracted using commercial kits (Invitrogen). Absorbencies were measured at a 260 nm wavelength, concentrations determined, and the ratio of mRNA to DNA calculated. Preliminary results do not reveal significant changes in either uridine uptake or rRNA populations in response to olfactory deprivation. Total mRNA levels show a slight consistent decrease on the side ipsilateral to the occlusion.

Work is underway to further clarify these results and to examine other time points post-occlusion.

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83. Mixture suppression of single fiber hamster chorda tympani responses to sucrose by quinine

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Previous research has shown that responses to sucrose in sucrose best units of the hamster parabrachial nucleus are suppressed by a mixture of sucrose and quinine.HCl (QHCl; Vogt and Smith, 1992). We have shown that multifiber chorda tympani (CT) responses to sucrose are also partially suppressed by mixtures of sucrose and QHCl (Formaker and Frank, 1992). Thus, part of the suppression seen in the central nervous system originates from the peripheral gustatory system. The present study examines the tuning characteristics of those peripheral CT units suppressed by mixtures of QHCl and sucrose. We currently have electrophysiological data in response to chemical stimulation of the anterior tongue from 12 single CT fibers obtained from six golden hamsters (*Mesocricetus auratus*). Single fibers were identified based on uniform spike amplitude and waveform distribution across time. A response was defined as the number of impulses in the first 5 s following stimulus onset minus the average 5 s pre-stimulus response rate for the recording session. Gustatory stimuli consisted of 0.03 M NaCl, 0.1 M KCl. 0.1 M sucrose, 0.03 M QHCl, and a mixture of 0.1 M sucrose and 0.03 M QHCl. Stimuli were presented at room temperature and applied via a gravity flow system at a rate of 2 ml/s. CT units were classified based on their responses to sucrose, NaCl and KCl. Responses to sucrose in sucrose best CT units were suppressed when sucrose was presented in a mixture with QHCl [$t(5) = 4.5$, $P < 0.01$]. Conversely, responses to QHCl in electrolyte sensitive CT units were unchanged when QHCl was presented in a mixture with sucrose [$t(5) = 1.1$, NS]. These results suggest possible interactions between biochemical mechanisms responsible for transducing sucrose and quinine information in the peripheral gustatory system. Sucrose and quinine stimulation may result in directly opposing effects within receptor cells responsible for transducing qualitatively 'sweet' information.

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84. Relationship between chorda tympani response and taste preference in inbred strains of mice

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Behavioral studies of C57BL/6J and DBA/2J mice suggest there may be major differences in the taste systems of these two inbred strains. C57s show greater preference for 'sweet' saccharin and sucrose, and DBAs show greater avoidance for 'bitter' sucrose octa-acetate and ethanol. These studies suggest an interaction between bitter and sweet behaviors in these strains. In an attempt to understand the neural basis of these differences in behavior, we recorded whole-nerve chorda tympani (CT) responses of the two strains. The nerve was exposed via the mandibular approach and looped over a Nichrome wire electrode for recording. Stimuli were presented for 15–20 s and distilled water rinses for 40–50 s via a gravity flow system at 1 ml/s. The stimulus set was composed of a test series (0.1 M NaCl, 0.3 M sucrose, 10 mM Na saccharin, 3 mM HCl, 3 mM quinine.HCl and 10% ethanol) and a concentration series for the same compounds. Amplified, rectified multi-unit neural responses were averaged for 200 ms periods and two measures of neural activity taken: peak phasic response, and tonic steady-state level measured 10s into the response. In general, the CT of C57s was more sensitive to sweet stimuli and the CT of DBAs was more sensitive to bitter stimuli. Among various effects observed, the pattern of phasic responses of the C57 and DBA CT across the test stimuli differed [$F(6, 108) = 3.30$, $P < 0.01$], with responses to 10 mM saccharin significantly larger in the C57 ($P < 0.01$). In addition, the tonic response of the C57 and DBA CT to the quinine concentration series differed [$F(4, 40) = 4.23$, $P < 0.01$], with responses to 1 mM, 3 mM, and 10 mM significantly larger in the DBA ($P < 0.05$). These neural data indicate taste differences occur at the peripheral level and suggest an interaction between sweet and bitter receptive mechanisms.

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85. Differential effects of cholecystokinin and bombesin on water intake of water deprived rats

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Cholecystokinin (CCK) and bombesin (BN) are peptides with well established effects on food satiation. Similarly, exogenous administration of CCK and BN have been demonstrated to induce satiety for salt solution intake in sodium depleted rats. We asked whether CCK and BN would also act as satiety agents for water in water deprived rats.

Adult, male Sprague–Dawley rats were overnight water (but not food) deprived on four separate occasions, separated by at least 2 days of water and food *ad libitum*. Following the overnight water deprivation, the rats were offered either water (HOH) or 5 mM saccharin to drink in a 2 h appetite test. Immediately prior to the appetite test rats were injected with isotonic saline (VEH), CCK (4 μg/kg, i.p.) or BN (4 μg/kg, i.p.).

With the exception of the BN/saccharin condition, intakes were not significantly different between the various conditions. At the concentration used, saccharin did not significantly increase the water intake. For the BN/saccharin condition, intakes were significantly reduced at 5, 15 and 30 min compared with all other groups, and remained lower than all other groups for the remainder of the test period.

Table 1 Cumulative fluid intake of water-deprived rats (mean ± SEM)

Group	5 min	15 min	30 min	60 min	120 min
VEH/HOH	11.3 ± 1.5	13.2 ± 1.5	15.0 ± 1.8	15.8 ± 2.5	16.4 ± 2.5
VEH/Sacch	10.9 ± 0.7	15.6 ± 0.8	16.8 ± 1.3	18.3 ± 1.7	20.0 ± 0.7
BN/HOH	9.0 ± 0.5	12.5 ± 0.8	14.1 ± 1.1	14.8 ± 0.9	18.9 ± 1.1
BN/Sacch	3.8 ± 1.1*	4.3 ± 1.3*	7.9 ± 2.3*	10.3 ± 3.0	12.1 ± 3.6

CCK was ineffective in reducing water intake in water deprived rats, as has been reported by others. BN was also ineffective in reducing water intake, unless a tastant was added to the water. Thus, the adulteration of water by a tastant revealed an inhibitory effect of BN on water intake in water deprived rats. The inhibitory effect of BN on intake appears to depend on salient taste cues.

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86. Gustducin knock-out mice exhibit reduced bitter taste sensitivity as evidenced by glossopharyngeal responses and conditioned taste aversion

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Gustducin is a guanine nucleotide binding protein (G-protein) that is taste cell specific. Alpha gustducin 'knock-out' mice were generated by homologous recombination. Mice homozygous for the null gustducin allele were shown to have reduced behavioral and chorda tympani responses to two bitter [denatonium benzoate and quinine sulfate (QSO_4)] and two sweet (sucrose and SC45647) compounds compared with wild-type mice. The present studies examine, in further detail, the role of gustducin in bitter taste responses. Glossopharyngeal nerve responses were obtained to a variety of bitter compounds for gustducin knock-out and wild-type mice. Glossopharyngeal responses were significantly reduced in knock-out mice compared with wild-type animals for all bitter compounds tested. These results are consistent with chorda tympani data. In the absence of gustducin, behavioral and electrophysiological bitter taste responses are diminished. However, it is not clear if gustducin affects intensity or quality of bitter taste. A conditioned taste aversion paradigm was used to address this issue. A bitter taste solution (QSO_4) was paired with an aversive stimulus (LiCl injection). Mice were then tested with a descending series of QSO_4. Since the degree of conditioning depends, in part, upon the intensity of the conditioned stimulus, it was hypothesized that if gustducin null mice perceive bitter tastants as less intense, then they would exhibit less of an effect of conditioning. Our results confirmed the prediction: following conditioning, gustducin knock-out mice showed significantly less aversion to QSO_4 than did wild-type controls. We conclude that gustducin plays an integral role in the transduction of bitter stimuli and, in the absence of gustducin, mice may perceive bitter stimuli as less intense.

87. Cross-cultural differences in scaling olfactory stimuli

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Interest concerning cultural influence on behavior and mental processes has increased dramatically in the last decade but studies relating culture and psychophysical variables are scarce. This work will study the differences between a Portuguese (n = 65) and a Spanish (n = 59) sample of university students on a classical magnitude estimation task studying: (i) direct responses (R) to the intensity of olfactory stimuli (S); (ii) three alternative monotonically increasing psychophysical models (linear, logarithmic and power fits); and (iii) three sensitivity measures. The cultural aspects, gender and habitat (rural versus urban) will be the independent variables having previously established the samples' structural, scalar and measurement unit equivalence.

Results show that there were significant differences between: (i) the direct responses given by Portuguese and Spaniards to each of the 14 stimuli presented ($F = 4.3$, $P < 0.04$). Portuguese responses were higher than the Spanish ones; (ii) responses given by males between countries ($F = 9.6$, $P < 0.002$). The Spanish males gave better responses according to the proximity criterium to the line S = R; and (iii) responses given by urban subjects between countries ($F = 5.9$, $P < 0.02$). Portuguese urban subjects gave better responses according to the same proximity criterium.

No significant differences between countries were found for the different fits to the data, nor for the three sensitivity measures studied.

Summarizing, these results show an incomplete gender (only in males) and habitat (only in urban people) difference in scaling olfactory stimuli between Spain and Portugal. Nevertheless, the same predicting models for responses can be used to describe all data and the cultural differences studied do not influence sensitivity.

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88. Correlative light and electron microscopic analyses of circumvallate taste buds in monkeys

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We are currently studying the circumvallate papillae of the *Macaca fasicularis* monkey in order to elucidate the ultrastructural basis for gustation in a species closely related to the human. Tissues were processed and embedded for electron microscopy. Structures of interest were selected using light microscopy of 2.5 mm thick

sections. These sections were then re-embedded, sectioned and examined with the electron microscope.

A typical monkey circumvallate taste bud is a small, teardrop-shaped structure containing cells that vary widely in their electron density. Apical projections of the cells possess microvilli that extend from the taste pore into the trench. Whereas other animals such as rodents and rabbits have circumvallate taste buds that line both walls of the trench, circumvallate taste buds of the monkey line only the inner wall of the trench.

Using the electron microscope, we have observed three putative cell types. The first cell type contains abundant filamentous material and apical dense granules. These type I cells vary in electron density from electron-lucent to electron-dense. These cells also have long, smooth endoplasmic reticulum and an abundance of mitochondria. Type II cells lack apical dense granules but are large, typically containing a round nucleus. This cell type also varies in electron density, although most of these cells tend to be more electron lucent. Cells resembling type III cells are also present. They possess a relatively electron-lucent cytoplasm and nuclei characterized by the presence of patchy heterochromatin. Occasionally, large, dense-cored vesicles are present in the cytoplasm of the nuclear region. Typical afferent synapses are present. Surprisingly, some synapses are associated with extremely electron-dense cells. Based on these results we speculate that more than one cell type may form synapses in taste buds of the monkey.

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89. Neuropsychological performance and cognitive olfactory event related potentials in the young and elderly

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The P3 event-related brain potential (ERP) reflects neuroelectric activity related to the speed of cognitive processing and allocation of attentional resources. The objective of the present study was to assess the relationship between the P3 and slow wave components of the olfactory event-related potential (OERP) elicited in a single stimulus paradigm with neuropsychological performance in the young and elderly. Studies relating neuropsychological measures with event related potential (ERP) recordings are often used in clinical and research settings. However, few studies have related neuropsychology with olfactory processing. OERPs were recorded monopolarly at the Fz, Cz and Pz electrode sites in 16 young adults (8 males, 8 females) and 16 older adults (8 males, 8 females) using inter-stimulus intervals (ISI) of 45, 60 and 90 s using amyl acetate, geraniol, and phenylethyl alcohol as odorants. P3 peak amplitude and latency were measured following the N1–P2–N2 complex and the Slow Wave (area under the curve) was measured from the offset of the N2 until return to baseline. Neuropsychological performance was assessed using the California Verbal Learning Test (CVLT), Trail Making Test (TMT), the Dementia Rating Scale (DRS), The Mini-Mental State exam (MMS), and the Blessed and the Ravens Progressive Matrices. Subjects produced magnitude estimations of odor strength, and producing these estimates appears to have elicited a P3. The results showed that the late cognitive OERP components change with age and are associated with changes in neuropsychological performance for all three ISI's and all three odors. Specifically, an increase in P3 amplitude, an increase in slow wave volume, and a decrease in P3 latency was associated with an increase in the number of items recalled on the CVLT. Furthermore, faster completion of the Trail Making Test was correlated with increased P3 amplitudes, increased Slow Wave volume, and decreased P3 latencies. These findings suggest that OERPs in conjunction with neuropsychological measures can be used in applied settings to assess and diagnose olfactory dysfunction and cognitive dementia associated with aging.

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90. Olfactory mucosal responses to the herbicide Alachlor

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Alachlor [(2-chloro-2′,6′-diethyL-*N*-(methoxymethyl)acetanilide)], a restricted-use pesticide, is used as a preemergent herbicide in the production of numerous crops including corn, peanuts, and soybeans. Chronic dietary exposure to alachlor is reported to be associated with the development of tumors of the olfactory mucosa in Long-Evans rats. The present studies were undertaken to gain insight into the mechanism underlying the development of alachlor-induced olfactory tumors, as well as the cell type of origin of the tumors. Male Long–Evans rats were administered 0, 14, 42 or 126 mg/kg/day of alachlor for 1, 4 or 28 days by i.p. injection. Rats were killed 24 h after the last dose and nasal cavities were prepared for light microscopic evaluation. Unlike other olfactory mucosal carcinogens such as nitrosamines and phenacetin, alachlor did not cause cytotoxicity in the olfactory mucosa under any of the dosing regimens used. Proliferating cell nuclear antigen immunohisto-chemistry revealed increased olfactory basal cell proliferation. Taken together, these two observations suggest that alachlor-induced tumors are not the result of non-specific high-dose cytotoxicity, but may reflect tissue-specific cellular alterations. In order to determine the mutagenic potential of alachlor in the olfactory mucosa, olfactory mucosal and liver S9 fractions were used in *in vitro* mutagenesis assays using *Salmonella typhimurium* strain TA100 and *Escherichia coli* strain WP2*uvr*A-. The preincubation approach was used, and for each strain and activation condition there were six plates for the negative (solvent) control, three plates for the appropriate positive control, and three plates for each of the same five quarter-log concentrations of alachlor (1500–15 000 μg/plate). All concentrations and conditions gave negative results in the *E. coli* assay. Preliminary results revealed a positive response with liver S9 at the two highest doses in the *Salmonella* assay. With olfactory mucosal S9, alachlor was cytotoxic to *Salmonella* at all but the lowest dose tested; however, a

40% increase in histidine revertants was observed at 1500 μg/plate. These results suggest the need for further examination of alachlor's mutagenic potential in the olfactory mucosa (underway). Further, the data suggest the possibility that alachlor is a genotoxic carcinogen in the olfactory mucosa.

91. Olfactory receptor spike activity reflects weak ion regulation

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Stimulus-evoked action potentials in amphibian olfactory receptor neurons have unusual time patterns. An initial burst of spikes is followed by a silent period, which is followed by a longer, lower frequency burst before the axon returns to the very slow, irregular firing characteristic of the resting state. If the stimulus is repeated, the same firing pattern is seen, but the number of evoked spikes in both bursts is usually smaller. If the stimulus concentration is increased, the initial burst occurs with shorter latency, the duration of initial burst is shorter, and, in the extreme, consists of a single spike. The following silent period increases in duration as concentrations increase.

After a few stimulus repeats, no spikes are evoked and resting activity is abolished. Recovery from this state requires tens of minutes.

The cause of this relatively unusual form of evoked neural activity is evident from the cellular ultrastructure of the olfactory nerve. The unmyelinated axons are densely packed. Extracellular space is minimal. Schwann cells are sparse, widely separated, and have a small volume of cytoplasm. An action potential will cause a decrease in the ionic gradient across the axon membrane which can only slowly be restored by membrane transport processes. The decreased gradient results in maintained depolarization, which inactivates sodium channels (the Hodgkin–Huxley *h* parameter). A maintained or repeated receptor current is less effective over time in evoking action potentials because partial inactivation is maintained. The structure and composition of the olfactory nerve serves to emphasize olfactory system responses to novel stimulus events.

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92. Induction of polymeric immunoglobulin receptor mRNA in the olfactory mucosa of virus-infected rats

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Mucosal humoral immunity is mediated predominantly by IgA, which is transported into secretions by a specific receptor/transporter, the polymeric immunoglobulin receptor (pIgR). We investigated the expression of pIgR mRNA, which is regulated by interferon-γ in a tissue-specific manner, in olfactory mucosae of virus-antibody-free (VAF) rats and rats infected with sialodacryoadenitis virus (SDAV) to initiate studies on the regulation of olfactory mucosal pIgR expression by cytokines. Riboprobes were synthesized from a 2.6 kb rat pIgR cDNA fragment subcloned into pBluescript SK- (kindly supplied by Dr Charlotte Kaetzel, Department of Pathology). *In situ* hybridization was performed with ^{35}S-labeled riboprobes on fixed frozen sections of rat nasal cavity. Total RNA isolated from nasal mucosae of VAF and SDAV$^+$ rats was analyzed by Northern blotting and ribonuclease protection assays with ^{32}P-labeled riboprobes. Dense clusters of autoradiographic silver grains were localized over Bowman's glands (BG) and their ducts in olfactory mucosa from SDAV$^+$ but not VAF rats hybridized with the antisense probe; hybridization with the sense probe resulted in a random, sparse distribution of grains in VAF and SDAV$^+$ rats. Northern blotting demonstrated upregulation of a 2.6 kb pIgR mRNA in SDAV$^+$ rats compared with VAF rats. Ribonuclease protection assays showed a distinct protected fragment of the expected size in RNA from SDAV$^+$ rats. Immunolocalization of the interferon-γ-inducible transcriptional activator interferon regulatory factor-1 in BGs with a strong hybridization signal for pIgR mRNA in SDAV$^+$ rats suggests that interferon-γ may induce olfactory mucosal pIgR expression during virus infection.

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93. Human olfactory receptor neurons and their central targets in the bulb differentially express BDNF and its receptor *Trk* B

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The aim of our study was to test the hypothesis that terminal differentiation and survival of human olfactory receptor neurons (ORNs) are dependent on trophic factor support from target neurons in the olfactory bulb. We investigated the expression of brain-derived neurotrophic factor (BDNF) and its receptor *trk* B in the olfactory mucosae and bulbs with immunocytochemical methods. Olfactory mucosae from two subjects of 19 (female) and 65 (male) years of age with PMIs of 4.5–5.5 h and olfactory bulbs from a 39 year old female subject with a PMI of 18 h were obtained at autopsy. Ten-micrometer-thick cryostat sections were processed for immunocytochemistry using streptavidin-biotin peroxidase kits and polyclonal antibodies to BDNF and *trk* B. Appropriate specificity controls were performed. In the olfactory mucosa, *trk* B was abundantly expressed in the soma, dendritic and axonal process of ORNs. In addition, a population of precursor cells located above the basement membrane also showed intense immunoreactivity. In the bulb, the most intense immunoreactivity for *trk* B was observed in the axons of ORNs in the olfactory nerve

and glomerular layers. BDNF immunoreactivity was most abundant in the mitral cells and to a limited extent in the juxtaglomerular neurons. In the olfactory epithelium, weak BDNF immunoreactivity was observed in few ORNs and occasionally in basal cells. Moderate immuno- reactivity was also observed in the supranuclear region of sustentacular cells. Our results demonstrate that ORNs and their immediate precursor cells express *trk* B, whereas their central target neurons, namely the mitral and juxtaglomerular cells, express, BDNF which suggest that the terminal differentiation and survival of ORNs is regulated, at least in part, by BDNF through its receptor *trk* B.

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94. Regulation of amiloride-sensitive sodium channels by extracellular sodium ions: sodium self-inhibition

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The contribution of amiloride-sensitive sodium channels (ASSCs) to the transduction of salts has been well-established. Recent work has shown ASSCs to be dynamic, that is, they are capable of being modulated by a number of extrinsic cues. In the present study, we are investigating the regulation of ASSCs in rat TRCs by extracellular Na ions using perforated patch-clamp and transepithelial current recording techniques. In isolated fungiform TRCs which contain ASSCs, we have recorded the current through ASSCs in response to voltage ramps while varying only the extracellular Na concentrations. Currents through ASSCs in mammalian TRCs are highly selective for Na (over K), yet at extracellular Na concentrations above ~50 mM the conductance through ASSCs decreases. This effect is consistent with an inhibition of ASSCs by extracellular Na ions. This process, termed sodium self-inhibition, develops with a time constant of ~8 s, much slower than the block of ASSCs by amiloride. Isolated fungiform TRCs which lack ASSCs and isolated rat vallate TRCs which do not have functional ASSCs do not demonstrate the sodium self-inhibition phenomenon. In both isolated TRCs and intact lingual epithelia, the sulfhydryl reagent, *p*-hydroxymercuribenzoate (*p*-HMB; 200 μM), removes inhibition by extracellular sodium, effectively increasing Na currents. Isolated TRCs and lingual epithelia which are amiloride-insensitive are unaffected by *p*-HMB. Interactions between *p*-HMB and amiloride suggest that the amiloride and Na binding sites may be linked. These data coupled with the fact that in rats salivary Na concentrations are ~60 mM, a concentration which is sufficient to inhibit ASSCs, suggest that sodium self-inhibition may be an important regulatory process in TRCs which contain ASSCs and, hence, in the transduction of sodium salts.

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95. Maintenance of an appropriate extracellular ionic/osmotic environment is vital to sustaining dendrite function in olfactory receptor cells

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The olfactory sensilla (aesthetascs) of the blue crab, *Callinectes sapidus*, each contain the highly branched outer dendritic segments (ODS) of up to 160 receptor cells. The 'exposed' portions of these dendritic processes are separated from the external environment by the thin, odor-permeable cuticle of the aesthetasc. Because the blue crab ranges from hypersaline lagoons to freshwater, the aesthetascs are subjected to a wide range of ionic/osmotic environments. A question of particular interest is how neural function is maintained in the 'exposed' ODS under such variable conditions. In this study we compared the effects of acutely exposing aesthetascs of seawater-acclimated crabs to low salinity in the absence and presence of mannitol (to maintain isosmotic conditions equal to seawater). Morphological and extracellular neurophysiological measures revealed that mannitol prevented the ODS from vesiculating and preserved the physiological response. We also examined the effects of removing the aesthetascs of freshwater-acclimated crabs in a freshwater medium versus a medium equivalent to hemolymph. In freshwater, the exposed ODS rapidly vesiculate whereas their structural integrity is maintained in the hemolymph medium. Finally, we found that crabs acclimated to intermediate salinities have ODS lengths that fall between those of freshwater- and seawater-acclimated crabs. These findings are consistent with our hypothesis that, at low salinities, ODS integrity is dynamically supported by a net efflux of ions from the hemolymph, and that the length of the 'exposed' ODS reflects the distance over which a suitable extracellular ionic/osmotic environment can be maintained.

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96. What is the functional significance of multiple bitter-sensitive taste receptors?

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The taste system of caterpillars is ideal for investigating the functional significance of multiple bitter-sensitive taste receptors. It contains 37–42 taste receptor cells (TRCs), which are borne in peg-like structures (sensilla) that project outside of the oral cavity. Each sensillum bears 3–4 TRCs, and each TRC has a different molecular receptive range. All sensilla appear to contain one TRC that responds best to compounds humans describe as bitter. Given that there are eight bilateral pairs of sensilla in a caterpillar, it follows that a caterpillar may possess as many as 16 bitter-sensitive TRCs. Stimulation of these TRCs is thought to elicit food-

rejection. However, little is known about the contribution of the different bitter-sensitive TRCs to the rejection response. If they have overlapping molecular receptive ranges, then they may respond in a concerted manner to the same bitter compound, and thus elicit a stronger and/or quicker rejection response than would a single TRC. I examined this hypothesis in *Manduca sexta* caterpillars in a series of five experiments. First, I identified three bitter compounds that elicited rapid rejection responses: caffeine, salicin and aristolochic acid. Second, I determined that only a subset of the sensilla contained a TRC that responded electrophysiologically to these compounds: the lateral (Lat) and medial (Med) styloconic sensilla, and the epipharyngeal (Epi) sensilla. Third, I found that surgically ablating the Lat, Med, and Epi sensilla completely eliminated the rejection response to the bitter compounds, demonstrating their central role in the rejection response. Fourth, I found that ablating only one bilateral pair of sensilla slowed but did not eliminate the rejection response, indicating that neither bilateral pair was completely necessary. Fifth, I discovered that ablating two bilateral pairs sensilla also reduced the rejection response, but not to a greater extent than observed in the previous experiment. In conclusion, surgical ablation of sensilla (and hence bitter-sensitive TRCs) significantly reduced the speed and magnitude of the rejection response. However, this effect did not increase with the number of sensilla ablated. This may reflect the fact that the ablations reduced the number of both bitter- and sweet-sensitive taste receptors. It is known that input from the latter class of receptors can modulate the rejection response.

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97. Transplantation of olfactory epithelial progenitor cells into the methyl bromide-lesioned rat

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The olfactory epithelium (OE) continually produces neurons and reconstitutes both neuronal and non-neuronal populations following experimental injury, including direct methyl bromide (MeBr)-induced damage. We have performed experiments to determine if rat OE progenitor cells can be transplanted into the OE of MeBr-lesioned hosts and give rise to cells that integrate into the host epithelium. Three days following a 6 h exposure to 330 p.p.m. MeBr, adult rats were perfused with Kreb's buffer. Following isolation and enzymatic dissociation of the nasal septum and turbinates, the suspension of viable OE cells was incubated with a retroviral vector encoding β-galactosidase as a heritable marker enzyme to label donor-derived cells. The cells were washed thoroughly and then transplanted by nasal infusion into an anesthetized, tracheotomized rat that had been exposed to MeBr the previous day. As a negative control to rule out labeling in the host OE due to passive transfer of the vector, donor cells were killed before incubation with retrovirus, washed and then infused into a host. One week or longer after transplantation, host rats were perfused with fixative, and the nasal septum and turbinates were processed to visualize β-galactosidase. Tissue was then embedded, cryosectioned and processed immunohistochemically to classify donor-derived cells as to type. Clusters of β-gal (+) cells were found in the OE of transplant recipients, but not in negative controls. Transplanted OE progenitors gave rise to neurons, identified by TuJ-1 immunoreactivity, and nonneuronal cells, which lack expression of neurotubulin. The results indicate that transplanted cells can integrate into the host epithelium and generate neurons during the reconstitution process. The method will allow us to transplant progenitor cells harvested from the OE of normal, bulbectomized or MeBr-lesioned animals and elucidate the ability of individual cell types to reconstitute the various populations of the OE. Furthermore, transplantation may eventually provide a modality for reversing the destruction or exhaustion of the neurogenic capacity of the OE that can occur clinically.

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98. The role of protein kinase A and C in adaptation of human olfactory receptor neurons to odor stimuli

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In previous biochemical investigations, protein kinase A and C (PKA and PKC) have been shown to play a role in the termination of the odor response, presumably via the phosphorylation of receptor proteins. PKA has been shown to specifically desensitize the components of signal transduction pathway mediated by cAMP, while PKC desensitizes the IP_3 pathway. To investigate the function of PKA and PKC in intact receptor cells, we obtained functional human olfactory receptor neurons from tissue samples from healthy volunteers. Olfactory tissue biopsies were enzymatically dissociated to yield olfactory neurons which were identified by their distinct morphology. To quantify odor responses, we loaded the cells with the calcium indicator fura 2 and measured changes in intracellular calcium as previously described (Restrepo *et al.*, 1993). Cells responded to odor mixes known to activate the cAMP (Mix A) or the IP_3 (Mix B) transduction pathway. When Mix A was presented together with a specific PKA inhibitor (H89), the odor response was enhanced and prolonged even after removal of the odorants and inhibitor, indicating that PKA was involved in adaptation to the odor. This effect was not noticed when Mix A was applied together with a specific PKC inhibitor (*N*-myristoylated EGF receptor). Conversely, the Mix B odor response was enhanced and prolonged when Mix B was co-applied with the PKC inhibitor but not with the PKA inhibitor. We are investigating these phenomena in further detail using dissociated neurons from biopsies as well as with cultured neurons from the adult human olfactory epithelium.

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99. Short-term synaptic plasticity in gustatory nucleus of the solitary tract induced by high frequency stimulation

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Gustatory stimulation of the tongue can elicit high frequencies of action potentials in afferent taste nerves. To examine what influence this high frequency activity has on inhibitory postsynaptic potentials (IPSP) we have made whole cell recordings from rostral nucleus of the solitary tract (rNST) neurons in rat brain slices. While recording from second order neurons, the solitary tract (ST) is stimulated at frequencies that mimic the in vivo firing rate of afferent taste fibers (5–50 Hz). Because the post synaptic potentials at the afferent synapse in rNST are mixtures of excitatory and inhibitory potentials, mediated by glutamate and GABA respectively, we isolated the GABA receptor response by using the glutamate blockers 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) and D-2-amino-5-phosphovalerate (APV, 50 μM). Recordings have been made from 58 neurons in 33 rats.

We found that high frequency stimulation (HFS) evoked two types of synaptic response. In the first type the HFS prolonged the decay time of the IPSP. Depending on the frequency, duration and magnitude of the ST stimulation, the decay time of the IPSP could be lengthened several hundred orders of magnitude compared with IPSPs elicited by a single shock to the ST. Thus, HFS can potentiate the IPSPs thereby increasing the time of inhibition. The second type of response was biphasic, with an initial hyperpolarizing IPSP which then became depolarizing and elicited action potentials. The depolarizing amplitude and the number of action potentials was dependent on the frequency, duration and magnitude of the stimulus. Thus, GABA activation, which is normally inhibitory, becomes excitatory at these high stimulation frequencies. These changes in synaptic activity produced by HFS demonstrate that rNST synapses are plastic. This short-term synaptic plasticity will influence the information processing and response molding that takes place in rNST and indicates the importance of inhibitory activity in the gustatory relay nucleus.

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100. Responses from sensilla basiconica on mosquito maxillary palps to behaviorally important chemical cues

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Electrophysiological studies of the basiconic sensilla on the maxillary palps of mosquitoes reveal that they are innervated by three receptor neurons. One neuron produces very large amplitude action potentials (300 V), and is sensitive to carbon dioxide (CO_2). This class of receptor neuron has response properties that could provide the afferent input required for some aspects of CO_2-modulated host-locating behavior. Step increases in CO_2 concentration elicits a phasic increase in action potential production, followed by a tonic pattern of discharge in this neuron. Interestingly, responses to step decreases in CO_2 concentration elicits a similar phasic–tonic pattern, but inverted in form. This symmetrical phasic–tonic response to rapid changes in CO_2 concentration may serve as an 'edge-sharpening' mechanism allowing the insect to discriminate very slight fluctuations in its CO_2 environment.

The other two receptor neurons in the sensillum produce smaller amplitude action potentials (50 V). Neither of these neurons responds to CO_2. However, one responds to stimulation with very low doses of another behaviorally relevant compound, 1-octen-3-ol. 1-Octen-3-ol has been shown to be attractive by itself, for several species of mosquitoes, and to behaviorally synergize the attraction elicited by CO_2 alone. The addition of 1-octen-3-ol to CO_2 does not alter the sensitivity of the CO_2-responsive neurons. In addition to 1-octen-3-ol, the neuron, producing the small amplitude action potential is sensitive to low doses of several other behaviorally relevant compounds. The mosquito maxillary palps contain highly sensitive olfactory receptor neurons that are sensitive to behaviorally relevant stimuli. Knowledge of the peripheral response capabilities of these receptor neurons may lead to the development of strategies designed to exploit these sensory capabilities and thus optimize the utility of bio-rational controls.

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101. One and two point estimates of chemical information content in a turbulent odor plume: what the American lobster (*Homarus americanus*) can and cannot use to locate a source

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We studied the dispersal patterns of chemical signals in a turbulent odor plume to assess their potential information value for an American lobster searching for the chemical source. Previous behavioral studies suggested that lobsters make instantaneous decisions based on the chemical signals downstream from the source. Other investigations indicated that temporal parameters of odor dispersal might provide the lobster with cues indicating the direction and distance of the source. We undertook this study to extend these single point measurement results using a pair or sensors separated by the same distance as lobster lateral antennules. We used these 'bilateral' recordings for the identification and statistical analysis of odor dispersal patterns in a single turbulent odor plume. The results demonstrate that temporal variations in simultaneous two-sample concentration profiles provide information that could be used to guide an animal to the odor source. This information supports four potential orientation mechanisms: (i) a form of klinotaxis based on the concentration peak parameters of slope, initial slope or (probably)

peak height; (ii) a form of klinotaxis which exploits the joint information content of a number of peak parameters simultaneously (peak shape); (iii) a series of olfactory search images or a 'map' of peak type distributions at different locations in the plume; and (iv) bilateral comparison of the signals arriving at each antennule to compute the direction to source from the delay between arrivals of similar peaks. We exclude the use of 'pure' tropotaxis, 'pure' klinotaxis and forms of tropotaxis based on peak duration, rise time or peak counting in our plume. We speculate about sampling constraints and general implications for animal behavior.

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102. Recovery from capsaicin desensitization during recurrent stimulation

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Capsaicin desensitization is currently thought to result from a long-lasting impairment of nociceptor excitability and, possibly, from depletion of neurotransmitter at central terminals. However, data from three psychophysical experiments will be reported that indicate desensitization is a temporarily reversible process. In the first experiment subjects rated the irritation produced on the tongue tip by 1.27 cm^2 filter paper disks treated with a 33 M capsaicin solution. The disks were presented in three blocks of 10 trials with 30 s between stimuli and 15 min between blocks. Subjects served in three sessions 24 h apart. Consistent with previous results significant desensitization was observed on the first trial of blocks 2 and 3. However, as stimulation continued within those blocks, ratings of irritation rose toward pre-treatment levels, i.e. desensitization was progressively reversed ('stimulus-induced recovery', SIR). Although desensitization reasserted itself between blocks, it did not carry-over from one day to the next. When a 10-fold higher concentration of capsaicin (330 M) was tested in a second experiment, SIR was again obtained across blocks. Moreover, the higher concentration caused significant desensitization across days which also exhibited SIR. A third experiment revealed that piperine could also produce SIR after either self-desensitization or cross-desensitization by capsaicin. In contrast, zingerone, which is capable of little or no self-desensitization, failed to produce SIR after cross-desensitization by capsaicin.

The results therefore demonstrate that reintroduction of the desensitizing (or a cross-desensitizing) stimulus can rapidly, albeit temporarily, reverse the effects of topical desensitization. This observation has implications for theories about the biochemical basis of desensitization, and for the use of capsaicin as a topical analgesic.

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103. Role of IP_3-sensitive calcium stores in salamander olfactory receptor neurons

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During olfactory transduction, Ca^{2+} enters the olfactory receptor neurons (ORNs) through cyclic nucleotide-gated (CNG) channels to mediate adaptation and other regulatory processes. Since prolonged high intracellular Ca^{2+} levels lead to cell death, intracellular Ca^{2+} needs to be tightly regulated through Ca^{2+} buffering and subsequent Ca^{2+} removal from the cytoplasm. Ca^{2+} stores play a critical role in the generation and termination of Ca^{2+} signals. In other cells, Ca^{2+} from stores can contribute to a cytosolic Ca^{2+} rise, while Ca^{2+} pumping into stores participates in termination of intracellular Ca^{2+} signals. Using the Ca^{2+} indicator fluo-3AM and confocal microscopy, Ca^{2+} stores in salamander ORNs were examined. At rest the intracellular Ca^{2+} level was generally low and appeared rather uniform throughout the ORNs except for various discrete organelle-like structures which showed a higher resting fluorescence level. These structures were normally seen at the rim of the large nucleus but were also found at the base of the dendrite and in more distal portions of the dendritic compartment. After stimulation with activators of the cyclic nucleotide pathway (8-br-cGMP or IBMX) these structures often increased in fluorescence, suggesting that they sequestered Ca^{2+} to prevent excessively high Ca^{2+} concentrations in the cytoplasm. We attempted to demonstrate pharmacologically the presence of IP_3-sensitive internal Ca^{2+} pools by examining the effect of thapsigargin (100 nM), which irreversibly depletes Ca^{2+} from IP_3-sensitive stores. Thapsigargin induced a rise in fluorescence at local sites within the distal and proximal portions of the dendrites. These data demonstrate that IP_3-sensitive stores exist in close proximity to the dendritic knob. Thapsigargin treatment did not abolish the Ca^{2+} response to activation of the cyclic nucleotide pathway. Instead, the main effect of thapsigargin as compared with control measurements was a longer delay in the dendritic and somatic Ca^{2+} increase with respect to the ciliary signals. This suggests that the Ca^{2+} stores in salamander ORNs do not directly interfere with the excitation process but rather have a role in sequestering cytoplasmic Ca^{2+} that has entered the cell after stimulation.

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104. Lesions of the parabrachial nuclei disrupt learned preferences for a flavor paired with NaCl

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Rats with bilateral ibotenic acid lesions of the pontine parabrachial nuclei (PBNX) fail to express an appetite for sodium when salt hungry. The current experiments were designed to determine whether PBNX rats could show a conditioned

preference for a flavor previously paired with NaCl when subsequently made salt hungry, and to verify that these rats could demonstrate a conditioned preference for a flavor paired with sucrose. Twenty rats received bilateral, electrophysiologically-guided, ibotenic acid (0.2 µl;20 µg/µl) lesions of the PBN and 14 rats served as SHAM controls. Food was freely available and 24 h fluid intake was monitored daily. Experiment I. Conditioned preference for a flavor paired with NaCl: All rats were given alternate 24 h access periods to two flavors of Kool-Aid for 6 days. One flavor was presented in water (referred to as the CS–) and the other flavor in 3% NaCl (0.15 M, referred to as the CS+). Following acquisition, the rats were given 24 h access to water, after which they were injected with furosemide (7.0 mg/0.7 ml s.c., 2×). The subjects were then moved to clean cages and placed on a Na+ free diet. Twenty-four hours later, the conditioned preference was evaluated using a two-bottle test in which both flavors of Kool-Aid were presented in water simultaneously for 24 h. Experiment II. Conditioned preference for a flavor paired with sucrose: The procedure was identical except that two novel flavors of Kool-Aid served as the conditioned stimuli, sucrose replaced NaCl as the unconditioned stimulus, and no injections were given. The results indicated that only the SHAM rats showed a reliable conditioned preference for the Kool-Aid flavor paired with NaCl, $P < 0.05$. Furthermore, this disruption was not simply due to an inability to make a taste–taste association because all rats (SHAM and PBNX) learned a conditioned preference for the flavor paired with sucrose. These findings demonstrate that an intact PBN is essential not only for the unconditioned, but also for the conditioned, expression of a sodium appetite.

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105. Purification of an arginine taste receptor from the channel catfish

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The high density of specific amino acid taste receptors make the catfish an ideal source of material for purification of taste receptors. Past studies indicate that the barbel membranes have specific binding sites for L-arginine (L-arg), and that L-arg stimulated channel activity is observed when these membranes are reconstituted into lipid bilayers. D-arg inhibited these responses. To purify this arg receptor, the lectin, *Ricinus communis* agglutinin I was bound to a CNBr-activated Sepharose column which was then used to selectively bind CHAPS-solubilized protein from a barbel membrane fraction. The bound proteins were eluted with D-galactose and reconstituted into lipid bilayers. A conductance increase of between 45 and 85 pS was observed in the presence of µM concentrations of L-arg (inhibited by D-arg), indicating the presence of the taste receptor. Electrophoresis of the eluted protein under reducing conditions (4–20% Ready Gels, Bio-Rad) showed a distinct band at 83 kDa. Polyclonal antibodies produced in guinea pig against the 83 kDa protein reacted with numerous small sites (~1 µm) within the taste pore of every taste bud when applied to fixed non-permeabilized barbels. This indicates that the antibody most likely reacts with an externally facing epitope of the putative arginine receptor. Electrophoresis under native conditions using a 1.5% agarose gel showed a broad high mol. wt band recognized by the antibodies produced against the 83 kDa protein. Reconstitution of the high mol. wt. protein into a lipid bilayer resulted in L-arg stimulated channels. Preliminary gel filtration chromatography (Sepharcryl S-400 HR) indicates that the high mol. wt protein is ~400 kDa. We propose that the 400 kDa protein may be the isolated L-arg receptor complex.

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106. Responses to alcohol in glossopharyngeal taste fibers of *Macaca mulatta*

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Responses of whole glossopharyngeal nerve as well as single taste fibers in rhesus monkey were recorded to taste stimulation of the posterior part of the tongue. Both foliate and circumvallate papillae were stimulated. Although our main goal was to investigate the effect of ethanol on the taste fibers we always used an extended array of taste stimuli: three salts, two umami compounds, three acids, three bitter compounds and 12 sweeteners. Having sufficient background about the response profile of the fibers, we stimulated them with 1 and 3 M ethanol alone or mixed with four basic stimuli: 0.07 M NaCl, 0.04 M citric acid, 5 mM quinine and 0.3 M sucrose. The whole nerve was stimulated with series of eight concentrations (from 0.3 to 8 M) of alcohol alone.

The summated response to ethanol showed mostly a tonic part in contrast to chorda tympani nerve response where a phasic and a tonic part were always recorded. 0.3 M ethanol was the lowest concentration tested and it did give response. The response–concentration relationship for ethanol fits to sigmoidal function (correlation coefficient 0.99).

The response profiles of 33 single fibers to 25 stimuli were subjected to hierarchical cluster analysis. Three major clusters of fibers, characterized by predominant sensitivity to sucrose, quinine and MSG were separated. At 1 M, ethanol stimulated ~60% of fibers in all three clusters of fibers; at 3 M it elicited a stronger response and stimulated ~80% of all fibers.

In 54% of the cases the responses to mixtures of alcohol and the basic four stimuli were larger than the responses to basic stimuli alone. The increases were the strongest and most often recorded in responses to sucrose-ethanol mixture in sucrose- and MSG-best fibers. In 30% of the cases the responses to mixtures of ethanol and basic four stimuli were suppressed. The strongest suppression occurred in response to quinine–ethanol mixture in quinine-best fibers.

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107. Amiloride and judgements of NaCl taste: no effects on tracked taste intensity

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Time–intensity tracking (visual feedback, 100 ms resolution, 8 s total duration) measurements of the effects of amiloride on NaCl were made in six practiced subjects over eight data collection sessions. Solutions flowed for 4 s through a closed delivery system over 39.3 mm^2 of the anterodorsal tongue tip region, preceded by 10 s H_2O and followed by 5 s H_2O. Stimuli were 100, 250 and 500 mM NaCl in H_2O (pH ~6), in 10 or 100 μM amiloride, or in caffeine controls. Each subject selected caffeine solution concentrations to approximate amiloride tastes: 33–100 μM caffeine for 10 μM amiloride; 8.33–12.5 mM caffeine for 100 μM amiloride. Time–intensity data for each trial of each subject, recorded at 100 ms after solution arrival at the tongue, at 200 ms after, through 8000 ms after, for stimulation with each NaCl–caffeine mixture, were subtracted from data recorded at corresponding times for NaCl–amiloride mixtures, then summed for 'pre-response' (100 ms through 600 ms), 'response' (700 ms through 6400 ms) and 'post-response' (6500 ms through 8000 ms) time domains. This yielded, for each subject for each corresponding trial of a pair of solutions (e.g. $NaCl^{100}$–caffeine and $NaCl^{100}$–amiloride), three numbers.

Results. No significant effects for treatment concentration during any time-domain or for any variable during the 'pre-response' time-domain. Individual subjects: 'response' time-domain, two subjects, [NaCl], $P \leq 0.048$. 'Post-response', one subject, $P = 0.008$ for [NaCl]. Across subjects: for the 'response' time-domain, [NaCl] was significant, $F(1,257) = 7.292$, $P = 0.007$. For 'post-response', similar results, $F(1,257) = 4.338$, $P = 0.038$.

Conclusions. Tracked taste intensity is not differentially affected by 10 or 100 μM amiloride mixed with 100, 250 or 500 mM NaCl in H_2O, when compared with matched caffeine controls. This agrees with our previous results for both time course of taste intensity and reports of salty taste (Halpern *et al.*, 1995).

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108. Separate populations of amygdala neurons express taste-elicited *c-fos* and project to the parabrachial nucleus

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The central gustatory neuraxis has a descending component that arises from forebrain regions associated with the motivational or appetitive aspects of feeding and projects to gustatory-related brainstem regions. As little is known about the anatomical organization and function of these descending pathways, the purpose of the present study was to anatomically characterize a feedback loop between the parabrachial nucleus (PBN) and the amygdala.

Six adult male rats were anesthetized and injected with a retrograde tracer (Fluoro-Gold, FG) into the central medial (CM), gustatory-responsive PBN. Following the appropriate survival period, awake, behaving rats were either stimulated intraorally with a taste solution [0.003 M quinine ($n = 3$) or 1.0 M sucrose ($n = 2$)] or unstimulated ($n = 1$). Standard techniques were used to prepare 40 μm transverse brain sections and to allow visualization of transported tracer and the FOS protein (FOS), a functional marker of sensory stimulation. Subsequent plotting and analysis of the tissue revealed a concentration of FOS-labeled cells in the central nucleus of the amygdala (CNA) in both groups of taste-stimulated animals. Specifically, more labeled cells were in the lateral (CeL) and lateral capsular (CeLC) subdivisions than in the medial subdivision (CeM). There were also fewer FOS-labeled cells in the CNA of the sucrose-stimulated than the quinine-stimulated animals. Few FOS-labeled neurons were noted in the CNA of the unstimulated control animal. In contrast to the c-fos expression in the taste-stimulated animals, most FG labeled cells were located in the CeM, with few labeled cells in the CeL or CeLC. Overall, there were very few cells double labeled for both c-fos and FG.

The lack of overlap between the distribution of taste-induced c-fos expression and efferent neurons projecting to the CM PBN suggests that this pathway from the amygdala is not a direct taste-activated feedback circuit. Neurons within the external medial PBN, however, are taste-responsive and are reciprocally connected to the same amygdala subdivisions expressing taste-elicited c-fos. This suggests a second, more direct taste-activated feedback circuit between the PBN and amygdala.

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109. Video images of dye coupling in the salamander olfactory bulb

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Reconstruction of salamander olfactory bulb sections after intracellular recording and staining often reveals that more than one cell has been stained. Although the staining of multiple cells could be an artifact, mitral cells in the rat olfactory bulb have been shown to be coupled to granule cells via gap junctions, through which the fluorescent dye Lucifer Yellow can pass in lightly fixed slices (Paternostro *et al.*, 1994). To investigate functional correlates of dye coupling, we have developed procedures for documenting the progression of Lucifer Yellow staining while recording from mitral/tufted cells in intact isolated salamander olfactory bulb preparations.

With these procedures, mitral/tufted cells are visually identified under bright-field illumination using a microscope equipped with a 40× water immersion objective and a CCD camera. A patch pipette containing 0.2% Lucifer Yellow CH and 1% Neurobiotin in whole-cell solution (Wellis and Kauer, 1993) is then positioned on the soma of a cell. The bright-field illumination is turned off, suction is applied to the pipette, the seal resistance is measured, and responses to voltage steps are recorded using PClamp and a personal computer. For up to 60 min, 640 × 480 pixel images of

fluorescence at 540 ± 25 nm are also acquired at regular intervals using Inovision and Silicon Graphics software and an Indy computer. Alternatively, images are stored on videotape for off–line analysis.

Our initial results indicate that salamander mitral/tufted cells are dye coupled. With 12 of 17 patched cells, we could distinguish one-to-four other fluorescent cells. We are presently investigating whether certain recording conditions, membrane properties, and response parameters are correlated with the number of fluorescent cells.

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110. Development of the olfactory epithelium in *Xenopus laevis*

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The olfactory system of *Xenopus laevis* is able to detect water-soluble and volatile odorants. In adult frogs separate chambers of the peripheral olfactory organ, equipped with flaps to close any of these chambers, are used in either aquatic or terrestrial surroundings. Sensory neurons responsible for the detection of airborne odorants are located in the main chamber, whereas olfactory neurons detecting water-soluble odorants are located in a lateral chamber. Recent studies have shown that *Xenopus* have two classes of genes encoding distinct olfactory receptor types, each class is spatially expressed in one of the chambers. In the development and in particular during metamorphoses of the *Xenopus* larvae, the olfactory organ undergoes a dramatic transformation from a single open pit to a complicated system of various chambers and diverticula. The state of the olfactory epithelium during these critical stages of development are characterized using scanning and transmission electron microscopical methods. Ultrastructural analyses revealed that microvillous and ciliated sensory cells are colocalized in the larval olfactory pit; this structure develops into the main chamber which in the adult animals has ciliated receptor cells only. We have begun to explore the temporal and spatial expression patterns of olfactory receptors during development applying *in situ* hybridization techniques in order to determine the onset of expression and the topographical location of receptor types in the morphologically changing organ. Our results show that different olfactory receptor types are already expressed in early developmental stages.

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111. PTC-avoidance polymorphism and other bitter-avoidance differences among mice in long-term preference tests

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Extended two-bottle preference testing at single concentrations produced marked differences in avoidance of bitter phenylthiourea (PTC) solutions among four inbred mouse strains. Duration of exposure interacted with concentration and strain to determine PTC-avoidance levels. The pattern of strain differences produced by propylthiouracil (PROP), a structural analog frequently used in human testing, was not the same. The PROP pattern was not duration dependent and more closely resembled the pattern found for quinine sulfate. Other bitter compounds, including sucrose octaacetate (SOA), caffeine and L-phenylalanine, each produced unique patterns of strain differences. The variety of patterns suggested multiple bitter perception mechanisms. The dissimilarity of the PTC and PROP patterns suggested differences between mice and humans in such mechanisms.

C3.SW SOA-taster and SOA-demitaster congenic mice and their parental strains were also tested. The pattern of strain differences produced by SOA in these mice, and observed with quinine sulfate and PROP, was not seen with PTC. The *Soa* bitter taste locus, thus, did not influence PTC avoidance, but did influence quinine and PROP avoidance.

The PTC phenotype of F_1 offspring of BALB/cByJ (avoider) and C3HeB/FeJ (non-avoider) mice closely resembled that of the avoider parent strain, indicating avoider dominance. The difference between the F_1 mice and the C3HeB/FeJ mice appeared large enough and consistent enough for use in segregation studies. Such studies should help to reveal the genetic basis for PTC-avoidance differences in mice.

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112. Associative olfactory learning in moths

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Moths use plant volatiles to localize host plants for oviposition and feeding. However, it was still a question whether the recognition of plant odours is exclusively innate or depends as well on learning. First experiments with restrained moths, *Heliothis virescens* and *Helicoverpa armigera*, have shown that they are indeed capable of learning to associate a flower odor with a food reward. The aim of the present study was to extend the behavioral experiments with *H. virescens* to get a survey of their learning ability. The following questions were addressed: (i) Can moths discriminate the compounds of a learned odour blend? (ii) Are some odours more potent to be learned than others? (iii) Can moths associate a smell of CO_2 with a food reward, as found in honeybees?

Like bees, moths extend their proboscis immediately after the tip

of the antenna has been touched with a drop of sucrose solution. This reflex has been used in the conditioning experiments. One-day-old moths were conditioned using 1 M sucrose solution as unconditioned stimulus (US) to extend their proboscis when stimulated with an odour (conditioned stimulus, CS). The US was applied immediately after a 3 s application of the CS. Three experiments were conducted to investigate the questions mentioned above:

(i) A group of moths was trained with a mixture of two compounds (α-pinene, pentan-1-ol). After 12 conditioning trials the animals were tested with the mixture and with the single compounds. The percentage of responses to the mixture was 57%. Most of the moths responding to the mixture responded to the single compounds as well.

(ii) In the second experiment two groups of moths were trained, each with one of the two compounds used for the mixture. After 12 conditioning trials the moths were tested with one unrewarded CS. The% of proboscis extension responses in the two groups were compared with see whether the two compounds have been learned with the same success.

(iii) In the third experiment CO_2 was used for CS. From electrophysiological studies it is known that moths have highly sensitive CO_2 receptors, located in the labial palp organ, however, the behavioral function of CO_2 is still not clear. After the first experiments it seems that CO_2 (0.3%) is a poor stimulus for conditioning experiments. To decide whether moths can learn to associate a CO_2 stimulus with a sugar reward, more experiments with different CO_2 concentrations are necessary.

113. Pheromone-evoked potentials and oscillations in the antennal lobes of the sphinx moth, *Manduca sexta*

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Coherent rhythmic activity patterns measured extracellularly have been observed in various regions of the vertebrate brain including the olfactory system and are thought to arise from temporal synchrony of neuronal discharge in groups of neurons. Possible functions of this activity include the integration of distributed processes in the nervous system and temporal coordination of activity within and between subsystems in the CNS. Recent studies in the locust olfactory system suggest that brain oscillations in the antennal lobes and mushroom bodies might be used in insects to encode information about general odorants.

In this study we demonstrate that oscillations measured extracellularly can be induced in a highly specialized olfactory subsystem, namely the macroglomerular complex (MGC) in the antennal lobe (AL) of male sphinx moth, *Manduca sexta*. The MGC is the first-order site of synaptic processing of sex-pheromonal information. By means of extracellular and intracellular electrophysiological recordings, activity of individual cells as well as neuronal assemblies was observed during antennal stimulation with the key components of the female sex pheromone. Pheromonal stimuli and not clean air resulted in evoked potentials and potential oscillations. The form of the evoked potentials was variable and dependent on the position of the recording site in the AL as well as on the composition of the pheromone blend. Typically the potential showed a short initial rising phase until it reached a sustained plateau which was maintained during stimulation with pheromone. After stimulation the potential slowly decreased to the prestimulus level. The amplitude of evoked potentials depended on the strength of the stimulus. Often fast oscillations were superimposed on the evoked potentials. Paired recordings of evoked potentials and intracellular activity of MGC projection neurons revealed that in individual neurons the action potentials were in phase with the oscillations. In neurons in which action potential generation was suppressed by hyperpolarizing current injection we often observed membrane potential fluctuations in phase with extracellular oscillations.

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114. Responses to alcohol in lingual proper fibers of *Macaca mulatta*

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The effect of alcohol on non-gustatory receptors on the tongue was studied. We were interested in how alcohol interacts with stimuli of different modalities. Each fiber was evaluated for its mechanical and thermal responses. This was followed by stimulation with eight concentrations (0.7–12 M) of ethanol at 33°C for 52 s.

We recorded five specific cold units, two of which were affected by high ethanol concentration. We recorded also 34 single nerve fibers which responded to mechanical stimulation, of which 21 also responded to ethanol; 8 of the 21 also responded to cooling. Based on their responses to mechanical stimulation the fibers were characterized as either slow or fast adapting units. Most fibers responding to ethanol were slow adapting. In contrast, most fast adapting units did not respond to ethanol. Thus there was relationship between the type of unit and its responsiveness to ethanol.

The response to ethanol was characterized by a regular impulse activity after a latency of 3 to >40 s. Some of the fibers responded to a very low concentration of ethanol. Thus, already 0.7 M elicited a response. This was a remarkably low stimulus concentration and unexpected. As expected the latency was shorter and the impulse activity evoked higher to higher ethanol concentrations. The impulse frequency peaked at some concentration. In some fibers the response to the highest concentrations (8 and 12 M) ceased before the end of stimulation.

Ethanol did not only stimulate non-gustatory fibers at remarkably low concentrations, it also affected the response to touch in mechanoreceptors and the response to temperature in thermoreceptors. These effects will be illustrated in the presentation.

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115. The Thy-1 allelic marking system shows reciprocal fiber penetration and host-to-donor neuron migration in mice with olfactory bulb transplants

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Ultimately, the functional significance of neural transplants is directly related to their ability to integrate with host brain. Ideally, a researcher should be able to clearly delineate all cells and their processes of host versus donor origin in order to determine the extent and appropriateness of fiber and cell integration. The membrane glycoprotein Thy-1 is a suitable marker since it: is present at high density on almost all neurons (except olfactory nerve); exists in two allelic forms in mice; and can be distinguished immunologically. Thus, neural transplantation of Thy-1.2 mouse tissue into a Thy-1.1 mouse strain, when combined with immunolocalization protocols, can be used to display reciprocal host and donor integration patterns. In addition, antibodies against olfactory marker protein (which is olfactory nerve-specific) can be used to show olfactory nerve penetration patterns. In this experiment, neonatal mouse pups (one to four days of age) had one olfactory bulb ablated and immediately replaced with a fetal, donor olfactory bulb (embryonic days 15–17). Previous experiments in our laboratory have shown that these transplants can be functional in rats. Our previous reports using traditional tract-tracing methods have demonstrated donor host fiber integration in rats but only of selected fiber pathways. Results from the present experiment indicate the following: (i) inter-strain transplants are viable; (ii) host olfactory nerve penetrates into donor tissue, entering glomerulus-like structures that are also penetrated by donor processes; (iii) donor fibers are able to penetrate appropriate (olfactory peduncle and olfactory cortex) and novel (subependymal cell layer and rostral frontal cortex) areas of brain; (iv) host fibers from the forebrain penetrate into the transplant; and (v) host neurons can migrate into transplant tissue and remain viable. Thus, the Thy-1 marking system is a powerful tool not only for the study of the integrative capabilities of transplants but also for the factors effecting their development. We are currently evaluating each of these findings in detail with the goal of better understanding the integrative capabilities of olfactory bulb transplants.

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116. Immunocytochemical localization of c-fos and nitric oxide synthase to von Ebner's Gland but not to posterior taste cells in rat tongue

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To further investigate cellular mechanisms potentially important to taste transduction, rat tongue tissue was probed immunocytochemically for the presence of c-fos, a transcription factor, or neuronal nitric oxide synthase (n-NOS), the synthetic enzyme for nitric oxide, a soluble neurotransmitter. Experiments were performed on paraformaldehyde fixed posterior tongue tissue containing circumvallate papillae. Immunoreactive product was visualized using commercially available antibodies, a biotinylated secondary antibody, and avidin labeled with either peroxidase or phosphatase, and subsequently developed with the chromagens diaminobenzidine (DAB) or *x*-phosphate/nitroblue tetrazolium (BCIP/NBT) respectively. Neuronal labeling in CNS tissue served as positive control for both antibodies. Omission of the primary antibody or application of inappropriate chromagen abolished all staining and served as negative controls. Positive reactivity for either c-fos or n-NOS was not observed in taste cells of the circumvallate papillae (n = 23 experiments). However, abundant immunoreactivity for both antigens was observed in cellular elements lying ventral to the circumvallate papillae. These cells were widely distributed, granular in appearance, and scattered in and around the connective tissue core under a narrow region of only the most distal circumvallate sections. They may be associated with von Ebner's gland. In addition, large ganglion cells, located at the base of the miD-CV core, displayed a prominent cytoplasmic distribution of reaction product to anti-n-NOS with either DAB or BCIP/NBT reactions. These ganglion cells have been previously described as postganglionic parasympathetic neurons of unclear function. Both c-fos and n-NOS may be important cellular elements relating to regulation of von Ebner's gland secretions though their cellular localization to taste cells is unlikely.

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117. A comparison of olfactory, tactile and visual stimuli as associated memory cues

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It has long been claimed that odors are the 'best' cues to memory. The purpose of the present research was to investigate what this statement really means. In two experiments, using a paired-associate incidental learning paradigm, the accuracy and emotionality of memories for pictures associated to cues presented in olfactory, tactile and visual forms were compared (e.g. the smell of an apple, the feel of an apple, the sight of an apple). Experiments were divided into two sessions (study, test) separated by 48 h. Subjects were never told that their memory for the pictures would later be assessed. During the study session, subjects were exposed to 12 different pictures projected onto a large screen, for 1 min each, while they simultaneously either smelled, felt, or looked at an associated cue. At the test session, subjects were re-presented with the same cues, and asked to recall what picture had been paired with it (memory accuracy measure), and to list and rate whatever emotions the cue elicited, as well as rating how emotionally intense their memory for the picture was (emotionality measures). In experiment 1, all the pictures were emotionally evocative. In experiment 2, emotional and non-emotional pictures were compared, to examine whether odors might be more associable to emotional than non-emotional material. Results revealed that odors, tactile and visual cues were equipotent for memory accuracy, but that odors elicited the most

emotion during recall, and memories that were more emotionally intense than visual and especially tactile cues. Additionally, emotional and non-emotional pictures were equally associable to the three cue-types, and were recalled to the same degree of accuracy, though emotional images were rated as more visualizable and elicited more emotional memories than non-emotional pictures. These data demonstrate that odors are not 'better' memory cues, if better means producing the most accurate recall. Rather, it seems that because odor-evoked memories are more emotionally potent memories than other cue-elicited memories, they produce the impression of being more real or 'better' memories.

This research was supported by a grant from the Olfactory Research Fund.

118. Species, sex, season and individual-specific chemosensory cues in urine discriminated by blind, subterranean mole rats (*Spalax ehrenbergi*)

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Breeding and non-breeding season mole rats from two chromosomal species ($2n$ = 58 and 60) of the *Spalax ehrenbergi* superspecies in Israel were tested in a tunnel T-maze to assess their responses to urine collected from breeding and non-breeding season conspecific and heterospecific donors. Differential responses (of groups compared using an independent t-test) indicate that mole rats can discriminate between species, sex and season-specific chemosensory cues in urine [species: males (t = 2.47, $P < 0.025$), females (t = 2.81, $P < 0.01$); sex: males (t = 1.95, $P < 0.05$), females (t = 1.97, $P < 0.05$); season: males (t = 2.62, $P < 0.001$), females (t = 4.40, $P < 0.001$)]. Mole rats were tested during the non-breeding season to determine whether they were able to discriminate differences in urine odors from same-sex individuals from their own and from the other chromosomal species. A habituation–discrimination apparatus was designed for use with blind, subterranean rodents. Animals habituated to the urine odor from one individual presented for 10 min at the middle sniff port in a 50 cm long Perspex tunnel. Significant differences (compared with paired t-tests) in the time spent sniffing the urine odor from a second individual versus the original individual presented at two other ports during a 5 min discrimination phase demonstrated successful discrimination between the odors. Mole rats of both species can discriminate between same-sex urine odors from conspecific (males: t = 4.242, df = 29, $P < 0.001$; females: t = 4.578, df = 29, $P < 0.001$) and heterospecific individuals (males: t = 3.214, df = 19, $P < 0.005$; females: t = 5.617, df = 19, $P < 0.001$).

Mole rats' ability to discriminate between these types of chemosensory cues in urine suggest that chemical communication could play an important role in social encounters, reproduction and species isolation in the *S. ehrenbergi* superspecies.

119. N and H chorda tympani fibers utilize separate taste transduction mechanisms

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An emerging model of taste reception for electrolytes in mammals portrays two different transduction mechanisms. One mechanism involves receptor cell depolarization by means of cation, predominantly sodium, entry through apical, amiloride-sensitive, ion channels. The other is based on field potentials set up by differential diffusion of cations and anions into the paracellular space between receptor cells. Our studies using single-fiber recordings of the hamster chorda tympani nerve strongly suggest that individual fibers exclusively receive input from taste receptors employing a single transduction mechanism. N fibers respond selectively to sodium salts and are blocked by amiloride while H fibers respond to a variety of electrolytes and are unaffected by amiloride. We have not observed fibers with intermediate characteristics. Quantitative differences between N and H fibers have also been found in their anion responses. In the present study, we found for the 0.1 M Na salt series NaCl, NaBr, Na acetate, Na benzoate and Na D-gluconate, a substantial decrease in H-fiber response across the series. N fibers showed the same trend, but to a smaller degree. Responses to Na D-gluconate were 19.5 ± 5.4% that of NaCl in H fibers and 59.3 ± 12.9% that of NaCl in N fibers. This difference is significant [$t(11)$ = 2.69, P = 0.02]. N fibers presumably receive their input from receptor cells having sodium-selective apical channels and are less affected by the nature of the anion. H fibers appear to be more sensitive to anion effects and less selective for cations. Since single nerve fibers are thought to innervate multiple receptor cells, the segregation of taste transduction mechanisms according to fiber type is noteworthy, and suggests important functional distinctions for N and H fibers. N fibers are probably involved in the regulation of sodium intake, but the role of H fibers is uncertain.

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120. Age-related changes in neuronal precursor cell dynamics in the vomeronasal epithelium of garter snakes

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Little is known of changes with age which may occur in the cell dynamics of neuronal precursors during postnatal neurogenesis. In the present study, precursor cells were labeled by [^{3}H]thymidine (^{3}H-T) autoradiography and by immunocytochemistry with an antibody recognizing an evolutionarily conserved peptide sequence (PSTAIRE) within a family of homologous cyclin-dependent kinases (CDKs). Young and adult snakes were examined for anti-PSTAIRE immunoreactivity (IR) in relation to ^{3}H-T labeling. CDKs have been shown previously to participate in the regulation of progression through the cell cycle in all cell types examined. Therefore, anti-PSTAIRE IR should identify all cells

within all phases of the cell cycle. After survival times of 1 h, 1 day, 1 week, 1 month and 2 months post-^{3}H-T injection for 2- to 3-month-old snakes, migration of ^{3}H-T-labeled cells is observed from the base of the VNE (1 h and 1 day) into the receptor cell columns (1 week–2 months). After 2 months post-^{3}H-T injection, some labeled cells are seen at the base of the VNE, which has not been reported previously in adults. One hour after ^{3}H-T injection, adults have significantly less ^{3}H-T-labeled cells/mm^2 (n = 5, 262.3 ± 44.8, mean ± SE) than 2- to 3-month-old snakes (n = 3, 982.9 ± 76.3, $P < 0.05$). Anti-PSTAIRE IR is only seen at the base of the VNE for all animals. Incubation of antiserum pre-absorbed with p34^{cdc2} results in an absence of IR. Three to four times more precursor cells are labeled consistently in young snakes by the PSTAIRE antibody than by ^{3}H-T. The number of anti-PSTAIRE IR cells/mm^2 is significantly less in 3- to 4-month-old (n = 8, 1520.7 ± 462.8), 5- to 6-month-old (n = 3, 1007.1 ± 211.3) and adult snakes (n = 5, 853.2 ± 101.3) than in 2- to 3-month-old snakes (n = 8, 2919.5 ± 282.9, $P < 0.05$ versus all groups). In sections double-labeled for ^{3}H-T and anti-PSTAIRE, all double-labeled cells are found at the base of the VNE. 85.7% of all ^{3}H-T-labeled cells are anti-PSTAIRE IR at 1 h post-^{3}H-T injection in 2- to 3-month-old snakes, suggesting that only a subset of the total precursor cell population is anti-PSTAIRE IR. Nearly all ^{3}H-T-labeled cells are anti-PSTAIRE IR in adult snakes. However, 23% of anti-PSTAIRE IR cells are also ^{3}H-T-labeled 1 h post-injection in both the 2- to 3-month-old and adult snakes. The data suggest that, from 2 months of age to adulthood, there is an ~75% decrease in both the number of neuronal precursors that are actively dividing during normal turnover and in the total number of neuronal precursor cells in the snake VNE.

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121. Number of fungiform papillae in nontasters, medium tasters and supertasters of PROP (6-*n*-propylthiouracil)

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The ability to taste the bitter compound 6-*n*-propylthiouracil (PROP) is genetically determined. PROP tastes intensely bitter to some individuals (supertasters), moderately bitter to others (medium tasters) and is nearly tasteless to a third group (nontasters). When the tongue is stained with blue food coloring, filiform papillae stain and can be discriminated from fungiform papillae, which contain taste buds and do not stain as well. Videomicroscopy can then be used to count both fungiform papillae and taste buds. The numbers of fungiform papillae and taste buds correlate significantly with the ability to taste PROP. The number of taste buds is also known to be affected by otitis media; however, the degree to which the number of fungiform papillae is so affected is unknown at present. Thus, the number of fungiform papillae may provide a measure of genetic PROP status that is more robust than either taste bud counts or psychophysical evaluation. In 29 female subjects, fungiform papillae were counted in two sample areas: a 3 mm square to the right of the midline at the tip of the tongue, and a rectangle at the tip of the tongue extending 6 mm back and 5 mm to each side of the midline. In addition, fungiform papillae were counted on the entire anterior tongue to a line 13.5 mm back from the tip; this measurement was expressed as both a count per tongue and a count per cm^2. All measures correlated significantly (at least $P < 0.05$) with the ability to taste PROP. However, one nontaster and one medium taster showed unusually dense clusters of fungiform papillae on the tip of the tongue. The only condition relevant to taste that could be identified in the medical histories of these individuals was otitis media. The existence of such pathologically induced clusters suggests the importance of sampling over a large area of the tongue.

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122. Amygdala lesions attenuate c-Fos induction in the nucleus of the solitary tract after conditioned taste aversion expression

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Acquisition and behavioral expression of conditioned taste aversions (CTAs) are mediated by a distributed neural network including both hindbrain and forebrain sites. The induction of c-Fos-like immunoreactivity (c-FLI) in the medial intermediate nucleus of the solitary tract (iNTS) after expression of a CTA is a neuronal correlate of CTA, and reveals one activated site within the neural network that may contribute to CTA expression. Schafe *et al.* (1995) demonstrated by hemidecerebration that forebrain connections are necessary for c-FLI induction in the iNTS by CTA expression. We have recently shown (Houpt *et al.*, 1995) that expression of a CTA also induces c-FLI in the central nucleus of the amygdala (CeA). Because the CeA and the iNTS are reciprocally connected, we hypothesize that activation of the amygdala is required for induction of c-FLI in the iNTS after CTA expression. The induction of c-FLI in the iNTS was therefore examined in rats with unilateral ibotenic acid lesions of the amygdala after CTA expression. Adult male rats (n = 8) were implanted with intraoral catheters. Rats were conditioned against sucrose by pairing intraoral infusions of 5% sucrose (6.6 ml/ 6 min) with LiCl injections (0.15 M, 12 ml/kg i.p. 30 min after sucrose infusions) three times over a week. All rats decreased their intake of the intraoral infusion of sucrose from ~6 ml to 0 ml during acqusition of the CTA against sucrose. Two to four weeks after CTA acquisition, ibotenic acid (1% in saline, 1 µl /5 min) was injected unilaterally into the amygdala, centered on the CeA. Contralateral vehicle injections were also made. Ten days after lesioning, rats received an unpaired intraoral infusion of 5% sucrose; 1 h later, the iNTS was processed for c-FLI. An additional five unconditioned rats received unilateral amygdala lesions and c-FLI was examined in the NTS 1 h after LiCl injections.

In 6/8 rats, the number of c-FLI-positive nuclei 1 h after CTA expression was attenuated or eliminated in the iNTS ipsilateral to

the ibotenic acid lesion. No attenuation in c-FLI was seen in any of five lesioned rats 1 h after LiCl injection. We conclude that forebrain sites in or near the amygdala or fibers perforating the amygdala are required for full neuronal activation of the iNTS during CTA expression.

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123. A subset of NST neurons that express gustatory-elicited Fos project to PBN

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Previous work in our laboratory has shown that sucrose or quinine stimulation elicits expression of the protein product (Fos) of the immediate-early gene, c-*fos*, in the rostral division of the nucleus of the solitary tract (rNST). Gustatory-elicited Fos is dominant in the rostral central subnucleus, where there is evidence for a rough chemotopy. The aim of the current study was to further characterize rNST neurons that express gustatory-elicited Fos by determining whether they project to the parabrachial nucleus (PBN). In anesthetized male rats, injections of the retrograde tracer, Fluorogold (FG), were made into the electrophysiologically-identified gustatory PBN and intraoral cannulas implanted for subsequent gustatory stimulation. After 10 days, rats were stimulated periodically with 1.0 M sucrose or 0.003 M quinine HCl over ~30 min, and 45 min later anesthetized and perfused with saline followed by 4% paraformaldehyde (sometimes with acrolein added). Frozen sections were reacted immunohistochemically for the Fos protein using nickel-enhanced DAB as the chromogen, and then for FG using naphthol/pyronin, resulting in brown or black Fos-labeled nuclei which contrasted well with pink FG-labeled somas. Light microscopic inspection of rNST revealed that Fos-and FG-labeled neurons were distributed similarly; both were dominant in, but not restricted to the rostral central subnucleus. Following either stimulus, a subset of neurons were double-labeled. In the two cases quantified, however, only a minority of the Fos-labeled neurons were FG-labeled (= 18.0 ± 9.92% SE) and vice versa (= 7.8 ± 1.83% SE). These results demonstrate that a subset of rNST neurons expressing gustatory-elicited Fos are part of the ascending taste pathway but further suggest that Fos expression is not restricted to PBN-projection neurons but also occurs in other cells, perhaps interneurons or neurons with local medullary projections.

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124. cDNA cloning and heterologous expression of mouse CYP2G1

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CYP2G1 is an abundant enzyme expressed specifically in the olfactory mucosa of mammalian species and is believed to have physiological functions important for the olfactory chemosensory system. The aim of this study is to determine the function of CYP2G1 in rodents. Previous studies from this laboratory indicated that purified CYP2G1 from rabbit olfactory microsomes is active toward several sex steroid hormones as well as odorants. However, substrate specificity of rodent CYP2G1 has not been determined even though a cDNA clone for rat CYP2G1 has been isolated (Nef *et al.*, 1989). In the present study, a cDNA of mouse CYP2G1 was obtained using PCR. Primers were designed based on conserved sequences between rat and rabbit CYP2G1 cDNAs, and RT-PCR was performed with RNA from the olfactory mucosa of C57/BL6 mice. The PCR products were subcloned and their sequences determined. A full-length cDNA, obtained with use of 5′-RACE and 3′-RACE techniques, contained an open reading frame for a protein of 494 residues. Sequence comparisons indicated that mouse CYP2G1 is highly homologous in deduced amino acid sequence to rabbit (82% identity) and rat CYP2G1 (94% identity). The coding region of the mouse CYP2G1 cDNA has been cloned into a baculoviral expression vector for heterologous production of the enzyme in cultured insect cells. The determination of the structure and activity of mouse CYP2G1 will facilitate future studies on the function of this unique enzyme.

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125. 'Sniffin' Sticks': new ways to test olfactory performance

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'Sniffin' Sticks' is a new test of nasal chemosensory performance based on pen-like odor dispensing devices. Thus, it was possible to create a portable, re-useable test of olfactory performance which is easy to be administered. Since the test may be used over a period of 4 months costs are kept at a low level. It consists of two major parts, a basic screening test, and a more sophisticated test suited for forensic or scientific questions. The screening test includes an identification task for both seven odorants and four tastants (in pen-shaped flacons) which is performed by means of a list of four items (forced choice). The advanced test is composed of three subtests, i.e. odor identification (16 odorants, multiple choice),

odor discrimination (triple forced choice, 16 triplets) and a butanol threshold test (triple forced choice, staircase method).

The following results were established: (i) In 98 subjects the basic screening test was compared with a version of the UPSIT (MODSIT); 'Sniffin' Sticks' exhibited a relatively higher coefficient of correlation with the subjects' age. In contrast to the MODSIT the 'sticks' demonstrated the women's superior olfactory sensitivity when compared with men. (ii) Threshold testing by means of 'Sniffin' Sticks' was compared with the CCCRC (squeeze bottles). There was a significant correlation between the two tests ($r26 = 0.49$, $P < 0.05$); regarding test–retest reliability the 'sticks' reached a coefficient of correlation of $r26 = 0.68$ ($P < 0.001$). (iii) The three subtests of the advanced part of the 'sticks' were investigated in 104 subjects at two different days where they exhibited a good test–retest reliability (th.: $r104 = 0.61$, $P < 0.001$; discr.: $r104 = 0.54$, $P < 0.001$; ident.: $r104 = 0.73$, $P < 0.001$). In addition, a significant life-span decrease of olfactory performance was observed.

126. Relationships between salivary responses and astringency, bitterness and sourness responses to aluminum ammonium sulfate

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Salivary PRPs (proline rich proteins) bind bitter compounds as well as polyphenols. Salivary buffering and lubricatory functions as well as the specific binding property of PRPs might induce correlations among astringency, bitterness, sourness and salivary responses.

In the first experiment it was examined whether PROP (propylthiouracil) taster status and salivary flow rate have any effect on suprathreshold responses to alum. Subjects were asked to rate sourness, bitterness, drying, roughing, puckering and astringency of 2.2, 2.8, 3.5, 4.4 and 5.5 mM alum, the bitterness of 0.032, 0.101, 0.320, 1.000 and 3.162 mM PROP and the saltiness of 0.10, 0.18, 0.32, 0.56 and 1.00 M NaCl. Accumulated saliva was collected following each series of five samples of the same compounds. Ratings were made on a labeled magnitude scale. Sourness ($r = 0.41$, $P = 0.01$) and astringency ($r = 0.39$, $P = 0.02$) responses to alum and bitterness ($r = 0.70$, $P < 0.01$) response to PROP were correlated with salivary flow rate. High flow subjects have higher sourness response to alum and higher bitterness response to PROP than low flow subjects. Effect of PROP taster status was significant in drying response ($F = 4.43$, $P = 0.02$) to alum. Nontasters have higher drying response than tasters.

In the second experiment it was examined whether there is a correlation between salivary protein concentration and the suprathreshold responses to alum. The procedure was the same as that of the first experiment and salivary protein concentration was measured by the Bradford method. Salivary protein concentration was correlated with bitterness response to alum ($r = 0.55$, $P = 0.01$) and bitterness response to PROP ($r = 0.67$, $P < 0.01$). The salivary protein concentration of nontasters was significantly lower ($F = 4.54$, $P = 0.05$) than that of the tasters. These results suggest that salivary buffering function might induce a correlation between sourness of alum and salivary flow rate. Salivary lubricatory function might induce a correlation between drying response to alum and the concentration of specific salivary proteins which are also related to PROP taster status. However, the specific protein was not identified in this experiment.

127. Odor identification in normally developing children and adolescents

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The perception of odors was investigated in normally developing children and adolescents using an odor identification task. The goal of this study was to identify odors from a battery of 33 common odors that are highly identifiable with 80–100% accuracy. These odors will then be incorporated in a child version of the Californian odor learning test (COLT), specifically designed for clinical purposes. One hundred and thirty subjects participated in the study. They were distributed in six age-groups (5–6; 7–8; 9–10; 11–12; 13–14; 15–16). During an identification trial, they attempted to identify an odor by recalling a name or pointing to a picture from a board. The experimental task also included a component of semantic categorization of odors since subjects were asked to cluster each odor into a semantic category. They were allowed to choose from a list of five categories: fruit, vegetable, candy, school and outdoor.

A series of ANOVAs revealed significant differences in odor identification performance across age-groups. The youngest subjects did poorly on the task compared with the oldest ones. There was a progression in odor identification performance by naming with increasing age. A comparison of subjects' mean scores of correct identification by naming per odor allowed us to identify six highly identifiable odors. Moreover, an overlapping of these same odors was found between groups. The results on semantic clustering of odors indicated significant differences between age-groups but also between semantic categories. The youngest subjects were less able to match odors with their appropriate semantic categories than did the oldest ones. However, in all groups, clustering scores were best for the semantic categories 'candy' and 'fruit', and worse for the categories 'school' and 'outdoor' which are more abstract in terms of semantic than the former ones. These results support the idea that associations between odors and their names are learned through a cognitive process of semantic categorization of odors in addition to language abilities.

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128. Parallel development of subclasses of vomeronasal receptor neurons

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Previous studies have shown that there are two populations of receptor neurons in the vomeronasal organ (VNO). The Giα2-expressing population is located in the middle layer of the vomeronasal epithelium and projects to the anterior part of the

accessory olfactory bulb (AOB). The Goα-expressing population is located in the deep layer of the vomeronasal epithelium and projects to the posterior part of the AOB. In the present study, (i) the relationship between the two populations of receptor neurons was investigated by immunocytochemical study of G protein expression in these receptor neurons during development; (ii) cell proliferation and migration in the vomeronasal epithelium were studied using BrdU labeling; and (iii) the distribution of dendrites of AOB neurons was studied by intracellular labeling with Lucifer Yellow.

(i) During opossum development, the Giα2-ir cells and Goα-ir cells appeared at the same time (P0-P3). The numbers of Giα2-ir cells and Goα-ir cells increased with development of the VNO. The Giα2-ir and Goα-ir cells were located in the middle and deep layers of the vomeronasal epithelium respectively. This pattern remained stable during later development and in the adult. Between P3 and P21, a layer of cells near the basal lamina could be identified which expressed neither Giα2 nor Goα. This may be a layer of precursor cells for Giα2-ir and Goα-ir neurons. In the AOB, a clear differential staining of Giα2 in the anterior part and Goα in the posterior part of the AOB could be seen at P10 and at older ages. These observations support the hypothesis that the Giα2-ir and Goα-ir neurons are developmentally parallel subpopulations.

In the mouse, differential localization of Giα2-ir and Goα-ir receptor neurons in different layers of the VNO, and Giα2-ir and Goα-ir terminals in different parts of the AOB could be found at birth (P0).

(ii) The proliferation and migration of vomeronasal cells were studied using BrdU-labeling in adult and developing young opossums. In adult opossums, the BrdU-labeled cells were located near the basal lamina 4 h after BrdU injection. After longer survival periods (7 and 14 days, and 1 month), BrdU-labeled cells appeared in both the middle (Giα2-ir) and deep (Goα-ir) receptor cell layers. An increased proliferation (increased BrdU labeling) was observed in developing young animals. This finding suggests that the proliferation and migration of cells in the VNO resemble that in the main olfactory epithelium and that the Giα2-ir and Goα-ir neurons are both generated by progenitor cells in the basal layer.

(iii) The dendritic distribution of AOB neurons was studied by intracellular injection of Lucifer Yellow in lightly fixed sections of the AOB. Preliminary data showed that the apical dendrites of most mitral/tufted cells in the AOB entered either the anterior or the posterior part of the glomerular layer. No mitral/tufted cell with dendrites entering both anterior and posterior glomerular layer was found. The secondary dendrites spread extensively in the mitral/tufted cell layer. Similar results have been obtained by Golgi impregnation. These observations suggest that Giα2-ir and Goα-ir vomeronasal receptor neurons synapse onto different mitral/tufted cells in the AOB.

129. Morphological analysis of a putative NO/cGMP signalling pathway in the crayfish olfactory lobe

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Accumulating evidence suggests that nitric oxide (NO) acts as a messenger in invertebrate nervous tissues. Recent biochemical studies indicate that NO can be synthesized from L-arginine by a NO synthase (NOS), and that NO stimulates the production of cyclic GMP. The distribution of NOS-containing neurons in invertebrates has been indirectly demonstrated by means of NADPH-diaphorase (NADPH) histochemistry. This technique was used in the present study to visualize putative NOS-containing neurons in the crayfish olfactory midbrain. Three distinct morphological types of interneurons with cell somata in cell clusters 9 and 11 (formerly the dorsal lateral cluster) expressed moderate to strong NADPH-d staining. Most intense staining was observed in a pair of large interneurons (cell somata 50–60 μm) with neurites that branched into the ipsilateral olfactory lobe. A second pair of less intensely stained large interneurons was also observed, as well as 55–65 globuli cells. To reveal potential NO-sensitive target cells in the brain, the NO-donor sodium nitroprusside (SNP) was applied *in vitro* and putative target cells were demonstrated by means of cyclic GMP immunohistochemistry. After stimulation with SNP (10 mM) for 10–15 min, a large number of cell somata in cell cluster 10 (formerly the lateral cluster) expressed cyclic GMP-immunoreactivity. No staining was observed in control brains. These data suggest that deutocerebral projection neurons may be putative targets for NO.

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130. Neural mechanisms of individual discrimination: roles of vomeronasal organ, orbital cortex, and medial and cortical amygdala

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We have previously identified five odors that hamsters use to discriminate between individuals and we have characterized how they discriminate individual odors in complex arrays of two or more individual's scents. In the present studies we used habituation techniques to assess discrimination of individual odors and lesion techniques to assess the importance of various neural structures. Males with complete removal of the vomeronasal organ (n = 10) failed to discriminate between individual odors using three of the four scents tested, but did discriminate with the fourth odor. Males that experienced sham lesion procedures (n = 10) discriminated all four individual odors tested. Animals with partial lesions of the vomeronasal organ showed intermediate results. Thus, the vomeronasal system has an important but not exclusive role in this type of social odor discrimination. Aspiration lesions of the orbital prefrontal cortex (n = 13), which have been shown to disrupt learned discriminations of arbitrary odors, did not affect discrimination of individual odors by female hamsters (and also did not disrupt sex discrimination and preference or scent marking behaviors). Control lesions in the medial prefrontal cortex (n = 15) also had no effect on individual discrimination or sex preferences, but resulted in increases in scent marking behaviors. Animals with sham lesions (n = 23) discriminated individual odors and sexes, and scent marked in response to odors. The effects of ibotenic acid

lesions of the medial and cortical amygdala are presently under investigation and will be discussed.

131. Perception of scent over marks: how do hamsters determine which scent is on top?

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After hamsters investigate partially overlapping scent marks of two other hamsters in 3–5 exposure trials, they remember the top scent better than the bottom scent and prefer the individual that deposited the top scent. These experiments demonstrate that they can tell which scent is on top; the current experiments continue our investigation into how they do this. Several experiments show that, counter to intuition, hamsters do not use relative scent age as a cue to which scent is on top (and therefore deposited most recently). However, they do use two other cues: (i) the spatial pattern of scent deposition, in particular scents that are continuous are remembered well (perceived as on top) but scents that are interrupted by another scent are not (perceived as on the bottom), even if there is no actual overlap of the two scents; and (ii) some as yet unknown information from the region of overlap, perhaps the relative strengths or apparent amounts of the two scents. We investigated this later mechanism in more detail by examining whether hamsters could determine which scent was on top from volatile cues alone, as one would expect if they used the strength of individual odors. First we showed that they could indeed discriminate between individual's odors by volatile cues alone, by preventing actual contact with the scents. When males investigated partially overlapping scents of two individuals across a layer of window screening, they still remembered the top scent but not the bottom scent. Thus they could still determine which was the top scent. Results from experiments in which hamsters investigate the overlapping scents from slightly greater distances are underway. The results so far suggest that hamsters determine the position of individual scents (top or bottom) on the basis of non-volatile cues. This could be done either by spatial analysis of continuous versus non-continuous scents or by relative strengths in the region of overlap.

132. Influence of laminin on neurite extension from rat olfactory receptor cells *in vitro*

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Prior studies have shown a differential expression of the extracellular matrix molecule (ECM) laminin in the olfactory pathway during development. This suggested that laminin may contribute to axonal outgrowth from olfactory receptor cells (ORCs). Related to this observation, it has also been shown that laminin provides a favorable substrate for the migration of neuronal precursors and neurons derived from the embryonic olfactory epithelium (OE). Collectively, these findings have led to the hypothesis that laminin may also provide an effective adhesion substrate for neurite/axonal extension. To test this hypothesis we have developed an *in vitro* assay in which neurite extension from ORCs can be quantified on different substrates.

ORCs harvested from the OE of E14 rat embryos were cultured for 48 h on coverslips coated with either poly-L-lysine (PLL) alone, PLL with a complete overlay of laminin (PLL/LN) or PLL overlaid with 5 mm spots of laminin (PLL/LN-S). Our analyses were conducted on cells immunoreactive to OMP (generously provided by Dr F.L. Margolis). In accord with the prior studies of Calof and Lander, cell density was significantly higher (33%) on PLL/LN substrates compared with PLL alone. This does not appear due to diffusible factors because cell density was equivalent on the PLL/LN-S and PLL/LN coverslips. Moreover, on PLL/LN and PLL/LN-S substrates neurons within the 48 h period extended significantly longer neurites (50.3 ± 7.3 μm) compared with those on PLL alone (33.9 ± 6.9 μm). Subsets of neurites fasciculated on PLL/LN and PLL/LN-S and few neurites left a laminin substrate to enter areas which were only PLL coated. In summary, these data strongly support the hypothesis that laminin provides a favorable ECM substrate for the outgrowth of rat ORC axons. We are currently exploring mechanisms via which laminin and ORC axons/neurites interact as well as the role of other ECM molecules in establishing favorable substrates for axonal extension.

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133. Descending projections from the gustatory responsive parabrachial nucleus to the medullary reticular formation in the rat

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The parabrachial nucleus (PBN) receives gustatory, intraoral tactile and visceral inputs from the medulla. Because decerebrate preparations express gustatory mediated ingestive responses, projections from the PBN potentially play a role in these functions. Little is known, however about projections from the PBN to medullary sites associated with ingestion. The purpose of the present study was to identify terminal fields within the medullary reticular formation (RF) originating from physiologically identified taste and intraoral tactile sites within the PBN. Under electrophysiological guidance, anterograde tracers (10% biotinylated Dextran: BD or 2.5% PHA-L) were injected into different subdivisions of the PBN. Following a suitable survival time, animals were perfused and the brains were sectioned and processed to visualize the tracers.

Injections into the gustatory responsive PBN, i.e. ventrolateral (VL), centromedial (CM) and waist area demonstrated projections to the parvocellular, intermediate and dorsal divisions of the RF. Sparse projections were also observed in the hypoglossal nucleus and the caudal solitary nucleus (NST). In contrast, an injection into the external medial (EM) subdivision of the PBN, responsive to posterior oral cavity taste and tactile stimuli (Halsell and S. Travers, 1995), produced few descending projections to the medulla. Significantly, ascending thalamic projections from the EM were similar to other PBN subnuclei that also receive rostral solitary nucleus input. Double labeling experiments (n = 2) combined BD injections into the rostral (gustatory) NST with 4%

Fluorogold (FG) injections into the RF. In these experiments, retrogradely labeled RF projection neurons within the VL, CM and waist area of the PBN were associated with anterograde terminals from the NST injections, further suggesting that PBN taste neurons project directly to the medullary RF. In addition, these double label experiments confirmed the paucity of connections from EM to the RF. These findings thus indicate a differential contribution of specific PBN subdivisions sensitive to intraoral stimulation, to medullary substrates implicated in the coordination of oromotor behavior.

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134. Intrinsic organization of the olfactory bulb glomerulus

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Olfactory receptor cell (ORC) axons terminate in the glomerular neuropil of the olfactory bulb where they make synaptic connections with target dendrites of mitral, tufted and periglomerular neurons. We have investigated the organization of the glomerular neuropil using antibodies or the lipophilic dye DiI to label constituents for analyses using confocal microscopy. In parallel, we have used electron microscopy (EM) to assess the distribution of synaptic appositions within the glomerulus. Sprague–Dawley rats, 30–50 days postnatal, were perfused with 4% paraformaldehyde and processed for immunocytochemistry of olfactory marker protein (OMP), synaptophysin (SYN), synapsin I (SYN-I), glial fibrillary acidic protein (GFAP) and/or MAP-2. Alternatively, crystals of DiI were implanted into the olfactory nerve for tract tracing. Equivalent rats were perfused with 4% paraformaldehyde and 1% glutaraldehyde and processed for routine transmission EM. ORC axons stained with DiI occupied restricted regions within the glomeruli. OMP staining confirmed that observation. Double labeling for OMP and MAP-2 revealed a distinct interdigitation of axonal and dendritic processes within glomeruli. Areas not occupied by either OMP or MAP-2 immunoreactivity (IR) were either IR for GFAP, indicating a glial process, or appeared to be a blood vessel. OMP staining, though present throughout the glomerulus, was strongest in the shell or outermost portion, an impression also gained from the DiI stained ORC axons. MAP-2 staining was less extensive and not apparent in the glomerular shell. SYN and SYN-I also showed differential labeling within the glomerulus. Reconstructions of the area occupied by ORC axons versus dendrites from EM montages revealed continuous islands of axons within the glomerulus, accounting for 28.24 ± 1.34% of the total area, with bundles of dendritic processes interspersed. The distribution of synapses within the glomerulus further suggested the segregation of axo- and dendrodendritic interactions. The results support the hypothesis of a subcompartmental organization of the olfactory bulb glomerular neuropil.

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135. Functional connectivity in the nucleus of the solitary tract of the rat

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Although anatomical and morphological characteristics of taste-responsive cells in the nucleus of the solitary tract (NTS) have been described in recent years, the functional interrelationships among these cells are unknown. To study the these relationships and their role in the neural code for taste, electrophysiologicial responses were recorded simultaneously from small groups of single units in urethane-anesthetized rats. A 2 × 3 matrix of etched tungsten microelectrodes, spaced 115 μm apart, was used. Taste responses from up to four different single cells have been recorded simultaneously from separate electrodes. Taste stimuli consisted of sapid solutions of NaCl (0.1 M), HCl (0.01 M), sucrose (0.5 M) and quinine HCl (0.01 M). For all pairwise comparisons among simultaneously recorded units, cross correlation functions (CCFs) were constructed to detect the presence of a functional connection. These CCFs provided evidence for excitatory and inhibitory interconnections and the presence of common input. Preliminary analyses suggest that excitatory interconnections, including those that indicate common input, may be stimulus specific. Three pairs of units showing an inhibitory interconnection have been recorded thus far. In each case, both units of the pair were recorded from the same electrode suggesting that these units are in close proximity to each other. Inhibitory showed lower response rates and were more broadly tuned than their target cells. Further analyses of the functional interconnections in the NTS may reveal the extent to which neural signals associated with various taste stimuli are segregated along the taste pathway and the particular roles of excitation and inhibition in this process.

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136. Messenger RNAs encoding neuronal nicotinic receptor subunits are expressed in the rat olfactory and trigeminal systems

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Electrophysiological responses of nasal trigeminal afferents and binding studies of the olfactory mucosa indicate relatively high sensitivity of both chemosensory inputs to nicotine in the rat. Thus, neuronal nicotinic acetylcholine receptors (nAChRs), for which the pharmacology is well-known, may be involved in the transduction events underlying olfactory and trigeminal responses to nicotine. To identify the nAChR subunits that are expressed in cells from the olfactory turbinates, olfactory bulb and trigeminal ganglia of adult female Sprague–Dawley rats, reverse transcriptase–polymerase chain reaction studies were performed. Multiple nAChR subunit mRNAs (α2, β2, α4, β4, α3, X255α5, β3,

α7 and α9) are heterogeneously expressed in these tissues with the greatest inter-animal variability being observed in the olfactory neuroepithelium (ONE). This finding suggests that nAChR subunit mRNAs in the ONE may be regulated by cyclic changes in ovarian hormonal levels and/or factors involved in neurogenesis. Additionally, we detected moderate levels of olfactory marker protein (OMP) mRNA in the trigeminal ganglia and olfactory bulb in comparison to high levels in the ONE. These results identify some or all of the nAChR subunits that may constitute the pentameric receptors involved in nicotine stimulation of the olfactory and trigeminal systems. These normative data will aid investigations of the possible regulatory effects of acute or chronic nicotine vapor exposure on nAChR and OMP gene expression and nAChR binding in cells of the olfactory and trigeminal systems.

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137. Human normosmic and anosmic sensory responses to propionic acid

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Attempts to understand responses to low levels of airborne odorants and irritants have been complicated by reports in the literature that: (i) trigeminal nerve activation is both necessary and sufficient for the perception of nasal irritation; and (ii) inter- and intra-individual human odor thresholds vary over two to three logarithmic units. To examine both questions, we used a computerized air-dilution olfactometer to present (at constant humidity, temperature and volume flow rate) four verified concentrations of propionic acid (85.0, 9.7, 1.1 and 0.2 p.p.m., v/v) to 31 normal and four anosmic subjects. Each subject underwent four identical, weekly 50-trial sessions. On each trial, respiratory measurements were recorded just before and during each 15 s clean air or odorant presentation. Ratings of the magnitude of odor (O) and nasal irritation (NI) were then entered on a computerized visual analogue scale. Our key findings were: (i) for normosmics, the average week-to-week spans in the concentrations required for half-maximal sensory magnitude averaged (in log units): normals , O/NI 0.4/0.2; anosmics, NI 0.3; (ii) over the dynamic range for normosmics the logarithmic 'distances' required to encompass 90% of the subjects decreased from ~0.85 to ~0.5 unit for O, and from ~1.8 to 0.5 for NI. In contrast, all four anosmics were contained within distances that increased from ~0.05 to ~0.3 with the growth in sensory magnitude; and (iii) threshold estimates averaged (p.p.m.): normals, O/NI 0.3/0.9; anosmics, NI 10.

We conclude that: (i) intra- and inter-subject variation in O sensitivity is considerably less than is commonly assumed; and (ii) normosmic NI perception, in response to stimuli not detected by anosmics, is the result of weak trigeminal stimulation and much greater olfactory activation. Considerable inter-subject variation in NI at these lower levels may reflect individual differences in the integration of olfactory and trigeminal inputs rather than variation in trigeminal sensitivity.

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138. Localization of olfactory brain areas using magnetic source imaging

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It is well established that the primary olfactory cortex projects to a number of neocortical areas, but very little is known about their location and the latencies at which these structures receive their inputs. For localizing these structures we used magnetic source imaging (MSI) with good temporal and spatial resolution.

Ten healthy volunteers (five male and five female subjects; 20–40 years) participated in the experiments. The olfactory stimuli (vanillin and hydrogen sulfide) were delivered within a humidified and temperature controlled constant air flow to the nasal cavity without altering the thermal conditions at the mucosa. The stimulus sequence consisted of 200 ms pulses once every 40 s. Cortical responses were recorded with a 37 channel neuromagnetometer (Krenikon^{R}, Siemens) in a magnetically shielded chamber. Additionally, to compare timing between magnetic and electric responses, olfactory event-related potentials (OERPs) were recorded from the vertex (Cz/A1). The functional MSI information was combined with anatomical data from magnetic resonance imaging.

The peak latencies of the olfactory event-related magnetic fields (OERMFs) corresponded to the ascending and descending slopes of the major electric deflections of the OERPs P1, N1 and P2. At these events we obtained consistent activation of parts of the insular cortex, the superior temporal plane and the superior temporal sulcus which is known for cognitive function. Our results show that cortical structures are activated bilaterally during the first 700 ms after stimulus onset and emphasize the role of the insula and temporal lobe structures in human olfactory function. They are in line with electrophysiological investigations in monkeys and reveal neocortical areas involved in olfactory processing.

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139. Expression of multiple cyclic nucleotide-gated channel genes in the rat olfactory bulb and cortex

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Cyclic nucleotide-gated (CNG) channels are critical components of the signal transduction cascade in olfactory receptor neurons (ORNs), where they mediate convergent second messenger signals induced by both odors and diffusible messengers (Leinders-Zufall *et al.*, 1995). These olfactory channels are distinguished from related CNG channels by the inclusion of particular subunits which confer unique kinetic properties and an increased sensitivity to cyclic AMP. We recently demonstrated that CNG channel distribution is not restricted to sensory neurons, but also includes

many other CNS neurons. This prompted us to examine centers of the olfactory system for the expression of CNG channel genes.

Primers specific for multiple regions of the olfactory receptor and rod photoreceptor CNG channel first subunits were combined with cDNA reverse transcribed from adult rat tissue in a polymerase chain reaction (PCR). PCR amplification with both olfactory and retinal primer sets yielded fragments of expected length, while control reactions excluding reverse transcriptase or cDNA did not. The identities of PCR products were confirmed with Southern blots.

In situ hybridization experiments with digoxigenin-labeled RNA probes for multiple regions of both CNG channel transcripts labeled mitral/tufted, granule, and juxtaglomerular cells of the olfactory bulb and both pyramidal and non-pyramidal neurons in olfactory cortex. At least some of these cell populations appear to express both retinal and olfactory channel subunits.

These findings suggest that central neurons of the olfactory system may use CNG channels to mediate the effects of both cAMP and cGMP, perhaps for functions similar to those described for ORNs. A working hypothesis is that CNG channels transduce signals generated by NO or other diffusible messengers to modulate central processing of odor signals.

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140. The effect of concanavalin A on the odor perception of the living rat

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The initial event in olfaction consists of interactions between an odorant and olfactory receptor neurons. These interactions are transduced into neural excitation patterns characteristic of each odor. Receptor cells have a fairly wide response spectrum; the typical cell is capable of responding to several odorants. The relative degrees of excitation are important in odor recognition. For certain odorants the amplitude of the rat electro-olfactogram, taken from the *in vitro* preparation, is reduced when the olfactory epithelium is treated with the lectin concanavalin A. In the present study it was tested, whether this effect is also shown in the odor perception of living rats. Therefore five 5-month-old and five 20-month-old rats were trained in an olfactory skinner-box (operant conditioning). The animals had to distinguish between either ethylacetate (EA 0.0001 vol%) and clean air or methacrylic acid (MAA 0.0005 vol%) and clean air. The odors were the rewarded stimuli. When the rat mastered the task with a minimum of 90% correct responses–i.e. collecting a reward after odor presentation, no reaction after the presentation of clean air–the olfactory epithelium was treated with concanavalin A (Con A) by intranasal perfusion. The Con A-concentration was 2 mg/ml. Then the odor discrimination tests were repeated. Con A did not affect the response to EA (number of correct responses before Con A treatment: 93.75%, after Con A treatment: 89.58%), whereas the ability to distinguish between MAA and clean air was reduced after treatment with Con A (before Con A: 91.25%, after Con A: 60.4%). In the 20-month-old rats there was a stronger Con A effect on MAA-perception (52.25%) compared with the 5-month-old animals (64.46%). These results suggest, that Con A inactivates one or more types of olfactory receptor molecules, which normally respond to MAA, while the EA-binding receptor molecules are not affected.

141. Amplification properties of amphibian olfactory receptor currents

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In amphibians and in rat, the olfactory receptor current has at least two components. First, cAMP gates ciliary channels that allow an influx of Na^+ and Ca^{2+}. Subsequently, Ca^{2+} accumulating in the cilium can gate a second set of channels, resulting in an efflux of Cl^- that amplifies the receptor current. The unit conductance of the Cl^- channels has been estimated to be just 0.8 pS. It is expected that such small channels should amplify the receptor current without substantially increasing the associated noise. I have verified this experimentally. In isolated frog olfactory cilia, currents were activated by adding cytoplasmic cAMP or Ca^{2+} over a range of concentrations. Ion substitution was used as necessary to distinguish the cationic and Cl^- currents. Steady-state current and current variance (noise) were measured at –40 or –50 mV. Current through the cAMP-gated channels, unblocked by divalent cations, gave variance-to-mean ratios averaging –164 fA. In the presence of external Ca^{2+} and Mg^{2+}, this ratio was reduced on average to –52 fA. Current through the Ca^{2+}-activated Cl^- channels showed the lowest ratio, averaging just –22 fA. It is possible to demonstrate both currents in sequence by adding cAMP to a single cilium. In this case the appearance of the second (Cl^-) phase of the cAMP-dependent current gave a 65% increase in mean current while increasing the variance by just 15%. It is concluded that (i) block by external Ca^{2+} improves the signal-to-noise ratio of the cationic receptor current; and (ii) amplification of this current by the Cl^- channels introduces little additional noise.

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142. Chorda tympani responses under lingual voltage clamp: implications for NH_4-salt taste transduction

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Rat chorda tympani (CT) responses to NH_4Cl, ammonium acetate (NH_4Ac) and ammonium hippurate (NH_4Hp) were studied while the lingual field potential was voltage-clamped. Although functional similarities exist at the level of nucleus tractus solitarius for NH_4^+ and K^+ salts, data reported in this study demonstrate

that differences between responses to both salts are discernable in the CT responses. The data suggest that differences in NH_4Cl and KCl responses result in part from specific shunt permeability changes. The characteristics of the chemically evoked neural responses for NH_4Cl manifest a biphasic non-linear relationship. Below 300 mM NH_4Cl, the CT response increases and then saturates. However, beyond 300 mM the responses again increase. This functional profile is Cl^- dependent and is not observed for NH_4Ac and NH_4Hp. This CT response function might arise from a concentration dependent increase in transepithelial conductance. This explanation was supported by observation. Unlike K-salt responses, Cl^- substitution in the case of NH_4Cl reveals different response properties including: lower threshold and fast onset CT kinetics for NH_4Ac or NH_4Hp, even under positive voltage clamp conditions. Unlike KCl responses, NH_4Cl neural responses are voltage sensitive suggesting a possible involvement of an apical membrane NH_4^+ transducing conductance. Amiloride (100 μM) partially suppresses CT responses to NH_4Cl within the concentration range 50–300 mM (percent suppression: 48–20%). In addition, amiloride treatment decreases the voltage sensitivity of NH_4Cl CT responses. In this regard, NH_4Cl responses are qualitatively similar to NaCl responses, however NH_4Cl voltage sensitivity is significantly less than that of NaCl. The data are consistent with two transduction pathways for NH_4Cl: an apical NH_4^+ ion conductance and one accessible via the paracellular pathway. The latter is especially dominant in the presence of Cl^- and when NH_4^+ concentration exceeds 300 mM.

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143. Effects of portal infusion of hypotonic- and hypertonic solutions on neuronal activity in the rat dorsal motor nucleus of the vagus

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Our previous investigation has shown that stimulation of the hepatoportal osmo (or sodium) receptors by hypertonic saline excited some neurons in the dorsal motor nucleus of the vagus (DMV) that innervate the abdominal viscera. The hepatic branch of the vagus nerve that conveys hepatoportal osmoreceptive signals to the CNS includes two types of neurons: one increases the discharge rate in response to increment of osmolarity in the portal vein; the other increases the discharge rate in response to decrement of osmolarity. However, the studies examined effects of portal infusion of hypotonic solution are few. The present experiment is designed to elucidate the response characteristics of DMV neurons to stimulation of the hepatoportal area by hypotonic solution in addition to hypertonic solution.

Eighty-one neurons that showed antidromic response to the ventral gastric or accessory celiac vagus were recorded in the left dorsal motor nucleus of the vagus (DMV) in urethane-chloralose anesthetized rats. Portal infusion of hypertonic saline (3.6% NaCl) and pure water were applied to those 81 neurons. Sixteen neurons increased their discharge rate in response to both portal infusion of hypertonic saline and water. Portal infusion of 0.9% NaCl produced no change in firing rate. Seven neurons increased their discharge rate in response to portal infusion of hypertonic saline but not water. The other 58 neurons did not respond to these stimuli. Neurons that increased their discharge rates in response to portal infusion of water also responded to portal infusion of hypertonic saline without exception. Jugular infusion of water produced no response. Therefore, the responses to portal infusion of water are derived from activation of the hepatoportal receptors. These results indicate that certain number of DMV neurons responded similarly to both portal infusion of hypertonic and of hypotonic saline. There might exist some reflex arcs that have similar effects induced by both increment and decrement of portal blood osmolarity.

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144. Mammalian olfactory–genetic–neuronal hormonal–behavioral reciprocity and human sexuality

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A five-step pathway that allows the environment to interact with the genetic substrates of mammalian behavior is gene → cell → tissue → organ → organ system. Mammalian pheromones are social environmental stimuli that appear to activate gene expression in gonadotropin releasing hormone (GnRH) neurosecretory cells of hypothalamic and of extrahypothalamic tissue in the brain—an organ that is essential to any organ system involved in behavior.

The integrative multidisciplinary literature review detailed in the text that accompanies this poster illustration supports the diagram of a neuroendocrine sequence that may allow human pheromones to influence human behavior. From this review comes evidence of the following:

The early prenatal migration of GnRH neurosecretory neurons establishes neural substrates. These substrates appear to enable human olfactory pathways to exhibit sexually dimorphic specificity to social environmental chemical stimuli and to exhibit the ability to transduce these chemical signals or pheromones. Human pheromones thereby appear to activate genes in GnRH neurons and to influence GnRH pulsatility and gonadotropin secretion in a manner that is consistent with other mammalian models, as evidenced by reports of menstrual synchrony, entrainment of hormone secretion in couples, coitus-induced ovulation and other phenomena.

GnRH pulsatility directs the concurrent maturation of the neuroendocrine, central nervous and reproductive systems via the hypothalamic–pituitary–gonadal and hypothalamic–pituitary–adrenal axes. Pheromonally induced alterations in GnRH pulsatility allow for a life-long causal linkage between human pheromones, olfaction, neurotransmission, autonomic responses, luteinizing hormone/follicle stimulating hormone ratios, steroidogenesis, synaptogenesis, synaptolysis, apoptosis and hormonally induced behavioral changes. Thus, via a conditioned stimulus–response cycle common to many species, human pheromones

appear to be the most likely link between the 'nature' and the 'nurture' of human reproductive sexual behaviors and of other behaviors.

145. Taste responses from the chorda tympani nerves in young and old SHR-SP rats

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To determine whether neurophysiological taste responses are different in young and old rats, recordings were made from the whole chorda tympani nerve. Female SHR-SP rats (stroke-prone spontaneously hypertensive rats) in two age groups were studied: 2–3 months and 11–13 months. Chemical stimuli included single concentrations of NaCl (0.1 M), KCl (0.1 M), sucrose (0.5 M), quinine hydrochloride (0.02 M), HCl (0.01 M), monosodium glutamate (MSG, 0.01 M) and L-glutamic acid (L-Glu, 0.01 M), and a concentration series (0.1 mM to 0.2 M) of NaCl solutions. Neural response magnitudes were calculated by dividing integrated response by spontaneous activity that precedes the response. Substantial neural response were obtained to all chemicals at both ages. Responses to KCl, sucrose, quinine hydrochloride, HCl, monosodium glutamate (MSG) and L-glu did not change, but the response to NaCl changed significantly with age. There were differences in the shapes of the response/concentration functions for NaCl with age, too—a much more abrupt response curve was observed in the young group than in the old one. In other words, the responses to the more concentrated solutions of NaCl than 0.1 M in the old rats decreased significantly compared with young rats. This result suggests that old SHR-SP rats cannot accurately recognize the difference in magnitude of highly concentrated NaCl solutions.

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146. Olfactory cell cultures, bulb tissue and mucosal tissue contain mRNA for the long and short forms of the D2 dopamine receptor

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Olfactory sensory neuron (OSN) axons transfer information to the olfactory bulb (OB) neurons. One subset of the OB neurons contacted, dopamine-producing interneurons, may affect this transfer by presynaptic modulation. The radiolabeled dopamine receptor (DAR) ligand, 3H spiperone, binds to only those regions of the adult rat OB that contain OSN axons. DARs increase the ability of the OB neurons to discriminate between odorants, based on effects observed after systemic application of spiperone. The effects of dopamine on OSNs could be further explored *in vitro*, but only if cultured OSNs express dopamine receptors. Pixley has developed a dissociated culture system (from newborn rat tissues) in which OSNs are generated. Functional studies may be aided by knowledge of the subtypes of DAR that are expressed by the OSNs. The subtypes are grouped into 'D1-like' DARs (D1 and D5) and 'D2-like' DARs (D2, D3, D4). The gene coding for the D2 DAR is alternatively spliced to yield a D2-long and a D2-short form. Using the reverse transcriptase polymerase chain reaction, mRNA for the D2-long, and in most cultures, the D2-short DAR subtypes was detected in 15 day, OSN-containing cultures. Olfactory mucosa and olfactory bulbs from newborn rats also contained the mRNA for the D2-long and D2-short DARs. Future studies will aim to explore cell localization and other DAR subtypes.

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147. Characterization of chloride channels in olfactory nerve axon membranes of the garfish, *Lepisosteus platostomus*

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The axons of olfactory receptor neurons are small diameter, unmyelinated nerve fibers (C fibers) which relay sensory information from the soma to the olfactory bulb. Their sub-micron diameter prevents the use of standard electrophysiological techniques such as intracellular recording or patch clamping to study axonal ion channels. Using the garfish olfactory nerve, we have begun to characterize the ion channels in olfactory nerve axons by reconstituting the channels into planar lipid bilayers.

Animals were killed by an approved protocol and olfactory nerve axon plasma membrane vesicles prepared. Planar bilayers were formed using a 2:1 mixture of the phospholipids, PE and PS.

We observed, in symmetrical 130 NMG-Cl, four types of Cl channels; none were Ca-dependent. Two types of channels had conductances of 18–25 pS and were DIDS-sensitive. One was linear and the other was outwardly rectifying. The third type of Cl channel had a conductance of ~122 pS, was linear, had substates, and was DIDS-insensitive. The fourth had a linear conductance of ~440 pS, had substates, and was DIDS-insensitive. These results indicate the presence of several Cl channels in the garfish olfactory nerve axons.

Characterization of ion channels in olfactory nerve axons provides a basis on which to understand the nature of excitability and possible modulation in these fine nerve processes.

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148. Olfactory thresholds in old aged Wistar rats

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In humans, experiments assessing threshold sensitivity have all demonstrated significant impairment in olfaction in old age. The causes for such age dependent decreases in olfactory sensitivity are not well understood. To test whether there are similar age related changes in olfactory sensitivity in rats, which might be used for further analyzing this open question, we established the thresholds of six subadult (2 months old at the beginning of testing) and six old (19 months) male Wistar rats. Ethylacetate was used as the solvent. The experiments were performed in an olfactory skinner box using operant conditioning. Before testing for olfactory thresholds was started, all animals were first familiarized with the experimental procedure and the individual learning speeds in the olfactory skinner box was established. In an additional experiment the learning speeds of two 32-month-old rats were tested. These additional experiments revealed no age related decrease in the learning ability.

In initial training a 0.1% (of vapor saturation) ethylacetate served as the positive stimulus, and air served as the negative stimulus. If performance accuracy was 85–100% for each animal, subsequent dilutions were made between 0.5 and 0.1 log unit steps. The olfactory threshold was defined as the lowest concentration in which performance of at least 75% correct responses was achieved. In all six young rats (age at the time of threshold establishment: 5 months) thresholds showed little individual differences and ranged between 3×10^{-6} vol% and 2×10^{-6} vol%. The thresholds for the old rats (age 31 months) were enormously wide spread ranging from 1×10^{-3} to 7.2×10^{-6} vol%. The difference between the two groups is statistically significant with $P < 0.001$. We, therefore, not only found an age dependent decline in the olfactory sensitivity, but also an increased variability in the thresholds in the old aged rats (similar to that described in humans).

149. Different parts of the diagonal band nucleus project to different layers of the olfactory bulb in the rat

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Projections to the main olfactory bulb from different brain structures are a part of the servomechanism of the olfactory system. The mode of centrifugal influences on intrabulbar processing is dependent not only on the origin of bulbopetal axons in the brain and the neurotransmitters used, but also on the sites of their termination. Cholinergic and GABAergic axons reach the olfactory bulb from the ipsilateral nucleus of the horizontal limb of the diagonal band (NHDB). Within the nucleus, cholinergic bulbopetal neurons are found mainly in its medial part, whereas those GABAergic are concentrated in the lateral part of the NHDB. The aim of our experiments was to localize, at the light microscopic level, termination fields of axons projecting to the rat olfactory bulb from medial and lateral subdivisions of the NHDB. Each rat received pressure injections of 10% biotin dextran amine (150 nl) into the medial (left hemisphere) and lateral (right hemisphere) parts of the NHDB through a glass pipette (tip diameter 40 µm). Following a 9–14 day survival period, specimens were transcardially perfused with saline and fixative, the olfactory bulbs and injected hemispheres were cut with a cryostat, and 30 µm transverse sections were processed in order to visualize the tracer. Varicose terminals of Anterogradely labeled axons, originating from the medial part of the NHDB, were found to surround olfactory glomeruli, whereas those arising from the lateral part of the NHDB were distributed in the granule cell layer. The results suggest that, in the rat, projections from different parts of the NHDB terminate in different layers of the olfactory bulb. Thus, cholinergic and GABAergic centrifugal axons may modulate different stages of signal processing in the olfactory bulb.

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150. Immunocytochemistry of taurine in the frog, rat and human olfactory bulb

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Taurine is one of the most abundant free amino acids in the brain and possesses multiple actions, including influences on brain development and differentiation, osmoregulation, chemical synaptic transmission, phosphorylation of membrane proteins, and activity of Ca^{2+} channels. The olfactory bulb contains the highest level of taurine in the brain but, despite this fact, little information is available about the cellular localization of taurine in this structure. The aim of the present study was to localize taurine in the frog, rat, and human olfactory bulb using immunocytochemical techniques. Light microscopic experiments were carried out using avidin–biotin and peroxidase–antiperoxidase methods; the postembedding immunogold technique was applied for electron microscopy. The following taurine-immunoreactive elements are found in the olfactory bulb at the light microscopic level: (i) olfactory receptor cell axons in the peripheral layers; (ii) short fragments and crossed cell processes (punctate structures) in all layers of the olfactory bulb; and (iii) a few cell bodies, which may be small neurons and/or glial cells, in the glomerular and external plexiform layers and more cell bodies in the granule cell layer. The rat and human olfactory bulbs have similar distribution of immunoreactivity, with much higher concentration of immunostained puncta in the rat external plexiform layer. At the electron microscopic level, taurine-immunoreactive cell bodies are observed in the frog's granule cell layer. These somata are either neurons or glial cells, and their morphological features allow to identify short-axon cells and oligodendroglial cells. The sites of biosynthesis and function(s) of taurine in the olfactory bulb remain to be investigated.

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151. A test for regional evaluation of taste function

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We developed a testing technique to quickly and accurately assess regional deficits in taste function. Four areas of the tongue are tested using 25 µl drops of suprathreshold solutions of sucrose, citric acid, NaCl and caffeine. The stimuli are applied using an Eppendorf™ pipette to specified right and left regions of the anterior tongue (CN VII), and to right and left regions of the posterior tongue (CN IX). Two presentations of each solution are made, in randomized order, to each of the four tongue regions, resulting in a total of 32 trials. Subjects are required to point to a response for each trial on a chart labeled 'sweet', 'sour', 'salty' and 'bitter', while their tongue remains extended to prevent solution contact with other tongue regions. Responses are forced-choice and patients are required to rinse their mouths with deionized water between trials.

Split half reliability for this test was determined using a sample of 369 subjects from the University of Pennsylvania Smell and Taste Center. Spearman–Brown corrected correlation coefficients for the total test and for each of the four test solutions are as follows: total test, 0.881; sucrose, 0.803; citric acid, 0.785; NaCl, 0.762; and caffeine, 0.757. This test allows for the assessment of regional taste functioning within a relatively short period of time. Issues of quality confusion and clinical correlations with regional identification deficits will be discussed.

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152. Influence of age on regional taste function

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Using a regional testing technique, taste function with respect to age was studied for identification of sucrose, NaCl, citric acid and caffeine. A total of 708 subjects from the University of Pennsylvania Smell and Taste Center, ranging in age from 15 to 84 years, were administered randomized trials of suprathreshold solutions to right and left anterior (CN VII) and right and left posterior (CN IX) tongue regions. Subjects provided forced-choice responses to each stimulus by indicating 'sweet', 'salty', 'sour' or 'bitter'. Logistic regression analysis revealed a steady age-related decline in correct identification of each stimulus type. Regional comparisons showed higher anterior than posterior scores for sucrose and NaCl at all ages. NaCl identification performance, however, declined at a faster rate posteriorly than anteriorly with age. Conversely, citric acid and caffeine scores were higher posteriorly than anteriorly, with both declining at a faster rate anteriorly than posteriorly with age. Women outperformed men for each solution at most ages. This study supports the perspective that sweet and salty identification is better anteriorly than posteriorly and sour and bitter identification better posteriorly than anteriorly. In addition, region-specific changes in tastant identification ability as a function of age are demonstrated.

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153. Characterization of *smell impaired* genes of *Drosophila melanogaster*

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We measured avoidance responses to benzaldehyde of 379 inbred lines of *Drosophila melanogaster*, containing single *P*-element (*P[lArBJ*) insertions. We identified 14 lines in which insertion of the transposable element resulted in aberrant olfactory behavior. *In situ* hybridization to polytene larval salivary chromosomes mapped these *P*-element insertions to different locations on the second and third chromosomes. To name these olfactory loci we adopted the nomenclature *'smell impaired (smi)'* followed by their chromosomal band designations. The paucity of candidate genes at these locations suggest that most, if not all, of our *smi* loci represent novel olfactory genes. Expression of 10 of these genes is controlled by olfactory tissue-specific promoter elements, as evident from the expression of the *lacZ* reporter gene of the *P[lArB]* construct. In addition, four of the *smi* mutants show a sexually dimorphic phenotype with larger impairment in the female.

P[lArB]-tagged DNA sequences were rescued as inserts in pBluescript after digestion of genomic DNA with HindIII. *In situ* hybridization of these inserts to polytene chromosomes from the *P*-element-free host strain and Southern blot analysis verified that they indeed derive from the original *P[lArB]* insertion sites. Whereas most rescued DNA fragments hybridize to a single chromosomal band, two *smi* sequences revealed additional hybridization sites. *P[lArB]*-tagged DNA from line *smi97B*, which shows major olfactory impairment and reporter gene expression in a restricted region of the antenna, hybridizes to several chromosomal bands in addition to band 97B, suggesting a gene family. DNA rescued from line *smi79E* hybridizes extensively to heterochromatin in addition to multiple euchromatic bands. *P[lArB]*-tagged DNA fragments rescued from the *smi* lines are currently used to identify and characterize mRNA transcripts from a *Drosophila melanogaster* head cDNA library. Finally, reversion of the *smell impaired* phenotype through excision of the *P*-element verifies that aberrant olfactory phenotypes indeed result from *P*-element insertions.

154. Relative odorant identification in the evaluation of hyposmia

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Tests to positively identify the causative factors in hyposmic patients have not yet matched the accuracy of those for dysfunctional patients in vision and audition. Chemosensory clinicians and researchers have difficulty in making even basic differentiations such as conductive versus sensorineural problems. The odorant confusion matrix (OCM), developed by Wright shows promise for answering this question. It has previously been reported that patients with olfactory losses due to head trauma, polyposis and colds produced unique OCM patterns of odorant identification and mis-identification. More recently, it has been suggested that odorant identification during a cold was a function of perceived odorant strength and odorant solubility. Strong odorants with low water solubility (mothballs) were much better identified than weak odorants with high water solubility (cinnamon).

A further examination of people with colds, the differential identification of mothballs/cinnamon is dependent on overall percent correct. That is, the smell of Mothballs is much better identified than cinnamon when the overall percent correct is between 40 and 80. At higher and lower percent correct, this effect tends to disappear. A similar effect is seen in patients with polyposis, who, in a small sample, consistently showed the mothballs/cinnamon differential. However, other disorders which might also be expected to show an olfactory loss based on congestion do not appear to show as robust a differential between mothballs and cinnamon. This later observation may, in part, be due to the presence of complicatings factors which often accompany the presentation of olfactory loss.

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155. Birth and death of olfactory receptor genes: lessons from large scale DNA sequencing in the human olfactory sub-genome

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The olfactory receptor (OR) gene superfamily occupies many clusters on several chromosomes, collectively comprising the human olfactory sub-genome. In evolution, the OR gene repertoire may have expanded by gene duplication. It may have also diminished by the elimination of OR genes or by their mutation into pseudogenes. The latter process, when manifested as inter-individual polymorphisms, is likely to be a source of specific anosmia. One of the OR gene clusters, on human chromosome 17 (17p13.3) has been described in detail (Ben-Arie *et al.*, 1994). We have now determined the full DNA sequence of a 40 kb cosmid (#39) at the center of this OR gene cluster. This was accomplished using shotgun subcloning, automated fluorescence-based sequencing, and computer assembly by the Sequencher software. Extensive computer analysis provided clear-cut examples of genomic reorganization within the cluster. It revealed an unexpected OR pseudogene (OR17–25) fused to a previously identified OR coding region (OR17–24). This gene doublet probably derived from illegitimate recombination between non homologous regions of two OR genes, resulting in the inactivation of one or both of them. Similar genomic rearrangements among visual opsin genes have been previously shown to result in color blindness. A second striking feature is the ancient duplication of a 14 kb segment, including a full OR gene, that may have been mediated by recombination between repetitive genomic DNA sequences of the MIR family. This led to the formation of two seemingly functional OR genes (OR17–40 and OR 17–228) that belong to the same subfamily. Such an event could be typical of the process by which the OR repertoire has expanded in mammals. The results also suggest that a functional OR gene may be ~14 kb long, containing 13 kb of non-coding sequences.

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156. Efferent projections of the main and accessory olfactory bulbs in the snake *Thamnophis sirtalis*

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Although the efferent projections of the main and accessory olfactory bulbs of snakes were the subject of previous studies using the lesion and degeneration technique, few recent studies have corroborated these data with the new and more sensitive tract-tracing techniques. We have reinvestigated the connections of the main and accessory olfactory bulbs in the snake *Thamnophis sirtalis* using the modern anterograde tracer biotynilated dextran amine (BDA). The main olfactory bulb (MOB) projects, as previously described, ipsilaterally to the olfactory tubercle and the olfactory gray, and bilaterally with ipsilateral predominance to the entire lateral cortex. This contralateral projection courses via the stria medullaris and crosses the midline through the habenular commissure. We also found an ipsilateral medial projection to the medial and dorsomedial retrobulbar formation, and a bilateral projection to the rostral amygdala.

The accessory olfactory bulb (AOB) projects, as previously described, to the ipsilateral hilus of the nucleus sphericus. We also found a projection to the ipsilateral nucleus of the accessory olfactory tract.

In addition to the previously described projections in snakes, our results reinforce the previous data from other reptiles concerning the presence of an ipsilateral medial projection from the MOB, a bilateral projection from the MOB to the rostral amygdaloid complex, and an ipsilateral projection from the AOB

to the amygdaloid formation. Apparently, there is no convergence of the two chemosensory systems in their secondary projections to the telencephalon.

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157. Alterations in geniculate ganglion protein synthesis following early postnatal receptor damage

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Damage produced to fungiform papillae of the anterior tongue in rats aged postnatal age 2 (P2) alters the development of gustatory terminal fields in rostral and intermediate divisions of the nucleus of the solitary tract (NST). We have shown that P2 damage alters concentrations of several specific neural proteins such as GAP-43, NF-160 and NF-200, glial proteins such as GFAP, and 14 additional proteins in the geniculate ganglia following P2 receptor damage. The present studies were conducted to determine whether early fungiform receptor damage alters protein synthesis in the geniculate ganglion. *In vitro* labeling with [^{35}S]L-methionine and conventional electrophoretic analyses of RNA were used to study protein synthesis in the geniculate ganglion following P2 receptor damage. Protein synthesis and RNA content were studied at various times following P2 damage. Additionally, synthesis was studied in rats that received late receptor damage at P40, because this damage occurs after critical periods of NST terminal development. A total of 55 proteins were analyzed in all groups; 21 of these proteins are preferentially synthesized in ganglion neurons. Preliminary results show both increases and decreases in neural protein synthesis (e.g. GAP-43; NF-160; NF-200) and increases in glial protein synthesis (e.g. GFAP; MBP) following P2 receptor damage, and these changes are related to the age at which synthesis is evaluated. Alterations in synthesis observed following P2 receptor damage can be distinguished from alterations observed following P40 damage. Following P2 damage concentrations of precursor and subunit rRNA (32S, 20S, 28S and 18S) increases in geniculate ganglia, however no reliable changes have been confirmed for tRNA. These results confirm that P2 damage affects protein synthesis pathways in both neurons and glia of the geniculate ganglion.

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158. Discrimination ability of squirrel monkeys for structurally related odorants

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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 esters. This group of substances comprises odorous compounds predominant in a variety of tropical fruits and thus is presumably of biological significance for these frugivorous primates.

Five squirrel monkeys were trained to distinguish isoamyl acetate (S+) from *n*- and iso-forms of other acetic esters (ethyl acetate to decyl acetate) and from other esters carrying the isoamyl group (isoamyl propionate to isoamyl capronate) as S–. Their performance in these tasks was compared with their discrimination ability in a control task [isoamyl acetate versus (–)-carvone] which was interspersed between the critical trials.

We found that all five 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 with the control and all other tasks: two of these (isobutyl acetate and isoamyl propionate) are direct neighbours of isoamyl acetate in the corresponding homologous series suggesting that carbon chain length may be correlated with perceived odor similarity, whereas the third odor (*n*-amyl acetate) is an isomeric form of the S+ suggesting that the two odorants were perceived as qualitatively similar despite different steric conformation of a functional group.

The results of this study provide evidence of well-developed olfactory discrimination ability in squirrel monkeys for aliphatic esters and support the assumption that human and nonhuman primates may share common principles of odor quality perception.

159. Context effects and the labeled magnitude scale

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The labeled magnitude scale (LMS) is a verbally anchored (quasi-logarithmically spaced) response scale. Due to its anchoring to common experience the LMS may facilitate comparisons between individuals of absolute sensory magnitudes. Three experiments examined whether the labeled magnitude scale showed context effects similar to those found with magnitude estimation and category scales. Two versions of the LMS were used, one anchored at the high end to the strongest imaginable sweetness and the other to the strongest imaginable oral sensation. In a simple contrast experiment, subjects judged the sweetness of a 10% sucrose fruit beverage in the context of a less sweet (5%) beverage or a more sweet (20%) beverage. Consistent with previous literature, the sweetness was judged more intense in the low context and less intense in the high context, for all scaling methods. In a second experiment, this effect persisted (although was smaller)

when the contextual item preceded the to-be-rated item, a so-called 'reversed pair' design. Once again, the effect was highly significant for all scaling methods. In a third experiment, the range effect was examined using wide and narrow ranges of concentration. Psychophysical functions were flatter in a wide context and steeper in a narrow context, consistent with previous observations on range-mapping bias. This result obtained for all scales. In three common contextual effects, the labeled magnitude scale behaved similarly to other scaling procedures. Its application to comparisons across individuals may be limited if those individuals have different experiential contexts within which they make their judgements.

160. Cyclic nucleotide-induced calcium transients in individual cilia and dendrites of salamander olfactory receptor cells

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Ca^{2+} plays an important role in adaptation of olfactory receptor neurons (ORNs) but its spatial and temporal dynamics in the transduction process are not well understood, mainly because of the inaccessibility of the cilia to imaging techniques. This prompted us to investigate intracellular Ca^{2+} signals in salamander ORNs using the Ca^{2+} indicator dye fluo-3AM (20 μM) and confocal imaging. To exclude movement artifacts from our measurements, the cells including the cilia were firmly attached to the substrate. Cells were always held in 4 μM TTX to prevent Ca^{2+} fluxes due to Na^+ channel mediated depolarization. At rest the fluorescence was generally low and appeared uniform except for various discrete subcellular structures. Individual cilia could often be clearly resolved. Stimulation of the cyclic nucleotide pathway with a one-minute application of IBMX (500 μM) caused a transient increase of the fluorescence signal in individual cilia with a peak after 8–10 s. The ciliary signal declined back to baseline levels after 20 s, whereas fluorescence signals remained high in the dendrite and cell body throughout stimulation. Temporal analysis revealed that the ciliary Ca^{2+} signal preceded the signals in the dendrite and soma, ruling out the possibility that Ca^{2+} backpropagated into the cilia from the knob. A similar response was found using 8-br-cGMP (100 μM). With lower concentrations of 8-br-cGMP (1 μM) the increase of the Ca^{2+} signal was restricted to the cilia and the knob of the ORNs. LY83583, a blocker of cyclic nucleotide-gated (CNG) channels, also blocked the increase in fluorescence induced by 8-br-cGMP, indicating that Ca^{2+} influx through the CNG channels represented the main source of the ciliary Ca^{2+} signal. In contrast, depolarization of the cells with high K^+ solutions induced very different intracellular Ca^{2+} responses. Directed at the cilia, 20 mM or 120 mM K^+ failed to elicit a significant Ca^{2+} response. These data demonstrate that we now can resolve Ca^{2+} signals in individual olfactory cilia due to stimulation of the cyclic nucleotide pathway. We therefore anticipate that odor-induced ciliary Ca^{2+} transients can soon be measured and experiments toward this goal are underway.

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161. Modulation by cyclic cGMP of the odor sensitivity of vertebrate olfactory receptor neurons

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Knowledge of olfactory transduction has become more complex than previously expected and much interest has recently focused on multi-second messenger systems in the olfactory receptor neurons (ORNs). Our finding that carbon monoxide (CO) acts as a potent stimulator of cGMP formation in salamander ORNs (Leinders-Zufall *et al.*, 1995) has led us to suggest a functional role of the CO/cGMP pathway in olfaction.

We have therefore tested the effects of exogenous cGMP and CO on odor responses of salamander ORNs. We found that cGMP modulates the odor sensitivity of ORNs ($K_{1/2}$ = 0.46 μM) and that CO simulates the effect of cGMP ($K_{1/2}$ = 1 μM). Sensitivity modulation is achieved through a regulatory chain of events in which cGMP activates a persistent background current carried through cyclic nucleotide-gated (CNG) channels. This in turn leads to sustained Ca^{2+} entry providing a negative feedback signal. One consequence of the Ca^{2+} entry is a shift to the right of the stimulus–response curves of odor currents and a reduction in maximum current at saturating odor concentrations. Thus, cGMP (through Ca^{2+} entry) functions to control the gain of the G-protein coupled cAMP pathway. This effect is in part reminiscent of the effect of Ca^{2+}-calmodulin on CNG channels in excised inside-out membrane patches (Chen and Yau, 1994). The effect of CO/cGMP is long-lasting and can continue for min after recovery of the primary odor response. The described mechanism helps to prevent saturation of the cell's response by adjusting the operational range of the cAMP second messenger cascade. The cAMP-mediated sensory generator current can be reduced by up to a 20-fold as calculated from the Hill coefficients.

These properties of the effects of CO/cGMP suggest a functional role in sensory adaptation of ORNs. A series of further experiments has identified a long-lasting form of adaptation that is indistinguishable from the effects of exogenous application of CO or cGMP. Long-lasting adaptation appears to have some properties similar to those underlying synaptic plasticity in central neurons.

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162. Intranuclear processing of gustatory information in the parabrachial nucleus of the pons

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Taste-responsive units in the parabrachial nucleus of the pons (PbN) can be classified into groups based on their response profiles, i.e. the relative response rates across taste stimuli. Because these groups appear to be well-defined, this type of classification scheme has been used to explain taste coding in this structure. However, although several studies have shown that response profiles may change under various conditions, the synaptic mechanisms that underlie these changes remain a mystery. In this context, the present study was designed to explore the stability of response profiles of taste units in the PbN following adaptation of the tongue to one of the prototypes of the four basic taste qualities (salty, sweet, bitter or sour). Electrophysiological responses to NaCl (0.1 M), sucrose (0.5 M), quinine HCl (0.01 M) and HCl (0.01 M) were recorded using a multi-wire array of tungsten microelectrodes (115 μm spacing). Responses to each tastant presented individually were measured initially. A target stimulus was then chosen 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. Cross-correlational analyses applied to the taste-evoked spike trains that have been recorded thus far have provided evidence of common input to pairs of PbN units. These data have demonstrated that PbN units with nearly identical response profiles and which receive common input nevertheless show different patterns of responsiveness across the various tastants following the adaptation procedure. This implies that the adaptation procedure is a useful technique for revealing differences among taste units in the PbN that would otherwise go undetected by traditional classification techniques. Further analyses will be directed at the description of synaptic interactions among PbN taste units and their role in the neural processing of gustation.

Sponsored by a grant from the Whitehall Foundation to P.M.D.

163. Clinical management of olfactory complaints in the late 19th century

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The last half of the 19th century produced an explosion of scientific and biologic investigation in Europe, mostly in Germany. To the existing knowledge of the upper nasal anatomy and cranial course of the olfactory nerves, anatomists added the detailed microscopic structure of the olfactory mucosa which would remain unchallenged until well into the 20th century. A basic clinical understanding of the physiology of olfactory function was also obtained. They understood that odorants were small particles which entered the nose with the airstream. A small percentage of this airstream went to the olfactory area along the roof of the nasal cavity. They also knew that the odorants needed to dissolve in the nasal mucus before interaction with the olfactory receptors could occur.

Based on this information, clinicians separated chemosensory receptions into taste, 'common sensation' (fifth cranial nerve) and smell (first cranial nerve), with separate neural input for each modality. In addition, they categorized the types of olfactory loss they saw into the following groups: dry mucosa (too little mucus), mechanical obstruction of olfactory airflow (such as with too much mucus, polyps, tumors or foreign bodies), congenital, loss of pigment (such as in Albinism), head trauma, excessive olfactory stimulation, olfactory nerve inflammation, tumors near the olfactory nerves, and disease of the central olfactory centers. Treatments included general remedies like '...nasal douches, sprays, and fumigations'. Specific therapies included inhaled strychnia powder, subcutaneous morphia, iodide of potassium (especially for syphilis) and direct current electrical stimulation. Thankfully some authors recommended no specific therapy.

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164. The role of chemical signals in the foraging behavior of the sea star *Asterias forbesi*

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The use of chemoreception in asteroids to locate prey in the littoral zone, while assumed, is not clearly understood. Utilization of chemical stimuli during foraging by the Forbes sea star, *Asterias forbesi* has yet to be definitively demonstrated. The experiments presented here were designed to determine the function of chemical signals in orientation to prey and the attraction or avoidance by the sea star to tissue and prey items. Behavioral assays were performed using various types of food items in a non-flow tank. Squid tissue was presented as a general food stimulus. Both living and freshly broken clams and mussels were used in trials as natural prey items. Turn angles, headings, average walking speed, and net to gross ratios of path length were compared between trials. The results show that odors from all tested potential food sources are attractive to *A. forbesi*. The walking behavior of the sea star is significantly altered by the presence of a chemical stimulus. The average walking speed decreased when the sea star was presented with a food item. Simultaneously, the path of the sea star was straighter in the presence of food, as determined by a ratio of the net distance traveled and the total path length. The walking behavior of *A. forbesi* significantly differs in the presence of prey, by slowing and walking a straighter path. Thus, it has been determined that *A. forbesi* uses chemical signals to detect prey in the absence of flow. Also, the sea star is able to use these chemical signals to locate the prey.

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165. Entry of aluminum into the CNS from inhaled soluble particles

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Olfactory receptor neurons have dendrites (cilia) in the nasal lumen and send axons through the cribriform plate to synapse within glomeruli of the olfactory bulb. This direct contact between the CNS and external environment has been hypothesized as a direct portal of entry to the CNS for inhaled toxicants. Because no evidence suggests that everything inhaled enters the CNS via this route, we have hypothesized a 'nose–brain barrier' that protects the CNS much as the blood–brain barrier protects from systemic toxicants. Previous work indicates direct entry of inhalants via this route exists, but the incidence is not well defined, nor are the properties of either inhalants or the epithelium that might affect entry well characterized. Our current research focus is to determine (i) the environmental relevance of this phenomenon; (ii) what properties of the olfactory epithelium control CNS entry; and (iii) what properties of inhalants control entry. We are examining the frequency of CNS entry following inhalation of relatively low concentrations of either soluble aluminum chlorhydrate particles or insoluble aluminosilicates. In two replications of 9 day inhalation exposures to aluminum chlorhydrate aerosols, 25% of exposed rats had elevated levels of aluminum in the olfactory bulbs. Rats with the olfactory epithelial lesions induced by methyl bromide pre-exposure did not differ from sham-exposed rats in bulb aluminum content following aluminum exposure. Olfactory bulb aluminum was independent of the concentration in the olfactory mucosa, and the presence of aluminum was often unilateral. These results indicate that aluminum inhaled as soluble particles can enter the CNS via this route, and that intact olfactory neurons are necessary for entry. Individual differences in the olfactory mucosa not readily observable at the light microscope level may result in the phenomenon occurring in a small, but consistent percentage of animals in a given exposure. Exposure to insoluble aluminosilicates is in progress.

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166. Mutual inhibition between sucrose and quinine or denatonium in cells of the hamster solitary nucleus

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Previous electrophysiological studies have shown that sucrose responses in cells of the parabrachial nucleus (PbN) are suppressed by the application of quinine hydrochloride (QHCl) to the anterior tongue. Often, when PbN cells are responsive to QHCl, application of sucrose suppresses this response. Recent electrophysiological data demonstrate a suppressive effect of QHCl on sucrose responses in sucrose-best chorda tympani fibers. Such a result implies a peripheral effect of QHCl, which could be due to its ability to block K^+ channels on the taste receptor cell. However, it is not clear that such an effect accounts entirely for the suppression seen in central taste neurons, nor could it explain the reciprocal effect of QHCl suppression by sucrose.

We have previously demonstrated that microinjections of GABA into the nucleus of the solitary tract (NST) have a profound suppressive effect on responses to sucrose. In order to investigate the possible role of GABAergic inhibition in the mutual suppression seen between sucrose and QHCl, we have initiated a study of this inhibitory interaction at the level of the NST. Cells were recorded extracellularly while the tongue was stimulated with 0.1 M sucrose, QHCl, NaCl or caffeine, 0.01 M citric acid or denatonium benzoate, and mixtures of sucrose with QHCl, denatonium or caffeine. Of these bitter stimuli, QHCl was the most effective, followed by denatonium; caffeine was not an effective stimulus. Robust responses to sucrose were often inhibited by QHCl and sometimes by denatonium, but not by caffeine. Responses to QHCl or denatonium were often inhibited by sucrose. Thus there is mutual inhibition between sucrose and these bitter stimuli. We are continuing these experiments using microinjection of GABA antagonists to study the role of brainstem inhibitory mechanisms in this gustatory interaction.

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167. Responses to monosodium glutamate and amiloride occur in single rat fungiform taste cells

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Monosodium glutamate (MSG) elicits umami taste. However, the mechanisms involved in the transduction of MSG are largely unknown. We examined the responses of isolated rat fungiform taste cells to MSG by patch-clamp and Ca^{2+}-imaging techniques. In whole cell voltage-clamp configuration (holding potential = –80 mV), bath application of 1 or 10 mM MSG induced three type of responses: a decrease in inward holding current; an increase in inward holding current and a biphasic response, with an initial increase followed by a sustained decrease in holding current. These data are consistent with previous studies of rat (Chen and Yau, 1994) and mice taste cells (Hayashi *et al.*, 1996), suggesting that MSG alters membrane conductance. Data from Ca^{2+}-imaging with fura-2 indicate that MSG increases the intracellular Ca^{2+} level in 31 of 64 cells tested. Since responses to MSG are enhanced by the presence of Na^+ salt (Ugawa and Kurihara, 1994), we examined the possibility that amiloride-sensitive Na^+ channel and glutamate receptors are colocalized to the same fungiform taste cells. A total of 96 taste cells have been tested with both amiloride (10 μM) and MSG. Of these, 55 cells (57%) responded to both amiloride and MSG, 26 cells (27%) had no response to either, 12 cells (12%) responded to amiloride only and three cells (3%) responded to MSG only. These results suggest that Na^+ may interact with the glutamate transduction mechanism.

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168. Ultrastructural analysis of circumvallate and fungiform taste buds from cross-reinnervated rats

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Previous studies suggest that differences in synaptic structure and taste bud morphology exist between circumvallate and fungiform taste buds in rodents. We are attempting to elucidate whether these differences are determined by the nature of the gustatory tissue or by the type of innervation. To test this hypothesis, we have carried out a cross-reinnervation study in which the IXth and VIIth nerves have been cut and cross-reinnervated in rats. Experimental data, normal control data, and data from taste buds in which the IXth and VIIth nerves were cut and the native stumps reattached, were characterized and compared by light and transmission electron microscopy. Using standard ultrastructural criteria, we identified electron-lucent type I, electron-dense type II and intermediate cells in normal taste buds. Taste buds in which the nerves had been cut and the native stumps reattached were similar in appearance to normal control taste buds. Normally, circumvallate type I taste cells contain apical dense-cored granules, while fungiform type I cells do not. In cross-reinnervated taste buds, the presence of apical dense-cored granules in circumvallate type I cells remained unchanged. There were, however, significant structural differences between the taste buds of normal and cross-reinnervated animals as well. Circumvallate taste buds reinnervated by the VIIth cranial nerve were smaller, had a distended appearance, and contained a cell with round nuclei and distinct, swollen electron-lucent vesicles adjacent to the cell membrane. Compared to controls, cross-reinnervated circumvallate taste buds contained significantly fewer nerves. Fungiform taste buds that were reinnervated by the IXth cranial nerve appeared to be in an 'atrophic' state, with taste pores lacking in most cases. Type I, type II and intermediate cell types were present. Nerve processes were large and abundant. Based on the results of this study, we propose that both the nature of the tissue and the type of innervation determine taste bud morphology.

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169. Rapid and accurate measurement of taste and smell thresholds using an adaptive maximum-likelihood staircase procedure

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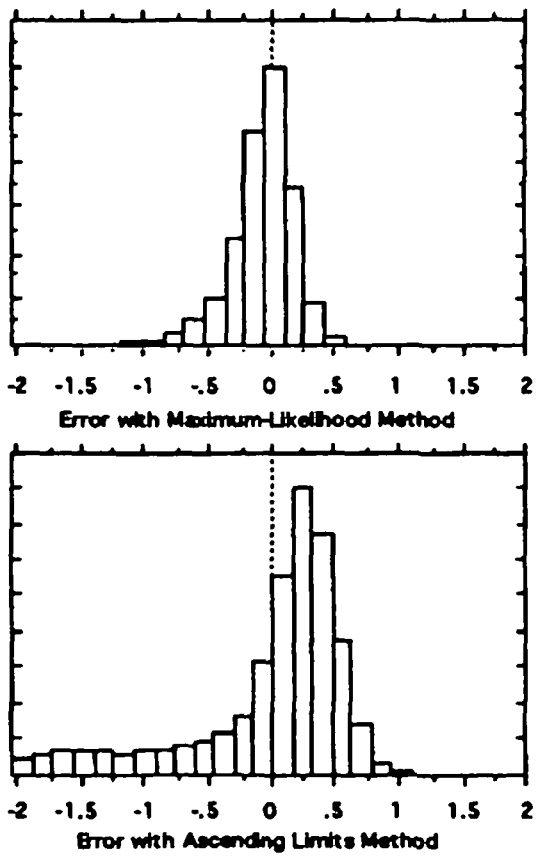
Compared with vision and audition, presentation of gustatory and olfactory stimuli require much more time per trial when measuring psychophysical thresholds. It is therefore important, especially with the testing of clinical patients, to use psychophysical methods that give the highest accuracy for the fewest number of trials. We have combined a maximum-likelihood method, originally developed in audition and vision, with a two-alternative, forced-choice procedure to assess taste and smell thresholds. The testing procedure is implemented in a program running on a Macintosh computer.

This program was used to measure detection thresholds for taste and smell with NaCl, sucrose, citric acid, quinine sulfate, and butyl alcohol as stimuli. The results show that thresholds can be estimated within a reasonably short time (10–15 min) and with a relatively small confidence interval. The median number of trials necessary to obtain a threshold was 11 for taste (n = 129) and 13 for smell (n = 76).

Monte Carlo simulations compared the maximum-likelihood method with other methods commonly used to measure



chemosensory thresholds quickly. The stimuli were 18 concentrations ranging from –4.0 to 0.25 log M units in 0.25 steps. The median error (estimated threshold minus true threshold) with the maximum-likelihood method was almost zero (–0.04, upper panel). The spread of errors using an ascending stimulus method, stopping with the stimulus that elicits five correct responses in a row, is considerably larger than with the maximum-likelihood method and the median error shows a significant overestimation of thresholds (0.18, lower panel). We conclude that the maximum-likelihood method provides a rapid, accurate and bias-free way to assess chemosensory detection thresholds.

170. Feedback regulation of neuromodulation in a model of olfactory bulb reduces overlap in the neural representation of olfactory stimuli

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Cholinergic modulation and noradrenergic modulation has been shown to have effects at various levels of the mammalian olfactory system. Behavioral studies have shown that cholinergic modulation is involved in olfactory short term memory in the olfactory bulb, as well as in olfactory learning in higher structures, more particularly the piriform cortex and the hippocampus. In the olfactory bulb,. NA modulation has been shown to suppress inhibition of mitral cells by granule cells, at least partly through suppression of the excitation of granule cells by mitral cells; ACh has been shown to suppress inhibition in mitral cells by granule cells while it seems to increase periglomerular cell firing and to decrease mitral cell responsiveness when applied to the glomerular layer. Here, we will show how the modulation of inhibition at the two levels of interneurons could play a role in the filtering and feature extraction role of the olfactory bulb.

In our model of olfactory bulb we analyze the putative role of feedback regulation of the two types of inhibition for olfactory bulb processing of olfactory stimuli: (i) feedback regulation of lateral inhibition in the glomerular layer ensures a constant average number of active mitral cells over the total input pattern space; (ii) feedback regulation of the modulation of inhibition mediated by granule cells in the deeper bulbar layers ensures constant average spiking probabilities of active mitral cells over the total input pattern space; and (iii) the combined effect of the modulation of both types of inhibition considerably decreases the overlap between pairs of output patterns thus enhancing the discrimination between largely overlapping olfactory input patterns.

171. Odorant-modulated K^+ conductances in rat olfactory neurons

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Olfactory neurons (ONs) respond to odorants with an elevation in the intracellular concentration of either adenosine 3′,5′-monophosphate (cAMP) or inositol-1,4,5-trisphosphate ($InsP_3$), and these two second messengers are known to directly gate non-specific and Ca^{2+}-selective second-messenger-regulated channels leading to a net influx of Ca^{2+} into the apical compartments of the cell. The subsequent elevation in $[Ca^{2+}]_i$ is known to cause opening of Ca^{2+}-activated K^+ and/or Cl^- conductances.

In order to study odorant-modulated conductances in rat ONs, we employed the perforated patch technique, which allows measurement of whole cell currents while minimally disturbing the intracellular environment. ONs were stimulated with two mixtures of odorants known to elicit increases in the concentrations of either cAMP (mix A) or $InsP_3$ (mix B) in biochemical experiments. As expected for cAMP-mediated responses, when ONs were bathed in Ca^{2+}-free Ringer, stimulation with mix A, or with IBMX elicited an outwardly rectifying current reversing near zero. However, when the ONs were bathed in Ringer containing a metabolic substrate (pyruvate), stimulation with mix A or mix B elicited stimulation of a K^+ conductance. Consistent with this observation, stimulation with either mix A or B under these conditions caused hyperpolarization when measured under current clamp. In contrast, when the cells were bathed in Ca^{2+}-free Ringerwith pyruvate, stimulation with odorants caused inhibition of a K^+ current under voltage clamp, and depolarization under current clamp. Modulation of the K^+ conductance by odorants disappeared when the patch membrane was ruptured to attain the whole cell configuration. Following patch rupture, stimulation with mix A elicited outwardly rectifying currents reversing near zero.

These observations indicate that odorants can elicit either depolarization or hyperpolarization in rat ONs. This might play an important role in quality coding in the olfactory system.

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172. Expression of neural cell adhesion molecule and L1 in outgrowing olfactory axons from the olfactory epithelium slice culture

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Early olfactory axons grow from the olfactory epithelium to the telencephalon along a specific pathway. Several extracellular matrix and cell surface molecules are present along the developing olfactory nerve pathway *in vivo*, indicating that these molecules might be involved in axonal guidance for early olfactory axons. To investigate potential mechanisms of olfactory axon pathfinding, we used an organotypic olfactory epithelium slice culture to analyze the expression of adhesion molecules [neural cell adhesion molecule (NCAM) and L1] in outgrowing olfactory axons from the olfactory epithelium slice cultures.

E13 embryos were taken out from timed pregnant rats. Embryonic heads were cut on vibratome. The olfactory epithelium (OE) slices were harvested from the sections and placed in the Milicell-CM culture plate inserts. Serum-free Waymouth's medium was added to the insert (0.7 ml) and the culture dish well (1.0 ml). The slices were maintained at 100% humidity in air/5% CO_2 at

37°C for 5 days. The medium was changed every 2 days. GAP43 or neuronal specific tubulin (NST) antibodies were used to identify the outgrowing olfactory axons from the olfactory epithelium slice cultures. A monoclonal antibody against NCAM (1:6) and the rabbit polyclonal antibody against L1 (1:1000) were used for analyzing the expression of NCAM and L1 in the outgrowing olfactory axons.

These processes were identified as axons by immunoreactivity with either NST, which is present in all neurons and axons, or with growth associate protein (GAP 43), which shows the growing neurons and axons. NCAM is strongly expressed by both the outgrowing olfactory axons and the olfactory receptor neurons (ORNs) in the OE slices. Some migrating cells also expressed NCAM, but we have not determined whether they are neurons or glia. The outgrowing olfactory axons strongly expressed L1, and the staining pattern of L1 is similar to that of NCAM. L1 positive axons formed more bundles than NCAM positive axons. This suggests that both NCAM and L1 are associated with the outgrowth of the olfactory axons and that L1 may also play a role in fasciculation of the olfactory axons.

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173. Nicotinic acetylcholine and capsaicin receptors in rat trigeminal ganglia

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Nicotine and capsaicin produce many similar physiological responses that include pain, irritation, and vasodilation. To determine whether neuronal nicotine acetylcholine receptors (nAChRs) are present on capsaicin-sensitive neurons, whole-cell patch clamp recordings were performed on rat trigeminal ganglion (TG) neurons. It was found that ~20% of the total number of neurons tested were activated by both 100 μM nicotine and 1 μM capsaicin. Other subsets of neurons were activated by only one of these compounds, whereas a fourth subset was not activated by either compound. These data indicate the existence of subsets of capsaicin-sensitive afferent neurons.

The peak current–voltage relationship of the nicotine-activated currents rectified, in that for voltages >0 mV, the current was essentially zero. In contrast, capsaicin-activated currents exhibited a linear current-voltage relationship. Other experiments showed that some nAChRs were inhibited by α-bungarotoxin, suggesting they comprise α7 subunits.

To complement these electrophysiogical studies RT-PCR was used to characterize the subtypes of nAChRs expressed the trigeminal ganglion of 17 female Sprague–Dawley rats. Although there was individual variability, cells within the TG expressed high levels of mRNA for the α3, β2, β3 and β4 subtypes, moderate levels for the α5 and α7 subtypes, and low levels for the α4 and α9 subtypes. mRNA for the α2 subtype was not detected. These data suggest that more than one subtype, and possibly combinations of nAChR subtypes, may be involved in the common chemical sense associated with trigeminal nerve stimulation.

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174. Cloning of a gene encoding chemoattractive protein from earthworm secretion

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We have previously isolated and characterized a protein, ES20, derived from earthworm shock secretion that is a chemoattractant for garter snakes. On the basis of its 15-residue N-terminal amino acid sequence, degenerative oligodeoxynucleotide probes were synthesized and used to generate PCR products as well as for screening cDNA library which was been constructed in sense orientation using UniZAP™ XR vector and XL1-Blue MRF' host. A gene of the chemoattractive protein, ES20, was cloned from PCR cDNA as well as from the cDNA library. The clone from the cDNA library could not be obtained by direct screening with the degenerative probes. We used a combination of our forward degenerative primer and T7 promotor instead of our reverse degenerative primer to obtain gene-specific DNA fragments. Based on the DNA sequences from these fragments, gene specific probes were synthesized and successfully used in screening the cDNA library. A clone was obtained. The gene from the library is ~700 bp long. Northern blot analysis yielded a single band of similar size. We therefore believe that a complete gene has been cloned. The DNAs from both the PCR product and a clone from the cDNA library were sequenced. The complete amino acid sequence of 214 amino residues has been deduced.

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175. A survey of neurotransmitters in the gustatory cortex of the Syrian golden hamster

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Our laboratory has begun to characterize the distribution of neurotransmitters in the gustatory cortex of the Syrian golden hamster (*Mesocricetus auratus*). We have tested for the presence of two excitatory (glutamate, aspartate) and one inhibitory (GABA) neurotransmitter. Antibodies to rat neurotransmitters (rabbit polyclonal anti-glutamate, rabbit polyclonal anti-aspartate, mouse monoclonal anti-GABA; Sigma) were cleaned with hamster liver acetone powder. Fixed brain sections were incubated with diluted antibodies for 24 h at 4°C, then incubated with the appropriate biotinylated goat anti-IgG. Antibodies were visualized with the avidin–biotin reaction (ABC Kit, Vector) using DAB histochemistry. Immunoreactive neuronal soma to all three neurotransmitters were observed in the gustatory cortex. Preliminary observations suggest that glutamate-like and aspartate-like immunoreactivity predominates in layers II/III and

V of the gustatory cortex. We are continuing to further characterize the results.

This work was supported by research grants number 2P50DC00168-14A1 and 5T32DC00025-09 from the National Institutes on Deafness and Other Communication Disorders, National Institutes of Health.

176. Evaluation of event-related synchronous brain activity following chemosensory stimulation

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Chemosensory event-related potentials (CSERPs) are useful for evaluating the phase-locked time series of brain activity following a chemosensory stimulus. In the event that a portion of the signal becomes shifted in latency, even slightly, the result of a CSERP analysis is usually an average of zero because of positive and negative oscillations. To avoid this effect in the present study, an adaptation of event-related desynchronization analysis was employed. Individual trials (4 s) were digitally filtered with a narrow bandpass filter (e.g. 4–7, 20–23 Hz). The resultant wave consisting of only limited frequencies was squared and averages constructed for each of the four stimuli and a blank condition. Fifteen subjects were used in the data analyses and eight frequency ranges from 0.5 to 31 Hz were evaluated. Chemosensory stimuli (two perfumes and two food extracts) were administered in a constant humidified and warmed air stream and coincided with inhalations. Stimulation lasted 0.25 s. Electrophysiological data were collected from 30 scalp sites and all trials were evaluated for eye movements and corrected if necessary. Results of these analyses indicated that most electrophysiological differences between chemosensory conditions occurred 1.5–2.0 s following stimulation. Differences were found in the 12–15, 20–23 and 24–27 Hz bands. Additionally, low frequency (0.5–3 Hz) activity was found to differ between chemosensory stimuli from 0.5 to 1.0 s following stimulation.

Supported by a grant from the Olfactory Research Fund.

177. Spatial taste loss associated with aging

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Suprathreshold whole mouth taste function appears stable with aging, although localized taste losses occur.

Methods. A spatial test was used to evaluate cranial innervation of taste and suprathreshold perception in 60 healthy older (65–95 years) and 43 younger (17–38 years) females in New Haven, CT. Older subjects were tested in their homes, and younger subjects at the Yale Taste Laboratory. Younger subjects with a history of otitis media or head trauma were excluded from the study. Suprathreshold concentrations of sodium chloride (1.0 M), sucrose (1.0 M), citric acid (0.032 M), and quinine hydrochloride (0.001 M) in deionized water were painted bilaterally on four discrete tongue and palate loci with a cotton-tipped applicator. Solutions were applied sequentially with water rinses prior to each stimulus. Perceived intensity was rated on a nine-point scale. The swallow test was performed to assess whole mouth perceived intensity of each stimulus. Statistical analyses included analysis of variance (ANOVA) and *t*-tests for planned comparisons.

Results. Quality- and area-specific spatial losses were exhibited in aged subjects (significance criterion $P < 0.001$ unless noted). For example, aging impaired taste function on the circumvallate loci for all qualities, but taste on the palate ($P < 0.01$), foliate, and frontal regions was diminished only for some qualities. In contrast to spatial taste, whole mouth perception was not reduced in the aged for any quality. Rather, older subjects assigned higher intensity ratings to sodium chloride ($P < 0.01$) and citric acid than younger subjects, which may reflect increased responsiveness to oral irritation in the aged.

Conclusions. Aged subjects exhibited localized taste losses. However, whole mouth taste perception at suprathreshold concentrations was not impaired with aging. These results support prior reports of stable suprathreshold whole mouth taste intensity perception, despite the existence of localized taste deficits in aged subjects.

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178. Dopamine modulates odor responses in rat olfactory receptor neurons

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We propose a model for dopaminergic modulation of olfactory signal transduction whereby dopamine (DA) present in nasal mucus binds to D_2 dopamine receptors on olfactory receptor neurons (ORNs). D_2 receptor activation reduces basal cAMP levels in ORNs (Mania-Farnell *et al.*, 1993). The reduction in basal cAMP increases the sensitivity of CNG channels to odor-activated increases in cAMP. In support of this model, we measured DA concentrations of 55.9 ± 53.1 nM ($n = 10$) in rat nasal mucus using HPLC. Our immunocytochemistry studies show specific labeling of cilia, dendritic knobs, soma, and axon bundles of ORNs with a polyclonal D_2 receptor antiserum (DR10). Nickell *et al.* (1991) showed that D_2 receptor mRNA is present in ORNs. Our EOG recordings on rat olfactory septum show a 14–20% increase of olfactory sensitivity to odors in the presence of 100 nM DA. The DA-induced increase in odor sensitivity is reversibly blocked by 100 μM haloperidol. Dopamine (100 nM) reversibly inhibits IBMX generated EOG responses suggesting that DA reduces basal cAMP production. At the single cell level, our fura imaging studies show that in the presence of DA, odor-activated increases in internal Ca increase by 28.3 ± 3.4% ($n = 5$). As in the EOG recordings, DA blocks basal cAMP production as evidenced by

reversible block of 65 ± 18% (n = 3) of the IBMX increase in internal calcium. We used inside-out macro-patches to record the effects of background levels of cAMP on the sensitivity of CNG channels to cAMP. We find that reducing the background level of cAMP increases the sensitivity of CNG channels to cAMP. These results provide a mechanism for modulation of odorant responses by DA at the level of ORNs .

This work was supported by NIDCD R29 DC02587-02.

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179. Dose–response analysis of the amiloride-sensitive portion of taste nerve responses to sodium and nonsodium salts in rats

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The present study demonstrates that low concentrations of amiloride and its two analogs benzamil and amiloride-5(*N*,*N*-dimethyl) (DMA) suppress taste nerve responses to various salt concentrations induced by 1 and/or 25 μM amiloride, benzamil and DMA was assessed in rats. We measured integrated chorda tympani nerve responses to 30, 75, 150, 300 and 500 mM concentrations of NaCl, KCl and NH_4Cl mixed with or without amiloride (1 and 25 μM), benzamil (1 and 25 μM) and DMA (1 μM) at 35°C. The drugs at a dose of 1 and 25 μM significantly suppressed taste nerve responses to a range of NaCl, KCl and NH_4Cl salt solutions (30–500 mM). Examining the magnitude of suppression across drugs for NaCl shows that the order of drug effectiveness was amiloride = benzamil > DMA. On the other hand, drug effectiveness on KCl nerve responses was 25 μM amiloride > 1 μM amiloride = benzamil = DMA. There was no difference between drug effectiveness on NH_4Cl nerve responses. Examining drug effectiveness across salts shows that the magnitude of inhibition produced by 1 μM amiloride and benzamil was NaCl = NH_4Cl < KCl. Differences in inhibition were not found across salts with 25 μM amiloride, while the magnitude of inhibition produced by 1 μM DMA was NH_4Cl > NaCl = KCl. Taken altogether, the results suggest that: (i) passive Na^+ channels in rat taste receptor cells have the capacity to pass K^+ and NH_4+ cations, and (ii) the Na^+/H^+ protein appears to be present on the apical membrane of taste receptor cells and possibly involved in NH_4Cl taste transduction.

180. Measurement of intracellular pH (pH_i) in isolated rat circumvallate papillae taste receptor cells (TRCs)

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The mechanisms for acid-taste transduction were investigated by measuring the effects of external pH (pH_o) on TRC pH_i in isolated fragments of taste buds, using the pH-sensitive dye, BCECF. In HEPES-buffered solutions (pH 7.4), mean TRC pH_i was 7.43 ± 0.03 (n = 23). pH_i responded rapidly to changes in pH_o between 6.5 and 8.0 with a slope close to unity. These data suggest that TRCs track external pH and demonstrate no spontaneous recovery of pH_i. The pH_o-induced changes in pH_i were insensitive to amiloride, MIA, EIPA, benzamil, NBD-Cl, bafilomycin A_1, SCH-28080, Na^+, K^+, Cl^-, Zn^{2+}, Ba^{2+} or Ca^{2+}. These data suggest that Na^+-H^+ exchanger, vacuolar type H^+-ATPase, H^+-K^+-ATPase, H^+ conductance via amiloride-sensitive Na^+ channels, putative divalent metal ion-sensitive H^+ channels, and Cl^-- HCO_3^- $(OH)^-$ exchanger do not have a dominant role in acid sensing. However, TRC pH_i was regulated. We observed reversible increases in TRC pH_i by (i) increasing extracellular Ca^{2+} concentration from 0 to 2.5 mM; (ii) treatment with 170 nM thapsigargin and 10 μM cyclopiazonic acid, drugs which release Ca^{2+} from intracellular stores; and (iii) treatment with 3 mM Zn^{2+} and 1 mM orthovanadate, inhibitors of protein-phosphotyrosine phosphatases. These data suggest a possible feedback control between intracellular Ca^{2+} and pH_i in TRCs and the involvement of protein-phosphotyrosine phosphatases in pH_i regulation. TRC pH_i was also decreased by 150 mM NH_4^+, propionate and acetate in the presence and absence of Na^+ (pH 7.4). These data suggest that NH_4^+ enters TRCs more rapidly than NH_3. The neutral weak acids also enter TRCs and decrease pH_i.

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181. Sweetener similarity in hamsters as determined by generalization of conditioned taste aversions

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Stimuli that are sweet to humans are not necessarily perceived similarly by other mammals. Behavioral and electrophysiological studies were used to test taste similarity of seven sweeteners in hamsters (*Mesocricetus auratus*). Test stimuli were the archetypal sucrose (0.1 M), and maltose (0.32 M), D-phenylalanine (0.032 M, D-Phe), Na-saccharin (0.0032 M), Ca-cyclamate (0.032 M), dulcin (0.01 M) and Na-nitrobenzenesulfonate (0.032 M). Stimulus concentrations were 1/2 log step above those preferred by 15% over water. The conditioned taste-aversion generalization paradigm

used 7 experimental groups (n = 8) and 1 control group (n = 11). Apomorphine (30 mg/kg) was injected i.p. after each hamster sampled a test stimulus or water, and after recovery test stimuli were presented for 1 h daily. The volume of each compound consumed by each experimental group and the control group was compared and a mean % suppression calculated. Patterns of suppression across test stimuli were used to establish similarities in perceived quality. Learned aversions [$t(17)$, $Ps < 0.001$] to all pairs of stimuli generalized [$t(17)$, $Ps < 0.05$] except Ca cyclamate and maltose, the two stimuli least similar to sucrose. D-Phe and saccharin were the most similar to sucrose. Preferences (n = 5–6) were determined with 48 h, 2-bottle preference tests. Each test stimulus was preferred over water by >30% [$t(4–5)$, $Ps < 0.02$], except Ca cyclamate, which was not preferred [$t(5) = 1.24$, NS]. Each test stimulus but maltose elicited a chorda tympani response ≥50% of the neural response to sucrose. Test stimuli that were both highly preferred and elicited strong chorda tympani responses were perceived to be similar to sucrose.

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182. Effect of human associated odours on dream content and sleep quality

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Humans respond to auditory, visual, tactile and to also to olfactory stimuli in sleep (Badia *et al.*, 1990). The question is whether human associated odours could influence dream content and physiological parameters (respiration rate, cardiofrequency). In the previous study significant effects of scatol and orange odour but not of the mixture of axillary and vaginal secretion on the physiological parameters could be observed. In contrast to that study we present vaginal secretions alone. Twelve male subjects sleep for four nights in the sleep laboratory. During the night following parameters are continuously measured: heart rate, respiration, EEG (C3,F4), EOG and EMG (Beckmann polygraph). The first night is to get accommodated to the sleep lab condition. In the following three nights 3-*trans*-methyl-hexenoic acid (Givaudan Roure) or vaginal secretion or control (air) are applied. The same stimulus is applied during the first deep sleep phase and the second and third REM phase. After 7 min the odour application is stopped, the subjects are awakened and interviewed (dream content, pleasantness of their dreams, mood, whether they notice an odour and its pleasantness. All subjects have no sleep problems, are non smokers, are aged 20–30 years and have a heterosexual orientation. At the end of the study both stimuli are presented again and the men are interviewed (pleasantness of the odour, what it reminds them on). The vaginal secretion were collected from four donors (age 24–29) at the time of ovulation and were pooled. The ovulation has been determined by a hormone assay. Under the influence of the hexenoic acid the subjects tend to sleep better and under the vaginal secretion they tend to sleep more restless.

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183. Dimorphism in the vomeronasal system of the gray short tailed opossum, *Monodelphis domestica*

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Previous studies in rodents indicate that components of the vomeronasal system are sexually dimorphic, being larger in males than in females. We investigated this dimorphism in a marsupial, Monodelphis domestica. Six pairs of 5-month-old littermates, one male and one female, each pair from a different mother, were used. Their body weights, brain weights and brain lengths (with and without olfactory bulbs) were determined. Left and right accessory olfactory bulb (AOB) volumes were determined for the nerve fiber layer and glomerular layer (NFL/GL) and mitral/tufted cell layer (M/TCL). In addition to a Nissl stained series, parallel series were stained with antibodies to olfactory marker protein (OMP) to distinguish anterior and posterior portions of the AOB and G protein, $G_{o\alpha}$ to reveal the boundaries of the M/TCL layer. In the opossum, the granule cell layer (GCL) of the AOB merges with the GCL of the main olfactory bulb, so that layer has not been measured.

The NFL/GL of the AOB volume was larger in males than in females ($P < 0.03$, one tailed).The anterior NFL/GL of the AOB of males was larger than that of females ($P < 0.008$, one tailed), but the volume of the posterior portions of those layers was not different in males and females. The M/TCL layer was significantly larger in males than in females ($P < 0.01$, one tailed). We are currently measuring the volume of the vomeronasal organ to determine if it is sexually dimorphic as well.

These results suggest that in addition to the M/TCL layer which has been previously described as sexually dimorphic in rodents, the anterior AOB of opossums is sexually dimorphic as well.

184. Topographic patterns of odorant receptor gene expression in the olfactory epithelium of the salamander, *Ambystoma tigrinum*

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Odorant transduction is thought to be mediated by recently cloned members of a seven-transmembrane spanning G-protein linked receptor family. Recent *in situ* hybridization histochemistry (ISHH) studies in rodents have characterized topographic patterns within the olfactory epithelium in the distribution of individual receptors.

We have used a PCR cloning strategy based on degenerate oligonucleotide primers derived from conserved regions of the family of rodent odorant receptors to extract olfactory receptor clones from salamander epithelia. Sequence analysis confirms that 18 of these novel sequences belong to the seven-transmembrane spanning G-protein linked receptor family. Two of these clones, AF02 and AE06, exhibit 35% identity in predicted amino acid sequence, indicating that they are members of separate families.

^{33}P-labeled antisense probes were transcribed for AF02 and AE06 and used for ISHH on salamander olfactory epithelia. For both probes, autoradiographically labeled cells were restricted to the epithelium with a distribution characteristic of olfactory sensory neurons (OSN). Approximately 1 and 2% of the total estimated number of OSN's expressed AF02 and AE06 respectively. Distinct topographic patterns were found in the distribution of labeled cells. Both receptors were predominantly expressed (75%) on the dorsal epithelial sheet. AF02 labeled cells were primarily restricted to the rostral and medial one-half of the dorsal sheet, whereas AE06 labeling extended the full rostra-caudal extent and included more laterally positioned cells. This topography was roughly approximated on the ventral epithelial sheet.

These data suggest that, similar to findings in the rodent, olfactory receptors in the salamander may exhibit topographic patterns in their distribution, although the zonal organization may not be as rigidly restricted.

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185. Unconditioned licking of quinine is increased by glossopharyngeal nerve transection in rats without presurgical stimulus exposure

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Previous research suggests that concentration-dependent licking of quinine during brief access trials and quinine detection thresholds (both measured using within subject experimental designs) are not disrupted by single gustatory nerve transection of the glossopharyngeal or chorda tympani nerves. However, a recent experiment in our laboratory indicated that rats with bilateral glossopharyngeal nerve transection (GLX) ingested more quinine than sham-operated controls (CON) when the taste solution was only presented after surgery (45 min single-bottle test). One of the principal differences between the behavioral studies that failed to find effects of GLX on responsiveness to quinine and our recent work is the presence or absence of presurgical exposure to the tastant. Rats that had experience with quinine before nerve transection did not show changes in responsiveness. In the present experiment we measured unconditioned licking to quinine in rats that had no exposure to quinine before surgery (between subject design). Male Sprague–Dawley rats were water-deprived and trained to lick water during 10 s trials in an automated gustometer. Following recovery from surgery, the water-deprived rats were presented with randomized blocks of seven concentrations of quinine-HCl (0.003 mM to 3 mM) and distilled water. Water rinses followed each stimulus presentation. The number of licks to each tastant was averaged over three days of testing (40 min sessions). The GLX rats (n = 12) licked more to higher concentrations of quinine relative to CON rats (n = 11). Thus, GLX alone can impair quinine sensibility in rats without presurgical taste exposure. These results taken with those from other studies suggest that experience before nerve transection may have a protective effect on taste-guided behavioral responsiveness to quinine in rats.

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186. Suprathreshold taste response and its relation to identification

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Measures of the ability to make absolute identifications of taste substances (single stimuli and mixtures) give promise of providing a means to assess deficits in taste perception (Gent *et al.*, 1995). In this earlier work, in order to match taste intensities, the concentrations of the unmixed test stimuli [sucrose, NaCl, quinine HCl, citric acid, KCl, monosodium glutamate (MSG) and aspartame] were selected primarily on the basis of taste matches previously reported in the literature. Concentrations in the binary mixtures of sucrose with NaCl, acid, and quinine were the same as those of the unmixed stimuli. The present study had 20 human subjects give magnitude estimates of the taste intensity of 36 stimuli. The stimulus set included several concentrations of the unmixed and mixed substances that we used in our previous work on taste identification. Average judgements given to stimuli used in the identification task varied by a factor of two, with 0.1 M NaCl, 0.1 M KCl, 3 mM citric acid, and 0.1 mM quinine HCl stronger than 3 mM aspartame, 0.3 M sucrose and 0.1 M MSG. Adjustment of stimulus concentrations by about one-half log step would eliminate the differences. However, the average ability of our previous subjects to identify the stimuli did not seem to be affected by this two-fold difference in average intensity judgments. Percentage correct identifications was 60.3% for the stronger stimuli compared with 57.5% for the weaker stimuli. In fact, stimuli that are judged stronger on average might be judged weaker by certain individuals; for example, a few subjects in the present study judged sucrose to be stronger than NaCl. It remains unclear whether such variation contributes to the difficulty that individual subjects may have in making absolute identifications.

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Reference

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187. Responsiveness of coyotes (*Canis latrans*) to simple taste cues

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Relatively little is known about the responsiveness of dogs to tastes, and nothing is known about the responsiveness of wild canids. In the present experiment, adult male and female coyotes were presented with 15 tastants (sweet, starch, sour, salty, bitter, protein) in one-bottle 6 h drinking tests. Animals then received plain water for 2 h, and were water-deprived overnight.

The results showed that both sweet (sucrose, fructose) and starch (polycose, corn starch) solutions were preferred relative to plain water and the other tastants. Protein taste (monosodium glutamate and inosine monophosphate) also was preferred to plain water. Coyotes were indifferent to salty solutions (sodium chloride, ammonium chloride, potassium chloride), and avoided sour (hydrochloric acid, citric acid, acetic acid) and bitter (quinine hydrochloride, sucrose octaacetate, denatonium benzoate) tastes. These results are generally consistent with previously reporting findings for dogs. They suggest that some canids, like rodents, perceive starch as a distinct taste.

This research was supported by USDA/APHIS/ADC. All procedures were approved by IACUC committees at both the Denver Wildlife Research Center and Utah State University.

188. Clinical assessment of olfactory function using the cross-cultural smell identification test in the district of Nagoya, Japan

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The cross-cultural smell identification test (CC-SIT: 12 odors) was developed as an olfactory test which can be used outside of the USA. This test has been found useful for clinical assessment of olfactory function in Japan. In this study, about one hundred normal volunteers and one hundred patients with complains of olfactory dysfunction, ranging in age from 20 to 80 years, were administered the CC-SIT and a quantified questionnaire about their ability to smell (full mark 30). In the normal group, the mean scores of the CC-SIT and the questionnaire were 9.8 and 29.4 respectively; corresponding scores in the patient group were 5.9 and 9.5. The percentage of correct answers for each item in the normal group was significantly higher than that in the patient group ($P < 0.05$). However, older persons (>65 years old) showed poorest performance in the CC-SIT (mean score: 8.1 in the normal group; 5.6 in the patient group). Interestingly, a relatively high correlation was present between the CC-SIT scores and the scores of the questionnaire (Spearman; $r = 0.80$).

189. Oral fat exposure enhances post-prandial triglyceride concentration in humans

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Chemosensory stimulation can elicit a wide array of physiological processes with potential health implications. Studies in rats indicate that oral exposure to dietary fat can prolong the postprandial triglyceride concentration relative to no oral exposure or stimulation with water. The present work examined this phenomenon in humans. Fifteen healthy adults were provided oral stimulation with a cracker, cracker with non-fat cream cheese, cracker with cream cheese or no stimulation after ingesting 50 g of safflower oil in capsules (to avoid oral exposure to fat) on four test days held at least two days apart. Oral stimuli were provided at 5 min intervals for the first hour and 15 min intervals for the second hour. All oral stimuli were masticated and expectorated. Blood was drawn at baseline and 2, 4 and 6 h after fat ingestion. Peak plasma triglyceride levels were significantly higher and remained elevated longer when the full fat cream cheese was sampled relative to the other treatments. No differences were observed between the other conditions. The effect cannot be ascribed to ingestion of fat since all stimuli were expectorated or to hedonic or cognitive influences because subjects were not able to discriminate between the two forms of cream cheese and they were not informed that two types were used or which was presented on a given day. These data suggest there is a mechanism for detecting fats, impurities in fats, fat constituents or fat by-products in the oral cavity of humans that influences lipid metabolism.

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190. Color-induced odor enhancement: the role of color intensity and appropriateness

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In 1990 Zellner and Kautz found that equally concentrated odorous solutions are perceived as smelling more intense when they are colored compared with when they are colorless. The appropriateness of the hue did not appear to matter. However, the colors used were not matched on intensity. This fact might have influenced the results as follows. Suppose color intensity and appropriateness both control perceived odor intensity. If so, an intense green and a weak red might produce similar odor intensity ratings.

The present experiment examines the contribution of both color intensity and appropriateness on the color-induced odor enhancement effect. During the first session, subjects indicated that a light pink solution was the most appropriate color for a pink lemonade odor and a dark red solution was the most appropriate color for a cherry odor. During the second session, subjects were assigned to one of two odorant groups (pink lemonade or cherry) and were asked to smell and rate the intensity of samples of the odorant. For any set of odors (e.g. the pink lemonade scent) three of the samples were colorless, three were colored light pink (the

color of pink lemonade and therefore appropriate for the pink lemonade scent), three were medium red, and three were dark red (the color of cherry Jello and therefore appropriate for the cherry scent). One of each of the four differently colored samples was odorless, while the other two contained the same concentration of odor.

The results suggest that both appropriateness and intensity of the color contribute to the odor enhancement effect. For the cherry odor, the dark red color was both the appropriate and most intense color and was rated strongest smelling. For the pink lemonade odor, the light pink was the most appropriate but the dark red color was the most intense. No enhancement was seen by any color in this group, possibly because the light colored ones were enhanced by being appropriate and the dark colored ones by being intense.

191. Synergism of a cabbage looper, *Trichoplusia ni* (Hübner), sex pheromone specialist neuron by three host-plant compounds: can host plant volatile emissions alter sex pheromone detection and perception?

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Several recent accounts link enhanced trap captures and modifications of noctuid moth sexual behavior with host plant aromas acting in concert with sex pheromones. Previous studies have demonstrated that compounds associated with host plant emissions interact with the sex pheromone specialist neurons usually by reducing the response to the pheromone. We asked whether or not aromatic host plant compounds might act through synergizing the HS(a) sex pheromone specialist receptor neuron of the cabbage looper moth, *Trichoplusia ni* (Hübner), to the most important component, Z7–12:Ac. Electrophysiological responses recorded from the specialist showed that the response to Z7–12:Ac was synergized by three typical aromatic compounds found in host plants. Induced wind tunnel behavioral responses confirmed the synergism. Whether or not the volatile emanations from host plants interact in this manner in nature is dependent upon whether or not the stimulus strengths that are necessary for synergism are the same as those that are found in the habitat.

192. Barnacle larvae of *Balanus amphitrite* (Darwin) specifically bind the settlement inducer mimic bradykinin

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Attachment of barnacle larvae to a surface must occur near conspecifics to enable reproduction of the sessile adults. Barnacle larvae locate conspecifics by detecting peptide pheromones. Pheromones are mimicked by peptides that have arginine at the carboxyl terminus preceded by several neutral amino acids. Two mechanisms of pheromone detection have been hypothesized: (i) pheromones are 'sticky' and barnacles settle where the glue they use to walk on surfaces 'sticks' the best; and (ii) pheromones stimulate chemoreceptors that induce attachment and metamorphosis. We tested these hypotheses with the vertebrate vasodialator peptide bradykinin which induces permanent attachment and metamorphosis of barnacle larvae. Barnacle larvae were incubated in various concentrations of [^{3}H]bradykinin with and without a 100-fold excess of unlabeled bradykinin for 1 h at 6°C. Larvae bind bradykinin in a specific manner. This supports the notion that there are receptors for pheromone. Studies are underway to determine the dissociation constant, the number, and the location of binding sites. Future studies will test other settlement inducers, as well as inhibitors, to see if these compounds act at the same sites as bradykinin does.

193. Response profiles of crayfish olfactory projection neurons to odorant stimuli

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We have recorded electrical responses to odorant stimuli from olfactory projection neurons (PNs) in perfused, isolated head preparations of the freshwater crayfish *Procambarus clarkii*. Sharp micropipette electrodes were used to penetrate processes of olfactory projection neurons within the olfactory lobe, while odorants were applied to the antennular filaments. Electrical stimulation of the antennules generated biphasic, excitatory–inhibitory response sequences in the PNs. Intense stimulation generated an initial large epsp and impulses, followed by a prolonged phase of inhibition, resembling responses of PNs to electrical stimulation of the antennular nerve in the spiny lobster (Wachowiak and Ache, 1994). Both response phases increased with stimulus intensity. Individual amino acids and their mixtures, a 0.02% tetramin solution, and sucrose solutions were used as odorant stimuli. Responses to odorants were of two types: (i) a brief train of inhibitory postsynaptic potentials; or (ii) an excitation-inhibition sequence that resembled the response to low-intensity electrical shocks. With an appropriately strong odorant, the excitatory phase of the response generated one or more action potentials. Self-adaptation occurred to all odorants tested and cross-adaptation was observed with some amino acid stimuli. Prolonged (>5′) periods of disadaptation were required to obtain the greatest responsiveness to odorants. All neurons were identified anatomically by injection with biocytin following the physiological testing procedures. Somata were within the lateral cell body cluster (cluster #10) and axons ran within the olfactory-globular tract. Most of the cells penetrated had multiglomerular distribution of dendritic arbors that were confined to the olfactory lobe. This distribution is functionally correlated with the broad-spectrum inhibition exhibited by most PNs in response to a variety of odorant stimuli.

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194. Developmental changes in the acceptance of protein hydrolysate formula and its relation to mothers' feeding habits

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Pediatricians have remarked anecdotally that although it is easy to introduce protein hydrolysate-containing formula to infants during the first weeks of life, it becomes extremely difficult to do so later in infancy. This early acceptance is often attributed to the young infant's 'lack of taste' perception since these formulas are reported to have an extremely unpalatable, offensive off-flavor. To investigate the age-related changes in the acceptance of a protein hydrolysate formula, Nutramigen, and to determine whether the infant's response to a novel formula is related to their mother's willingness to try novel foods, we tested healthy infants, who were either 1–2 or 7–8 months of age.

The data revealed that there was a significant effect of age on the infants' intake of the protein hydrolysate formula relative to their regular formula ($P = 0.00004$). Infants <2 months of age could detect the difference between the Nutramigen and their regular milk- or soy-based formula as evidenced by the slight, relative depression in intake ($P = 0.04$). However, these infants clearly were willing to accept substantial amounts of the protein hydrolysate formula. In marked contrast, virtually all of the older (7- to 8-month-old) infants rejected the Nutramigen within a few min and, based on the videotape analysis by a panel of adults, this was evident within the first min of a feed. Finally, there was a significant correlation between the mothers' eating habits as determined by the questionnaires, and their young (1- to 2-month-old) infants' response to Nutramigen. Mothers who exhibited a greater willingness to consume novel foods ($P = 0.003$) or less food neophobia ($P = 0.04$) had infants who consumed relatively more of the Nutramigen.

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195. A functional activity map of the zebrafish olfactory organ: a new method for activity dependent mapping of odor sensitivity using an agmatine specific antibody

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Decarboxylated arginine (1-amino-4-guanidobutane, agmatine, AGB) is a cationic guanidine compound that passes through non-selective cation channels, including photoreceptor CNG channels (R. Marc, unpublished data). Polyclonal IgG antibodies against a glutaraldehyde-fixed AGB/albumin conjugate allow quantification of AGB flux through non-selective cation channels. Using this approach we have begun to map odor-evoked activity in the olfactory organ of zebrafish. Anesthetized zebrafish were secured in a recording chamber and EOG methods confirmed the viability of the nose. To label ORNs the solution bathing the nose was switched from normal AFW to AGB-AFW (3 mM NaCl replaced with 3 mM AGB sulfate). Fish were stimulated with odors (100 μM cysteine, 1 μM taurocholic acid), 10 μM forskolin or an AGB control five times at a 2 min interstimulus interval. The odor and forskolin-evoked responses were monitored using EOG technique. The odor response in an AGB-AFW background was 70% initially and 15% after 60 min of the response in AFW. After odor stimulation fish were fixed in 2.5% glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer, embedded in Epon 12 and sectioned at 0.5 μm. After incubation in primary antibody, the label was visualized using a nanogold secondary antibody followed by silver intensification. In taurocholic acid and forskolin–stimulated (presumed to activate the CNG pathway) preparations numerous labeled cells were distributed throughout the sensory region. Fewer labeled cells were noted in the AGB-control and cysteine-stimulated (presumed to activate the IP_3 pathway) preparations. We are currently optimizing the stimulation protocol to minimize control labeling.

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196. Evidence for distinct receptors and transduction pathways for amino acid and bile acid stimuli in the zebrafish olfactory system

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The olfactory system of the zebrafish is sensitive to amino acid and biles acid stimuli. Electro-olfactogram (EOG), whole cell patch clamp and calcium imaging techniques were used to characterize the underlying molecular receptors and transduction pathways. Fourteen of the more effective amino acid and bile acid stimuli comprised the odorants tested. A reciprocal cross-adaptation protocol suggested that each of the 14 amino acid and bile acid odorants tested use distinct molecular receptors. Based on a cluster analysis of a correlation matrix generated from the mean percent adapted responses in each of the odor backgrounds the amino acid and bile acid odorants could be divided into two distinct groups. Within the amino acid group distinct subgroups comprising neutral, basic and acidic amino acids were identified. Measurements of changes in $[Ca^{2+}]_i$ using dynamic imaging and Fura-2AM revealed that odorants presumed to use distinct molecular receptors evoke increases in Ca^{2+} in single olfactory receptor neurons. To investigate the transduction pathways we compared EOG responses of representative amino acid and bile acid stimuli in normal AFW with those obtained in the presence of a variety of pharmacological agents that perturb various points in potential transduction cascades. Inward currents ranging from <10 pA to >100 pA were activated when either IP_3 (10 μM) and cAMP (500 μM) was included in the pipette solution indicating that the two transduction cascades are present in isolated ORNs. Collectively these findings suggest that amino acid and bile acid

stimuli are transduced via the PLC and AC pathways respectively. We are working to confirm that the correlation between odorant group used and transduction cascade activated observed using EOG methods holds for single ORNs. Supported by NIH DC-01418.

197. Rats discriminate and prefer axillary odors of ovulating human females

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Human odors have been shown to change in a cyclical fashion, since axillary secretions from one female can be used to entrain and synchronize the menstrual cycles of other females. However, little research has been undertaken on the effect of these cyclical alterations of odor on animal subjects. The present study suggests that laboratory rats may indeed be affected by cyclical changes in the odor of their human caretakers.

Donor females wore gauze pads for 24 h daily taped under the arm during an entire menstrual cycle. During this time, the donor did not use deodorant under the arm, but did wash daily. Gauze pads were sealed in plastic bags and stored in a freezer until the time of testing. During the open field tests, rats were placed in an open field divided into 16 squares, 18′ (45.6 cm) on a side. Gauze pads from the same female donor were placed in diagonally opposite corners of the open field. One of these was from the time of menstruation, and the other was from approximately the time of ovulation (days 14–20 of the 28-day cycle). A clean gauze pad was placed in one of the remaining two corners, and a gauze pad saturated with boar pheromone was placed in the fourth corner. Animals were introduced into the open field equidistant between two corners, and were allowed to explore freely for 30 min. Recorders who were blind to the conditions of the experiment recorded the amount of time the animals spent in each corner of the open field. Results indicate a strong and significant preference for the corner containing the pad worn at about the time of ovulation.

198. Anterior gustatory papillae and human taste perception

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Anatomical definition of gustatory papillae on the anterior part of the human tongue requires both structural and behavioral qualifications. This presentation examines the relationship between taste perception and anatomical features of lingual papillae. Taste perception and vital anatomy are combined to identify which papillae yield taste responses in (n = 67) living human subjects with either microdrop or regional stimulation with chemicals. In another series of experiments, human cadaver tongues (n = 24) are sectioned to study the anatomical features by light microscopy from papillae that are similar those on the living tongues. The objective is to identify what anatomical features of anterior gustatory papillae comprise the *sine qua non* for human taste perception. It was observed that most individual, 'fungiform' papillae respond with definitive qualities only to high concentrations of stimuli. Some fungiform papillae do not contain taste pores or yield taste responses. Individual 'fungiform' papillae are relatively insensitive to chemical stimulation. Gustatory papillae (those with taste buds) are not easy to characterize by a simple set of anatomical features. Responsive papillae include both low, flat structures and also papillae with more typical 'mushroom' shapes. Only papillae with taste pores produced typical taste responses. Living papillae ranged in diameter from 0.27 to 1.65 mm (average 0.70 ± 33 mm, n = 79) and in height from 0.17 to 1.51 mm (n = 79). The number of taste pores/ living papilla ranged from 0 to 14 (average 4.95 ± 3.5, n = 79). In fixed tissue, the epithelial thickness of papillae with taste buds was thinner than in papillae without taste buds (t = 5.27, df = 79, $P < 0.001$). In general, living gustatory papillae are larger and more pink than unresponsive filiform and conical papillae. The minimal requirement for taste perception in humans seems to be a single gustatory papilla with one taste bud. However, most gustatory papillae contain between one and five taste buds, and the stimulation of several papillae is required generally to produce reliable taste responses.

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199. Purification and characterization of a taste specific phosphodiesterase from bovine taste tissues

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Gustducin and rod transducin are guanine nucleotide binding proteins (G proteins) that are expressed in taste cells. Bitter and sweet taste transduction is thought to utilize G protein coupled seven transmembrane helix receptors. G proteins regulate effector enzymes such as phosphodiesterase (PDE) and phospholipase to mediate taste cell changes in intracellular second messengers (e.g. cAMP, cGMP, IP_3, Ca^{2+}). Transducin's role in phototransduction is to couple the receptor rhodopsin to an intracellular PDE. Since gustducin and transducin are very similar (~80% identical), and since gustducin can also activate retinal PDE, we expected that a taste cell PDE would be the intracellular target of gustducin and/or transducin in taste transduction.

We set out to identify taste specific PDEs which are regulated by gustducin or transducin. Using conventional and high performance liquid chromatography including ion exchange, gel permeation, hydrophobic interaction and affinity purification we fractionated PDEs from bovine taste and non-taste tissue. We then assayed PDE-containing fractions for stimulation by a transducin derived peptide that mimics the effect of activated transducin (transducin's 'effector interaction peptide'). We have identified a transducin responsive taste specific PDE present in both circumvallate and fungiform papillae of bovine tongues. The holoenzyme migrates on nondenaturing PAGE similarly to bovine retinal PDE. However, western analysis indicates that the taste PDE is distinct from retinal cGMP PDE. Physical characterization and molecular cloning of this enzyme is in progress. Recent studies from our laboratory with the gustducin knock out mouse have

demonstrated that gustducin is involved in both bitter and sweet transduction. The taste PDE that we have isolated may be the effector enzyme that gustducin activates in bitter or sweet taste transduction.

200. Neurite outgrowth from sensory ganglia into embryonic rat tongue in a co-culture system

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Gustatory papillae appear on the rat tongue between embryonic days 14 and 15 (E0: dam is sperm positive), in a patterned distribution on anterior and posterior tongue. We have reported previously on an organ culture system of the E13 or E14 rat tongue in which the fungiform papillae form in rows on the cultured tongue, in the absence of an intact sensory or posterior tongue, autonomic innervation. To study the role of sensory innervation in papilla maintenance and in taste bud development, we have now established a co-culture system of sensory ganglia in combination with a piece of embryonic tongue. E16 embryos are obtained from an anesthetized, pregnant Sprague–Dawley rat. The embryonic tongue and sensory ganglia (petrosal and/or trigeminal) are dissected. Tongues are cut into anterior pieces containing fungiform papillae and posterior pieces containing the circumvallate papilla. One sensory ganglion is placed on a Millipore type HA filter and a tongue piece is closely apposed to the ganglion; the co-cultures are maintained in standard conditions in DMEM/F12 plus 1% fetal bovine serum and B-27 supplement (Gibco). Co-cultures are fixed after 7–14 days and processed for immunocytochemistry. Antibodies to neurofilaments, choline acetyltransferase and tyrosine hydroxylase are used as markers of cultured neurons and their neurites. Tongue explants and ganglia survive in co-culture without necrosis. Gustatory papillae and surrounding lingual epithelium continue to differentiate from E16 stages and neurites extend from the ganglia to reach the epithelium of co-cultured tongue. In current experiments we are manipulating age of tongue tissue at time of explant with E16 ganglia. Our results demonstrate the viability of embryonic tongue and sensory ganglia in a co-culture system, and the extension of sensory neurites into the tongue in vitro, with a potential for establishing neural connections. The system will allow study of the neural regulation of gustatory papilla morphogenesis and maintenance, and of taste bud development.

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201. Sensitivity to the basic tastes: the effects of age

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Twenty-one elderly subjects (age 60–75) and twenty-one young subjects (age 18–33) rated the intensity of ten tastants, representing the basic tastes (two per basic taste), on a 9-point category scale. Sodium chloride, potassium chloride, sucrose, aspartame, acetic acid, citric acid, caffeine, quinine chloride, mono-sodium glutamate and inosine-5′-monophosphate were dissolved in water and in product system, in each case in five suprathreshold concentrations. For cross-modal matching loudness of sounds on five levels was intermingled with the taste stimuli and rated on a similar scale.

Analysis of variance showed a significant age effect for all tastants in water. The elderly perceived the intensities as less intense than the young. In the product system an age effect was found only for NaCl, KCl, sucrose and aspartame, where the elderly rated the intensities as lower than the young and for caffeine, where the increase in intensity with the increase in tastant concentration was perceived as greater by the young. This result is most probably influenced by the choice of a different product per basic taste. The young women gave higher and the elderly men gave lower intensity scores in general.

In order to compare the results of intensities in water and product we calculated the ranking of subjects' mean rank. We found a significant positive correlation between the perceived intensities in water and in product for the salt (NaCl and KCl, tomato soup), sweet (sucrose and aspartame, ice-tea) and sour (acetic and citric acid, mayonnaise) tastants, but not for the bitter (caffeine and quinine, chocolate milk) and umami (MSG and IMP, broth) tastants. In general this study showed a decreased taste acuity for the elderly. However compared with the change in smell acuity with age found in the literature, the magnitude of the change in taste sensitivity is relatively small.

202. The MU chamber: a new method to record electrophysiological responses of taste receptor cells to gustatory stimuli

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The breadth of tuning and the organization of the chemical sensitivities of taste receptor cells (TRCs) remains one of the central questions in gustatory physiology. Both the investigation of TRC specificity and mechanisms of taste transduction have been hampered by the difficulty of applying physiological stimuli in an *in vitro* setting. Electrophysiological studies at the single cell level in most cases preclude the use of hypertonic taste stimuli (e.g. salts and sucrose at effective concentrations) and do not restrict the stimulus to the TRC apical membrane. While the recording of transepithelial potentials in Ussing chambers, field potentials in the tongue or activity from taste buds *in situ* maintain the polarity of the TRCs, these techniques are comparatively less sensitive than patch clamping and do not reveal information at the level of the individual TRC. We have attempted to combine the sensitivity of patch clamping with the benefits of transepithelial current recording, similar in some respects to an approach used by Béhé *et al.* (1989). Epithelia containing fungiform taste buds from hamster were attached to a silicon O-ring (5/16″ i.d.) with cyanoacrylate

ester and mounted in a modified Ussing (MU) chamber. In this apparatus, the mucosal surface lies in a closed chamber (0.1 ml volume) and up to 16 stimuli may be applied while the basolateral membrane of a TRC is patch clamped in the open, saline-containing serosal chamber. Multiple taste buds are accessible in this configuration. In preliminary experiments we have recorded from TRCs in current clamp mode while applying an array of stimuli. In some cases, individual TRCs appear to be sensitive to more than a single stimulus; they are depolarized by salts (NaCl, KCl) and acids (HCl, citric acid) following water rinses. Sucrose-responding cells have not, to date, proven to be sensitive to taste stimuli representing the other basic tastes. We anticipate using this technique to map the chemical sensitivity of individual taste receptor cells.

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Béhé *et al* (1989) *Xth International Symposium on Olfaction and Taste*, p. 271.

203. Differential localization of ionotropic glutamate receptor subunits in the developing rat olfactory bulb

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Previous studies using antibodies (Abs) to subgroups of ionotropic glutamate receptors (GluRs) have suggested specific laminar, cellular, and subcellular localization of the GluRs in the adult rat olfactory bulb (OB). The localization of GluRs in developing rat OB, however, remains to be elucidated. To address this question, we studied the immunoreactivity (IR) of Abs to several ionotropic GluRs in the OBs of rats at embryonic day 18 (E18), postnatal day 1(P1) and postnatal day 6 (P6). The rats were anesthetized, killed and the brains immersion fixed with 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M PBS. Twenty micron tissue sections were cut on a cryostat, slide mounted and then incubated for 48 h in primary antibody and processed with Vector Labs ABC Kits. IR for all of the receptor subunits studied was present by E18, although some regional variation was noted within the OB. IR for GluR4 (Chemicon) and GluR5/6/7 (Pharmingen) mirrored the adult pattern. GluR4 IR localized to mitral cells (MCs) and large processes in the external plexiform layer (EPL), consistent with MC processes. In the developing OB, just as in the adult OB, GluR5/6/7 IR was noted predominantly in MCs, large EPL processes and scattered granule cells (GCs). In contrast, the IR for GluR1 (Chemicon) and GluR2/3 (Chemicon) differed slightly from the adult pattern. In the adult OB, GluR1 IR was localized to periglomerular cells (PGs), short axon cells (SAs) and large EPL processes. In the developing OB, however, GluR1 IR was also noted in MC somata. In the adult OB, GluR2/3 was localized exclusively to GCs. However, in the developing OB, IR was also noted in MCs and large EPL processes. These results support the hypothesis that different subsets of GluRs localize to different cell populations and subcellular compartments within the developing as well as the adult OB. This further suggests that the different GluRs may be differentially participating in OB local synaptic circuits. In addition, the appearance of GluR IR at such an early stage of OB embryogenesis is consistent with the notion that glutamate may have an inductive or morphogenic role during development.

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204. Olfactory sampling in lobsters: chemical dynamics during flicking and recovery in the Maine and spiny lobster

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For many organisms, the generation of fluid flow is necessary to bring chemical signals from the environment to microscale environment of the receptor cells. Crustaceans have various mechanisms that enhance the movement of water, and thus, chemical signals, near chemosensory appendages. For example, the flicking by the lateral antennule of lobsters is thought to enhance the delivery of odorant molecules to the sensory aesthetascs on the antennules. By coupling detailed video analysis of antennule movement with microscale electrochemical measurements, it is possible to examine how flicking and recovery influences fluid flow and chemical dynamics in the local environment around aesthetascs. We have mounted a IVEC probe on the lateral antennules of both the Maine, *Homarus americanus*, and spiny, *Panulirus interruptus*, lobsters and used dopamine as a model for an odorant molecule. Lateral antennules with electrodes were then mounted onto complete carapaces with a mechanical device was constructed that allowed us to 'flick' antennules to simulate the flick of a live lobster. Models were placed in a flow tank and an odor plume of dopamine and fluorescein was released upstream. Video records of flicks were synched with 50 Hz IVEC chemical recordings. Results show that flicking increases the probability that the antennule will encounter odor patches. Flicking increases the concentration detected by the electrode and odorant arrives at the IVEC probed located amongst the aesthetascs after the flick is completed. The results from these studies are critical to the understanding of the chemical dynamics that occur during olfactory sampling and may lead to new insights into the physical design of olfactory appendages.

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205. An olfactory P300 from a single olfactory stimulus

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Event-related potential (ERP) recordings of the visual and auditory systems are widely used today in clinical and research settings. Relatively few studies have been done on olfactory ERPs (OERPs) because techniques for recording them have only recently been developed due to problems with control of the stimulus and simultaneous stimulation of the auditory, somatosensory or trigeminal systems. In this study OERPs were recorded monopolarly at the Fz, Cz and Pz electrode sites in 16 young adults (8 males, 8 females) and 16 older adults (8 males, 8 females) using amyl acetate as the odorant. The P300, in the auditory and visual domains is usually elicited by performing an oddball paradigm in which two stimuli are present, one frequent, and one infrequent. The participant is to raise a finger when the infrequent stimulus is heard, a cognitive task which elicits a P300. Resent research in the auditory modality has shown that a P300 can also be elicited by a single auditory stimulus, where the frequent tone is replaced by silence. In the present experiment a single olfactory stimulus with the participants rating the intensity of each odor trial was used to elicit the olfactory P300. The inter-trail interval used was 45 s, similar to that used in the single stimulus auditory P300 paradigm. The resulting olfactory P300s had similar characteristics to P300s of the auditory and visual systems: firstly, latencies between the P200 and P300 were ~100 ms; and secondly the distribution across the scalp was similar, with the P300 being about the same size as the P200 at the Fz location, but increasing in size to the Pz location. This was true for both young and older adults. Future studies will compare the traditional P300 oddball paradigm using olfactory stimuli to the single-stimulus olfactory P300 paradigm, and also compare auditory and visual ERPs to olfactory ERPs.

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206. Cell-type specific representation of olfactory sensory neurons onto the olfactory bulb of channel catfish

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The primary olfactory projections of channel catfish *Ictarulus punctatus* have been examined by *in situ* axon tracing using lipophilic fluorescent dye DiI. Pieces of crystal DiI were placed in either the dorsal or ventral part of the paraformaldehyde-fixed olfactory bulb. Following 14–17 days postapplication, nasal rosettes were cut into 50 mm serial Vibratome sections or each lamella of the rosettes was prepared for flat mount specimens. For ultrastructural study, nasal rosettes were processed for photo-conversion from fluorescent dye DiI into *in situ* DAB precipitations, followed by routine treatment for electron microscopy.

Dye was deposited into each of four quadrants of the olfactory bulb: dorsal, ventral, medial, and lateral. Detailed analyses were performed in cases of either the dorsal or the ventral quadrant of the bulb. Dye application to the dorsal quadrant of the bulb exclusively labeled short olfactory sensory neurons whose whole cell bodies were situated in the superficial half of the olfactory epithelium. Their axons traveled down basally to leave the olfactory epithelium. Structures of their apical dendritic tips were not well resolved under a fluorescence microscope, but seemed to show a tiny crown-like appearance. In contrast the cases of the ventral quadrant produced primarily tall olfactory sensory neurons whose cylindrical cell bodies spanned the entire thickness of the olfactory epithelium. Their nuclei were situated basally and their apical dendritic tips exhibited cilia-like structures. The epithelial surface structures of the two types of olfactory sensory neurons were verified by electron microscopy. Short olfactory sensory neurons had microvilli; tall neurons had cilia.

The patterns of distribution across the epithelium of labeled short and tall olfactory sensory neurons were diffuse in either sectioned or flat-mounted nasal lamellae. No regional segregation such as the dorsomedial and ventrolateral portions of the olfactory epithelium, was observed.

The present study has demonstrated the clear separation of the two types of olfactory sensory neurons on the basis of target domains of the olfactory bulb. That is, microvillar olfactory sensory neurons preferentially project to the dorsal part of the olfactory bulb while the ciliated neurons project to the ventral olfactory bulb.

207. The role of chondroitin sulfate proteoglycans in the *in vitro* morphogenesis of circumvallate papillae

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Gustatory papillae form from epithelial placodes on the dorsal surface of the tongue. The formation and differentiation of similarly placode-derived epithelial specializations, e.g. teeth, whisker follicles, feathers and scales, involve epithelial–mesenchymal interactions, often mediated by extracellular matrix molecules. Distinct temporospatial distributions exist for chondroitin sulfate proteoglycans (ChSPGs) that correlate with stages in the patterning and differentiation of circumvallate papillae *in vivo*, suggesting that these molecules play a role in papillae and possibly taste bud morphogenesis. Papillae development occurs *in utero*. Therefore, any manipulation of their development to determine causal relationships between the distributions of extracellular matrix molecules and events in papillae morphogenesis, is impracticable. To allow such manipulation, we have been examining the validity of an *in vitro* system for circumvallate (CV) papillae development. Early gestational day 11 embryos are partially dissected to produce explants that include the mandibular, hyoid, third and fourth arches and portions of tissues dorsal to the arches that include the trigeminal, geniculate, vestibulocochlear and petrosal ganglia. These explants are cultivated in roller tube culture in BJG$_b$

medium with 10% FBS. In this study, explants were removed after 24, 48, 72 and 96 h of culture and processed for either histological evaluation or immunofluorescent-staining for ChSPGs and NCAM. Small tongues form in the explants after 24 h of culture that are similar in size to gestational day 12 tongues *in vivo*. Histological examination of the explants demonstrate that the sequence of events leading to CV papilla formation in branchial arch explants is similar to those observed *in vivo*. After 24 h a large invaginating placode is observed histologically in the posterior tongue. *In vitro*, as observed *in vivo*, immunofluorescent-staining demonstrates the absence of ChSPGs within the placode epithelium, although they are abundant in adjacent dorsal epithelium. By 48 h of culture, a large nerve bundle, as revealed by NCAM immunostaining, is observed nestling under the placode. After 72 h in culture, the presumptive CV papillae are slightly elevated. Large, NCAM-positive nerve bundles are immediately subjacent to the papillary epithelium and at some points appear to engulf, or surround the epithelium, but do not penetrate into it. By 98 h in culture, the papilla is well elevated; the nerve occupies most of the papilla, although some mesenchymal organization is apparent. Immunofluorescent-staining for NCAM demonstrates that nerve fibers have now penetrated the epithelial basement membrane of the presumptive taste bud region. As is observed *in vivo*, ChSPGs are absent from the apical epithelium of the papilla, although they are present within the adjacent papillary epithelium. The results of this study demonstrate the validity of this *in vivo* system for the study of CV papillae development and provide further support for a role for ChSPGs in papillae morphogenesis.

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208. Cell number and the formation of ectopic glomeruli

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The glomerulus is a spheroid neuropil wherein olfactory and vomeronasal organ (VNO) receptor axons synapse with second order dendrites of olfactory bulb neurons. Previously, we showed (Morrison *et al.*, 1995) that whole neonatal VNO transplanted into littermate cerebral cortex form, in the absence of target neurons, fibrous plexuses which contain distinct glomerular-like structures. To determine if transplant cell number affects the formation of these glomerular-like structures, we cut neonatal VNOs into thirds and transplanted one fragment each into littermate parietal cortex. Twenty to 60 days after surgery, hosts were injected with bromodeoxyuridine (BrdU). Approximately 16 h later, they were perfused with saline and Bouin's fixative, the brains were embedded in paraffin and sectioned for light microscopy. Sections were processed by a rapid Golgi technique or by immunocytochemistry. The transplanted tissue remained neurogenic since some BrdU(+) cells could be identified as neuronal by morphology. Transplant axons identified by morphology or NCAM labeling, grew into the host brain. Ectopic glomeruli were not seen in any host that received a single VNO fragment. A second host group received VNO which had been sectioned into thirds, then all three fragments of the same VNO were transplanted into each host. At least one fiber plexus developed in several hosts each of which contained one or more glomerular-like structures. These results suggest that the number of receptor neurons in the transplant influence, at least indirectly, the formation of ectopic glomeruli.

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209. Beyond liking to intake: assessing acceptance by the pattern of drinking beverages

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Most human psychophysics involving taste and smell use direct measures of acceptance, such as ratings of liking, or choice tasks such as paired comparisons. Both the literature on animal taste preferences and observations of product successes and failures in the marketplace reveal that stimuli which are highly preferred in a short term taste test are necessarily consumed more than stimuli which are less preferred. In order to delve more deeply into the relation between liking and intake we constructed an instrument which combines a scale with a computer. The device records the moment to moment weight of food on it in 3 s intervals, and thus monitors and measures intake.

The experiment involved four different beverages: water, cola, orange juice and ice tea. The same 20 individuals were presented with glasses comprising of 16 oz. of the beverage, on four separate days, one day for each beverage, with the order of beverages randomized. Each individual was told to drink as much of the beverage as he wanted. There was no prescribed length of the session, but a session tended to finish within 6 min.

The curves reveal radically different drinking patterns. Cola was drunk at a fairly constant rate, but with the lowest slope (i.e. most slowly). There did not appear to be a leveling off point for cola. Orange juice was drunk most quickly, but leveled off beyond 2 min. Water and iced tea showed intermediate patterns.

The method provides the investigator with a new tool to understand food and beverage preferences. The instrumentation enables the study of solid foods as well as liquids. Furthermore, the instrumentation has been enhanced to allow the panelist to look at a video of the beverage instead of the actual beverage, and record expected intake by pressing a button. An analog cup on the computer screen shows the amount 'ingested' by this conceptual drinking. Finally, a questionnaire on the sensory attributes of the beverage has been incorporated into the research protocol in order to provide data using more traditional methods.

210. Olfactory mucus enhances chemosensory ligand responses in vomeronasal bipolar neurons

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Chemosensory ligands reaching the vomeronasal (VN) organ must first travel through the nasal passages. By analogy to the main olfactory system where an odorant binding protein delivers various odor molecules to the main olfactory receptors, we hypothesized that the nasal mucus might also contain a binding protein which delivers chemosensory ligands to the VN organ. We studied this hypothesis by comparing the effects of a chemosensory ligand alone or a chemosensory ligand mixed with mucus on the electrical properties of VN bipolar neurons.

Bipolar neurons were dissociated from mouse VN organs as previously described and whole cell voltage clamp recordings were obtained. A series of depolarizing and hyperpolarizing voltage steps (–80 to 80 mV in 20 mV steps for 200 ms) elicited a transient inward current following by a sustained outward current. The chemosensory ligands, dehydro-*exo*-brevicomin, 2-(*s-butyl*)-thiazoline, and lactol were puffed directly onto the dendritic knob of the VN neuron as was KCl and bath solution. At steady state current conditions and a holding potential of –70 mV, the application of any of the three chemosensory ligands evoked an outward current while the application of KCl induced an inward current. Bath solution had no effect.

Nasal mucus was collected from female mice by lavage of the nasal passages with a small volume of bath solution. When nasal mucus was mixed with the chemosensory ligands, the amplitude of the current response was increased. Pre-boiling the mucus prior to mixing with chemosensory ligands eliminated this potentiation. These findings support the notion that nasal mucus contains an agent that facilitates the binding of chemosensory ligands to receptor sites on VN neurons.

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211. Cloning and molecular characterization of two components of the IP_3 pathway from lobster olfactory organ

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Physiological and biochemical evidence implicates inositol 1,4,5-trisphosphate (IP_3) as well as adenosine 3',5'-cyclic monophosphate (cAMP) as olfactory second messengers (Breer and Boekhoff). Analogous to the cAMP transduction pathway, where the major molecular components have been cloned, sequenced and localized to the transduction zone, the IP_3 pathway has been minimally characterized at the molecular level, although recently Abogadie *et al.* (1995) characterized an olfactory phospholipase C from catfish. Previous work in our lab has implicated a plasma membrane-localized IP_3R and a G_q protein in olfactory transduction in lobster (Fadool and Ache, 1992; Fadool *et al.*, 1995; Estey *et al.*, 1996; Munger *et al.*, 1996). We have now fully cloned and sequenced cDNAs encoding an IP_3R and a G_q protein from the spiny lobster olfactory organ. The IP_3R cDNA has an 8409 bp open reading frame coding for 2803 amino acids (a computed 320 kDa protein), and is homologous to known IP_3Rs (50–61% amino acid identity). The message has been localized to both olfactory organ and brain (Munger *et al.*, 1996) The G_q cDNA has an open reading frame of 1059 bp (353 aa); the sequence is homologous to known G_q proteins (70–83% amino acid identity). The computed molecular weight of the protein is 41.5 kDa, consistent with that of the native protein (Fadool *et al.*, 1995). Northern analysis demonstrates a 4.5 kDa G_q message in olfactory organ. We are in the process of localizing these molecules to the transduction zone.

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212. Late (cognitive) components of the olfactory event-related potential are more sensitive to aging than early components

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The present investigation explored the relative effect sizes for age-associated differences in amplitude and latency of the early and late components of the olfactory event-related potential. Methods similar to those of Kobal and Hummel and Murphy *et al.* were employed to record OERPs in 16 young and 16 elderly subjects. Stimuli were presented olfactometrically in a stream of heated (36.5°C) and humidified (80% RH) air 45, 60 and 90 s ISIs. Stimulus rise time did not exceed 20 ms. EEG activity was recorded from the Fz, Cz and Pz electrode positions of the international 10/20 system, amplified, and filtered using a computer-based recording system. Electro-ocular activity also was monitored. Breathing was restricted to the mouth during trials, keeping nasal flow rate constant. Subjects produced magnitude estimates to monitor odor strength, and producing these estimates appears to have elicited a P3. Age-related differences in latency from stimulus onset as well as amplitudes of N1, P2, N2 and P3 of the OERP were assessed, using the $\eta 2$ partial after ANOVA. In addition, detection thresholds for odor were measured using a two-alternative, forced-choice method. ENT examinations ruled out nasal sinus disease. Neuropsychological testing screened out dementia and provided the opportunity to assess the relationship

between OERP measures and neuropsychological function. Overall, older subjects had smaller amplitudes and longer latencies than young subjects. Computation of effect sizes showed these differences to be largest for the later components and particularly for the P3. Females tended to show larger amplitudes than males and this difference was greatest in the elderly. Older men required longer ISIs to produce maximum amplitudes. Interestingly, there was a statistically significant difference in amplitude between young and elderly on the first trial taken alone. Threshold showed a smaller age effect than the OERP, but thresholds correlated withearly events of the OERP. Relationships between neuropsychological variables and P3 amplitude and latency suggest age-related degeneration in frontal and temporal lobe structures.

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213. Investigation of two potassium currents in neurons of the rostral nucleus of the solitary tract

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Neurons of the rostral, gustatory area of the nucleus of the solitary tract (rNST) can be separated into four different types based on their intrinsic firing properties followed current injection pulses. To develop an ionic current model of these neuron types, properties of the different ion channels of rNST neurons are being measured and then used to construct models. We are initially modeling type II neurons, which respond to a depolarization witha regular repetitive discharge pattern, but a prior hyperpolarization either delays the occurrence of the first action potential or increases the length of the first interspike interval. To study the different ionic currents we have made whole cell recordings in a horizontal slice preparation of the rNST in rats. Once isolated, the type was determined in current clamp mode and then currents were measured in voltage clamp. Ionic channel blockers and different voltage protocols were used to isolate and characterize two potassium currents, I_D and I_A. These two currents are responsible for a delay in the onset of firing in response to long depolarizing stimuli. While I_A is a fast inactivating current and can cause just brief delays, I_D is a slowly inactivating one and can cause much longer delays. I_D was blocked by 30 μM 4-aminopyridine (4-AP). I_A was blocked by 5 mM 4-AP. In type II neurons this block resulted in the disappearance of the delay at the onset of repetitive firing that normally follows brief membrane hyperpolarization. Voltage clamp records were analyzed according to a general Hodgkin–Huxley description of whole cell currents. Measurements of the half-activation potentials, reversal potentials, steady-state values of gating variables and reciprocals of the slope of activation curve at the half-activation potential were used to find a parameter set that best fits the experimental data. By using mathematical modeling for the different currents, we can propose explanations for the existence of the four types of neurons and unify their description.

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214. Putative salt taste receptors in the toad skin

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Toads obtain water by osmotic flow across their skin. Dehydrated toads (*Bufo punctatus*) exhibit stereotyped behavior termed the water absorption responses (WR), in which the ventral skin is pressed onto a surface wetted with pure water. When an animal is placed on a NaCl solution, the WR is suppressed. However, if 10 μM amiloride is added to the solution, the WR is resumed. The spinal nerves innervating the ventral skin of toads (*Bufo marinus*) respond to NaCl and KCl solution, and amiloride suppresses only neural responses to sodium salt. These observations have lead us to search for cutaneous receptors that toads may use to detect osmolality or salt taste. Fluorescent dye dil was applied to the spinal nerves (5–7) of toads (*Bufo alvarius*) fixed with 4% paraformaldehyde and 0.5% glutaraldehyde to label the fibers and target cells for innervation. Skin tissues were examined in 200 μm sections with a confocal laser scanning microscope (Zeiss LSM 410). The peripheral nerve fibers passed into the epithelium of the toad skin. Occasionally, brightly fluorescent cells occurred in a small cluster with each cell isolated. Single fibers reached those solitary cells, showing transneuronal diffusion of the dye. Surprisingly, no cell was directly faced to the surface of the skin, but located in the deeper layer i.e. germinativum cell layer. The nerve fibers expanded in germinativum cell layer or even deeper layers. Few fibers extended to superficial granulosum cell layer. Therefore, either labeled cells free nerve fibers, or both, may be a transducer element in detecting Na^+ flowing through amiloride-blockable channels in the toad skin.

215. Olfactory sensitivities of foraging procellariid seabirds in the Aleutian Islands

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During the spring of 1995, we conducted a series of shipboard experiments designed to examine what role olfaction might play in the behavior of procellariid seabirds foraging in the Bering Sea. This work compliments earlier studies conducted on Antarctic procellariids (Nevitt *et al.*, 1995). To create an odor trail for birds to follow, an odor-saturated wick mounted on a floating buoy was deployed and allowed to drift 150–200 m off the bow of the vessel. Teams of observers recorded numbers, behaviors and species identities of birds in a 100 m circle around the odor buoy for a 30 min observation period. To control for any attraction birds might show to the buoy alone, birds' responses to unscented buoys were also recorded. Four candidate prey-related odors were tested: fish oil, ammonia, dimethyl sulfide (a metabolic byproduct of

phytoplankton) and pyrazene (a primary krill aromatic). Each of the four odors was tested five times against five controls at five different locations, all in the vicinity of Unimak Pass, AK. Preliminary analysis suggests that local procellariid species including short-tailed shearwaters, sooty shearwaters and Northern fulmars showed a greater tendency to respond to fish oil and ammonia than to control buoys. Fulmars were more strongly attracted to these odors than any other bird tested. Responses to dimethyl sulfide and pyrazene were qualitatively much less robust. These results contrast strikingly to responses of Antarctic procellariids tested in the vicinity of South Georgia Island in the South Atlantic. We speculate that these differences reflect a plasticity in the foraging strategies that procellariids exploit in areas of grossly different food availability or distribution. Further analysis will explore these differences in greater detail.

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216. Progressive decline in olfactory sensitivity in older adults with Down's syndrome

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Neuropathological changes similar to those found in patients with Alzheimer's disease (AD) have been observed in older individuals with Down's syndrome (DS). These changes, consisting of neuritic plaques and neurofibrillary tangles, are particularly marked in olfactory pathways and olfactory impairment has been reported in both AD and older DS subjects. In a previous study by Murphy *et al.* a comparison between DS and matched normal controls revealed that DS subjects showed significant impairment in both odor threshold and odor identification tests. In AD it has been noted that progression of the disease is mirrored by progressive olfactory decline. We were interested in assessing the rate of olfactory decline in DS. The present study compares baseline measures to follow-up testing. A total of 35 subjects participated in the follow-up assessment: 19 with DS (mean age = 30.9 years old), and 16 controls (mean age = 36.1 years old). A global measure of cognitive status was assessed using the dementia rating scale. Threshold for butanol was assessed using the forced-choice ascending method with a criterion of five correct choices at a given concentration level for threshold determination. Two bottles (stimuli and blank) were presented, and the subject's task was to choose the bottle that smelled stronger. A 2 × 2 analysis of variance indicated a significant interaction: sensitivity of the DS subjects was significantly poorer than that of the controls initially, and showed significant decline from baseline to follow-up, while control subjects showed no significant decline over time. DS has been suggested as a high-risk condition for dementia of the Alzheimer's type. Follow-up studies of this nature with DS subjects can be invaluable for providing a more crystallized understanding of the time course of the disease.

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217. Neurotrophins in the tongue and its taste buds; mRNA expression pattern, bioactivity and appearance in BDNF and NT3 null-mutated mice

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Innervation of taste buds and tongue papillae has been a matter of interest in the past century. Many studies have been undertaken to demonstrate the dependency of taste bud survival on intact innervation. Different neurotransmitters from the nerves have been suggested as factors for taste bud survival but relatively few studies have dealt with the development of taste bud innervation. In a part of this study, using *in situ* hybridization histochemistry, we now show that NT3 and BDNF mRNA are expressed in a specific pattern in the taste buds, tongue papillae and lingual epithelium during development and that expression persists into adult hood. Using an *in vitro* bioassay, we also show that tissues from different parts of the tongue, related to different taste bud subpopulations, have neurotrophic competence and that the neurite outgrowth from a set of different chick embryo ganglia could be interpreted and charted to explain the results according to expression of different neurotrophins. In the last part of the study, we present preliminary data from BDNF and NT3 knockout mice. Despite null-mutation of the genes for these two constantly present neurotrophins of taste buds and tongue papillae, the taste buds develop, become innervated and persist as long as the animals are alive. Further studies are needed to determine if taste bud-morphology and/or innervation is disturbed in null-mutated animals. We suggest that the neurotrophins BDNF and NT3 are involved in the initiation and maintenance of gustatory and lingual sensory innervation respectively. Extracts from different parts of the tongue with different taste bud subpopulations are indeed bioactive and give rise to neurite outgrowth from different ganglia. The neurite outgrowth scoring results are indicative of a range of bioactivity for several of the known neurotrophic factors and might include several unknown factors as well. Existence of taste buds in both BDNF and NT3 knockout mice also indicates that the sensogustatory innervation of the tongue is complicated and suggests that other known and/or unknown factors may play important roles in both initiation and maintenance of taste buds and their innervation.

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218. Temporal aspects of pheromone-sensitive receptor neuron responses are differentially affected by pulsed stimuli in the adult cabbage looper moth, *Trichoplusia ni*

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Many male moths, including the cabbage looper moth, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae), fly upwind in response to stimulation with pheromone blends produced and released by 'calling' conspecific females. Previous studies have suggested that the stimulus plume downwind from a calling female moth is discontinuous and composed of discreet packets of pheromones. The orientation of the male to the female moth is characterized by a pattern of direct upwind flight interspersed with 'casting' flight (perpendicular to the direction of the wind). Specialized sensilla on the male antenna contain highly sensitive and specific olfactory receptor neurons which respond to stimulation with these olfactory signals, by producing a phasic-tonic pattern of action potential discharge. To evaluate the importance of pulsed stimuli on the temporal components of the neural response, we stimulated pheromone-sensitive sensilla with short pulses (200 ms) of the major component of the pheromone blend, *Z*-7, dodecen-1-ol acetate (*Z*-7,12:AC) at a dosage approximating the concentration released by a calling female moth. Different stimulus protocols, in which pairs of pulses were separated from each other by varying intervals, were tested and evaluated. Intervals between stimulus pulses, as short as 30 ms, reduced the total response to the second pulse by >50%. This decrement was graded, smaller reductions occurred with longer interpulse intervals. When the interval between stimulus pulses was longer than two s the responses elicited by the two pulses were similar. In addition, the decrement in total response observed at any interval preferentially involved the initial phasic component of the discharge. Implications for male orientation in natural, female-produced, pheromone plumes are discussed.

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219. BDNF null mutant mice have disrupted gustatory epithelia

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We obtained heterozygous brain derived neurotrophic factor (BDNF) knockout mice from Jackson Labs. We bred them to generate progeny homozygous (–/–) for functionally inactivated BDNF genes. This absence of BDNF throughout development was associated with some defects in the vallate papilla, reduced innervation and fewer taste buds. To rule out sluggish development, we have structured our analysis to examine pairs of BDNF –/– null mutant and BDNF +/+ (wild type) mice matched for age and crown-rump length. On average the vallate papilla of BDNF –/– mice aged 7 days (P7) was 25% narrower than P7 wild type mice. One neonatal P7 BDNF –/– mouse had only one of two vallate trenches. Its stunted vallate papilla lay at the base of a pronounced cavity in the tongue.

Fungiform taste buds presented a range of defects. Some were seemingly normal, but others were extensively vacuolated. P7 BDNF +/+ mice averaged more than 100 vallate taste buds. One P7 BDNF –/– mutant had 17 sparsely innervated vallate taste buds. Another P7 BDNF –/– had only five vallate taste buds.

Hence, the absence of BDNF can lead to defective gustatory papilla, sparse gustatory innervation and a nearly complete loss of vallate taste buds. Our working hypothesis is that the neurotrophin BDNF acts to support developing taste neurons which in turn are required for the formation of vallate taste buds. NT-3 or other compensatory processes can account for residual innervation and taste buds in BDNF null mutants.

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220. Responses of mudpuppy taste receptor cells to denatonium: $[Ca^{2+}]_i$, ionic current and feeding behavior

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Several mechanisms for bitter taste transduction have been proposed, including: (i) release of Ca^{2+} from internal stores (Akabas *et al.*, 1988; Spielman *et al.*, 1994); (ii) activation of phosphodiesterase via a G-protein coupled receptor (Ruiz-Avila *et al.*, 1995); and (iii) direct block of apical K^+ channels (Cummings and Kinnamon, 1992). In this study, responses of isolated mudpuppy taste receptor cells to denatonium benzoate (DN) were studied by Ca^{2+} imaging with fura-2, whole-cell recording and feeding behavior. In most taste receptor cells, DN (5 mM) increased $[Ca^{2+}]_i$ by 50–150%. The response began in the apical tip of the receptor cell. The response to DN was present in a Ca^{2+}-free external solution and was blocked after depletion of internal Ca^{2+} stores with thapsigargin (1 μM), a Ca^{2+}-ATPase inhibitor. Ryanodine (10–200 μM) had no direct effect on $[Ca^{2+}]_i$ and failed to block the DN responses. These results suggest that DN increased $[Ca^{2+}]_i$ by release from thapsigargin-sensitive, ryanodine-insensitive Ca^{2+} stores. Whole-cell recording showed that DN (1–5 mM) potentiated K^+ and Cl^- currents in most taste receptor cells. The current response to DN was blocked by inclusion of GDP-bS in the pipette. The $[Ca^{2+}]_i$ response to DN persisted in pertussis toxin-treated cells, suggesting the involvement of a pertussis toxin-insensitive G-protein. The $[Ca^{2+}]_i$ response to DN was blocked by U73122 (5 μM), a phospholipase C inhibitor, suggesting that the $[Ca^{2+}]_i$ response is due primarily to an IP3-dependent increase of $[Ca^{2+}]_i$. Mudpuppies were presented with gelatin cubes containing either 1 mM DN or minnow extract (0.1 g/ml) to determine the behavioral significance of DN to mudpuppies. Gelatin cubes containing minnow extract were not rejected by any mudpuppies (n = 10), while >90% of animals rejected gelatin cubes containing DN (n = 12). The behavioural and physiological data suggest that the mudpuppy is an

appropriate model for examining mechanisms involved in DN transduction.

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221. Evidence that developing olfactory axons are attracted to their targets

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The targeting of olfactory sensory axons to the olfactory part of the brain and subsequently to individual glomeruli during development is a complex and poorly understood phenomenon. A complete understanding of the phenomenon awaits a molecular description of olfactory coding. In our ongoing studies of the development of olfactory glomeruli in the brain of the moth *Manduca sexta*, however, we recently have discovered potentially significant features of the early olfactory projection in this species. First, if the earliest developing olfactory axons are experimentally misrouted so that they encounter the brain at an ectopic location, they frequently course over the surface of the ectopic region toward the antennal lobe (the insect's equivalent of the olfactory bulb) and only upon reaching the lobe do they dive in to form glomeruli. Secondly, in normally developing lobes, as axons home in on an individual developing glomerulus, they can arrive from many directions via many loosely organized fascicles. Their growth cones can be complex in the nerve layer and in the nascent glomerulus. All of these features suggest that the earliest axons to innervate the lobe are attracted to a target, rather than following a prescribed path; later-growing axons may then fasciculate with the earlier ones. As ingrowing olfactory receptor axons arrive in the lobe, their terminals coalesce to form 'protoglomeruli,' which become surrounded by glial cells. If the number of glial cells is experimentally reduced during development, the newly formed axonal protoglomeruli quickly disintegrate. This indicates that glial cells normally constrain axonal branching to protoglomerular clusters, and also suggests that the influence attracting axons to a particular destination may be ephemeral.

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222. Lateralization of odor recognition

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In an odor recognition experiment, we investigated the difference between the right and left nostrils. Two principal questions were pursued. (i) Would monorhinically presented odors be recognized differently via the left nostril compared with the right? Several studies indicate that the right nostril stands out as superior at least for some types of olfactory functioning. Three aspects of performance were investigated in this study: correctness, latency, and confidence of response. (ii) Would odor recognition be sensitive to a shift of nostril from study to test? That is, would an odor studied via one nostril be differently remembered if presented later to the same versus the other nostril? Sixty-four right handed young adults (half males, half females) were presented with six (target) odors, monorhinically, in a study phase and were asked when they had smelled them last. In a subsequent test just minutes later, the target odors were presented ipsilaterally or contralaterally to the nostril used at study. In the test phase, subjects were presented with 12 test odors (six targets and six lures) and asked to press a button when they knew whether a test odor had been presented during the study phase. After giving their answers ('yes' to old and 'no' to new), subjects also gave confidence ratings to indicate how sure they felt that their answers were correct. In a final phase, participants were asked to identify the odors encountered as targets and lures. Latency of response tended to differentiate between the nostrils. Participants responded faster when odors were presented to the right nostril at test (averaging 0.260 log s) than to the left (0.313 log s). Furthermore, there was a marked difference between the two groups that encountered the odors on the same side and on different sides between study and test. Ipsilateral recognition was much more accurate than contralateral. The results are discussed in terms of olfactory lateralization of hemispheric functioning.

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223. Capsaicin suppresses responses of rat chorda tympani nerve fibers to NaCl

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Single- and multi-fiber preparations of the rat chorda tympani nerve were used to study the mechanism of action of the pungent component of red peppers, capsaicin, on salt-taste transduction. Capsaicin (328 μM) suppressed the responses to NaCl of all N-type fibers (sodium-specific and non- or 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). In both N- and E-types, 5% ethanol (the vehicle for capsaicin) reduced the response to 100 mM NaCl. However, the suppressing effect of capsaicin on the response of the N-type fiber to 100 mM NaCl was significantly stronger than was the effect of 5% ethanol. The suppressive effect continued for at least 20 s after the simultaneous application of 100 p.p.m. capsaicin-100 mM NaCl. The mechanism of the effect of capsaicin on the salt taste transduction in the CT nerve fibers will be discussed.

224. Development and regeneration of chemosensory neurons in the rat vomeronasal organ

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Neurogenesis of chemosensory neurons occurs during development and throughout the life span of most mammals. Neurogenesis and regeneration of chemosensory neurons also occur during recovery from injury. In this study we used immunohistochemical methods to identify chemosensory neurons at different stages of development and compared them to neurons undergoing regeneration following nerve transection. Results suggest that chemosensory cells can be classified into four stages. The first stage was characterized by nestin single positive cells. Nestin positive cells were observed at early stages in development and after 6–10 days of recovery from nerve transection. During this first stage there was a rapid increase in cell number and epithelial thickness. Nestin-single positive cells may represent the precursor cells for chemosensory neurons. In the second stage cells were nestin and neural cell adhesion molecule (N-CAM) double positive. Cells at this stage may represent a transition from precursor to immature neurons. In the third stage cells were N-CAM single positive cells. After nerve transection, N-CAM positive cells degenerated and most disappeared by 4 days. Newly regenerated N-CAM positive cells reappeared after 8 days of recovery and subsequently increased in number. The final stage of cell development was characterized by olfactory marker protein (OMP) and N-CAM double positive cells. These cells represent mature sensory neurons. This study suggests that immunohistochemical markers can be used to identify cells at four stages of development and could play an important role in future studies of neurogenesis and regeneration in chemosensory systems.

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225. Amiloride effects on human taste quality: methods of magnitude estimation

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The effects of amiloride on human taste perception are conflicting. Some studies have shown a suppression of NaCl saltiness by amiloride; others show no effect on saltiness but a significant reduction in sourness. In studies that demonstrate a reduction of *saltiness* subjects estimated only saltiness. Many salts elicit not only a salty taste but also one or more side tastes. In studies that showed an amiloride effect on *sourness* (for NaCl, Na-gluconate and LiCl) and not saltiness, subjects estimated all qualities on each trial. This suggests that the apical Na^+ channels mediate the sourness but not the saltiness of Na^+ and Li^+ salts.

The present study examines the role of the psychophysical method in these conflicting results. First we used a modified profile method; instead of rating total intensity and dividing this estimate among the appropriate qualities, subjects estimated the taste qualities of five LiCl concentrations independently, after water and after amiloride. Another group estimated only the total intensity in proportion to the same modulus (0.1 mM QHCl). The sum of the quality estimates of the first group equaled the total intensity estimate of the second. A third group estimated only the saltiness of LiCl, and a fourth only its sourness. These latter experiments showed an amiloride suppression of LiCl saltiness and sourness respectively. When subjects estimate only one of these qualities, amiloride reduces it, but if subjects attend to all four qualities, amiloride specifically reduces the sourness of LiCl.

To reveal the effect of amiloride on a specific quality of a salt, the psychophysical method should force subjects to focus their attention on all qualities on each trial. Attending to a single quality may yield an estimate more comparable to the total intensity of the salt.

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226. Multisapophoric molecules—an investigation into the solution properties and taste of D-glucono-1,5-lactone, D-glucosamine hydrochloride and sodium saccharin

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Multisapophoric molecules give rise to more than one basic taste. Three different examples are now structurally examined in solution.

D-Glucono-1,5-lactone hydrolyses rapidly in water to D-gluconic acid. D-Gluconic acid predominates in the solution and gradually lactonises to the D-glucono-1,4-lactone. At the start of hydrolysis, the mixture tastes sweet and bitter and then rapidly changes to sour. Glucono-1,5-lactone, apparent specific volume (ASV) = 0.61 $cm^3\ g^{-1}$, has a pyranose structure and is expected to taste sweet/bitter. Glucono-1,4-lactone (ASV = 0.63 $cm^3\ g^{-1}$) is also sweet/bitter. Gluconic acid has both sweet and sour sapophores but tastes sour as it gives rise to protons in solution. Moreover, the ASV of 0.51 $cm^3\ g^{-1}$ places the molecule in the acid range. Apparent specific volumes fall from 0.609 to 0.579 $cm^3\ g^{-1}$ during the first one and a half h of hydrolysis as the solution becomes more acidic. Isentropic apparent molar compressibilities also fall from -1.307×10^{-3} to -2.004×10^{-3} $cm^3\ g^{-1}\ bar^{-1}$ as the solution becomes less compressible.

D-Glucosamine hydrochloride has potential sweet, salty and bitter sapophores. Its apparent specific volume (0.61 $cm^3\ g^{-1}$) places it in the sweet range but it possesses more than one basic taste. Substitution of –OH group at carbon number 2 by $NH_3^+Cl^-$ may affect the orientation of the molecule at the sweet receptor, hence causing a lack of sweetness. The salty taste is probably due to the dissociated form of the salt which accedes rapidly to the salt receptors. The ionic nature of glucosamine hydrochloride is also reflected in the large negative compressibility value (Partial

isentropic compressibility = -3.7×10^{-3} cm^3 mol^{-1} bar^{-1}) which is caused by the disturbance of the H-bonded structure of water by electrostriction around the ion.

Sodium saccharin (ASV = 0.57 cm^3 g^{-1}) is sweet with a bitter aftertaste and can be assumed to be almost completely dissociated in solution. The saccharinic ion therefore gives rise to a large negative isentropic partial molar compressibility value (-3.4×10^{-3} cm^3 mol^{-1} bar^{-1}) in solution, and this presumably governs the taste characteristics.

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227. Intakes and patterns of ingestion by the rat for emulsions of sucrose or NaCl with corn oil

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Smith *et al.* have described intakes and patterns of intakes of sucrose and NaCl. Little information exists regarding the intake of fat in liquid form by the rat. One group of rats was tested with emulsions of various concentrations of corn oil mixed with sucrose and a second group received the corn oil mixed with NaCl. All tests were 24 h two-bottle tests with food and water always present. We showed that as the concentration of sucrose in solution increased the intake of the solute (not solution) increased. Since the intake of Purina Chow decreased as the concentration of sucrose increased, the overall consumption of calories remained relatively constant. We found that when corn oil was added to any of the sucrose solutions, the amount of the mixture ingested did not decrease significantly as the percentage of fat in the emulsion increased, leading to a significant increase in total intake of calories. Water intake was negligible when either the sucrose or the sucrose–fat mixtures were available. NaCl solution intake in the second group of rats peaked at isotonic level and was lowered at higher concentrations. When the corn oil was added to isotonic and hypertonic solutions the mixture intake increased significantly, hence, calorie intake increased. Water intake was high when either the NaCl or the NaCl–fat mixtures were available. Counterbalancing the presentation of the various sucrose–fat or salt–fat mixtures indicated no order effect.

To further investigate the effects of the corn oil emulsions on the eating and drinking behavior of the rat, 16 rats were placed in an apparatus that measured the number and duration of food and water bouts. When the rats received the sucrose–fat or the salt–fat mixtures, the number of drinking bouts decreased and the duration of the bouts increased when compared with baseline water drinking.

The rat, either by the oral cavity or post-ingestional processes, seemed to be able to recognize and regulate for sucrose or salt solutions, but failed to do so with the addition of fat. To ascertain the role of taste in the intake of these fat solutions, additional short term taste tests were conducted.

228. Taste sensitivity and preference in the frail, institutionalized elderly

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On average, gustatory function declines with increasing age. With a few exceptions, studies have used healthy community-dwelling subjects, and nursing home residents have been avoided because, in that population, age and poor health tend to be confounded. Although this may be good science, it is unfortunate from a practical point of view: The healthy, younger community-dwelling group has good nutrition and relatively few food complaints whereas the nursing home population is at greater nutritional risk and has more food-quality grievances. Institutionalized elderly subjects (EI) and community-dwelling elders (EC) rated intensity of sucrose, NaCl, citric acid, quinine sulphate and urea solutions using the rapid screening test developed by the Monell-Jefferson Taste and Smell Disorders Clinic. Overall, the EI group had significantly lower ratings than the EC group. The EI group also had significantly more taste-identification errors and differed in preferred level of tastants in a beverage or soup. Therefore, the chemosensory functioning of the community-dwelling young elderly should not be seen as representative of that of frail, elderly residents of nursing homes. The EI and EC groups differed in bothhealth indices and in age. However, a healthy, non-institutionalized, age-matched control group also had significantly better taste functioning than the EI group.

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229. Olfactory dysfunction in head trauma patients presenting to a smell and taste center

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To explore influences of head trauma on olfactory function in a smell and taste center referral population, we evaluated UPSIT scores for 268 consecutive patients presenting to our center with complaints of trauma-induced olfactory loss. Sixty-six percent were anosmic, 20% microsmic, 13% normosmic and 1% normosmic with responses indicative of malingering. No meaningful relationship was present between UPSIT scores and the time since traumatic episode ($r = 0.06$, $P > 0.20$). Retesting of 66 of the patients (35 males, 31 females), at individual test–retest intervals ranging from 0.5 to 13 years, revealed a slight improvement between test occasions [respective means = 18.82 and 20.58; $F(1,64) = 5.21$, $P = 0.026$; test–retest $r = 0.81$, $P < 0.001$]. Interestingly, non-anosmic patients (UPSIT scores ≥19) with initial UPSIT scores ranging from 19 to 29 showed more improvement than did non-anosmic patients with UPSIT scores ≥30 [$F(1,23) = 4.182$, $P < 0.05$]. Seventy-one percent of patients with initial UPSIT scores ranging from 19–29 improved in performance, whereas only 46% of those with UPSIT scores ≥30 did so. Of 169 patients for whom relevant data were available, 39% received the

primary impact to the occipital region, 38% to the front of the head, 11% to the left temporal region, 10% to the right temporal region and 2% to the crown. Frontal impacts produced less dysfunction than occipital or temporal impacts (respective UPSIT values: 19.9, 15.83 and 16.31; $Ps < 0.05$). No relationship was present between the duration of a patient's unconsciousness and his or her olfactory test score ($r = -0.05$, $P > 0.5$). In general, patients with greater olfactory deficits evidenced greater depression, as measured by the Beck depression inventory ($r = -0.31$, $P < 0.001$). Overall, these data indicate that most individuals presenting to a smell and taste center with head trauma-related olfactory loss have total or near-total anosmia and that very little return of function can be expected over time.

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230. Cytokeratin immunoreactivity in taste buds of sodium-restricted and control rats after denervation

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Following regeneration and maintenance on a sodium-restricted diet (0.03%), CT responses to sodium salts are selectively and profoundly reduced. Within days after section of the contralateral CT, the uncut CT also exhibits highly attenuated sodium taste function. Rats must be placed on the sodium-restricted diet by 14 days following nerve section to promote reduced sodium taste responses by the regenerated CT. In contrast, sodium restriction must be initiated within the first 7 days following contralateral nerve section to observe the functional alterations exhibited by the uncut CT. Given that there are discrete periods after CT section when the environment influences taste receptor function, we proposed that there are also changes in taste receptor structure during this time that differ with respect to dietary condition. Tongues were examined for cytokeratin 19 (CK19) immunoreactivity at 0 (i.e. uncut controls), 2, 5, 7 and 14 days after rats received unilateral CT section and were placed on the sodium-restricted diet or maintained on a control diet. CK19 (monoclonal antibody 4.62) is expressed in fusiform taste cells, and is postulated to be a marker for functional receptor cells. While there was no loss of CK19 positive buds from the intact side of the tongues of either restricted or control rats at any time point examined, rapid decreases in CK19 immunoreactivity occurred in denervated taste buds. Within the first 7 days following nerve section, only ~11% of taste buds from control rats were CK19 positive. However, ~35% of taste buds from sodium-restricted rats remained immunopositive at the same time point. Thus, during the first week after denervation, sodium-restriction protects a portion of taste buds from degeneration.

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231. Unilateral naris occlusion and calbindin-immunoreactivity in the developing rat olfactory bulb

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Early naris occlusion has profound consequences on the subsequent morphological and physiological development of the ipsilateral olfactory bulb. These changes include reduced bulb volume, cellular metabolism and protein synthesis, and increased cell death. Although the changes are diverse, perhaps alterations in intracellular calcium may be a common mechanism accounting for the effects. Calcium is involved in synaptic transmission and in a variety of intracellular second messenger signaling pathways. The present study begins to address the putative role of calcium in bulb development by characterizing the intensity and distribution of calbindin D-28k, a calcium binding protein, with ABC immunohistochemistry. Rat pups were occluded on the day after birth (P1) and killed on P10, P20 or P30. In addition, bulbs were examined from rats occluded on P30 and killed on P60. In control bulbs (contralateral to the occluded nares), labeling intensity increased steadily until P60. Immunoreactivity was most prominent in somata within periglomerular and superficial external plexiform regions, although cells were occasionally observed in deeper layers. Labeled periglomerular processes typically formed a dense plexus within glomeruli. Following 30 days of occlusion, however, there was a profound decrease in both the intensity and number of labeled periglomerular cells in experimental bulbs. This result was seen in pups occluded on either P1 or P30. Furthermore, there was minimal labeling of periglomerular cell processes in experimental bulbs. The reduction of calbindin immunoreactivity in experimental bulbs may reflect a change in intracellular calcium buffering and a deregulation of calcium homeostasis. This suggests changes in calcium-mediated events may be associated with the consequences of naris occlusion.

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232. Computer modeling and odorant random repertoire docking in human olfactory receptors

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Receptor proteins are believed to provide the molecular basis for human olfactory sensitivity and discrimination. Recently, genes coding for olfactory receptors (ORs) have been identified, cloned and sequenced from the human genome (Ben-Arie *et al.*, 1994). The inferred protein sequences allowed us to attempt computer models of the putative receptor structure. We report here analysis of five human OR protein models. Such models (Singer and Shepherd, 1994) are based on coordinates for bacteriorhodopsin and rhodopsin, two proteins widely used as templates for

G-protein-coupled receptor modeling. All of these proteins share a seven transmembrane domain structure, forming a functional pocket where ligands are believed to bind. The five putative human OR structures are used to perform simulated ligand docking, using the program DOCK, which combines geometrical and energetic considerations. This simulation is done for a large number of compounds from the Cambridge small molecules structural database. The approach is based on the notion that odorant–olfactory receptor interactions are probability-based phenomena (Lancet *et al.*, 1993). The results allow us to generate comparative binding profiles for individual ORs, and to identify potential ligands. In parallel, we are using expressed and purified human OR proteins (Gat *et al.*, 1994) to screen phage display random peptide libraries. Such strategy may lead to the isolation of specific peptide mimetics that could serve as 'lead compounds' for identifying odorant ligands for individual OR proteins. This approach could be used in the future to study a large number of OR gene products from different clusters in the human genome.

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233. Male responses to their mates' body odors: the 'desire for more' variable

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Recently I have introduced the variable 'desire for more' of an odor stimulus in the context of pair-bonded men sniffing Q-tip samples of their mates' body odors (Poran, 1995). My findings indicated that this response peaked for odors collected around ovulation.

To explore this variable further, its correlation with six other variables was studied with 10 couples and two body odors: underarm sweat and vaginal secretions.

Results indicated that on the average, the 'desire for more' score for vaginal odors was ~20% higher than for underarm sweat. For both odor sources this variable correlated strongly with the perception of odor pleasantness and mildly with perception of odor familiarity and sweetness. A weak negative correlation with odor pungency was found. No relationship emerged between the 'desire for more' and perceived odor intensity nor the perceived duration of stimulus lingering.

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234. Sweetness as an olfactory quality: relationship to tasted sweetness

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Certain odors are reliably described as possessing the quality of 'sweetness', despite the fact that they do not directly stimulate sweetness receptors. Some (if not all) of these odors also enhance sweetness if included in a sucrose solution as a flavor. We examined the relationship between the smelled sweetness of an odor and the degree of enhancement of sweetness in solution. Thirty subjects rated a variety of odors—food, non-food, and a concentration series of odors rated as being moderately sweet (lychee and water chestnut)—for sweetness, sourness, overall intensity, liking, familiarity, and whether they were considered food or non-food odors. Subjects then rated the same odors again as flavors in a solution of 0.3 M sucrose.

The degree to which an odor smelt sweet accounted for 60% of the variance in sweet taste ratings. Two odors enhanced, and three odors suppressed, sweetness when compared with 0.3 M sucrose alone. Odor sweetness was not related to the intensity of the odor, as it remained fairly stable across the two concentration series. Overall, odors classed as food related smelled sweeter the non-food odors. We have previously shown that odors can be made to smell sweeter by pairing them with sucrose. The present data provide further support that associative mechanisms underlie the development of odor sweetness. We argue that odor sweetness forms a dimension of olfactory experience that parallels the properties of sweet tastes, hence its ability to enhance sweetness in solution. This ability to perceive as odor as smelling sweet may be an example of an olfactory induced taste synesthesia.

235. A human axillary odor is carried by apolipoprotein D

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Human axillary odor is important both commercially and biologically; axillary extracts can alter the length and timing of the female menstrual cycle. Axillary odor consists of a mixture of C_6–C_{11} straight-chain, branched and unsaturated acids. In males, the most abundant component is E-3-methyl-2 hexenoic acid (E-3 M2H). The odor arises through the interaction of non-odorous apocrine secretions with the cutaneous axillary microorganisms. 3 M2H is carried to the skin surface in apocrine gland secretions, bound to two proteins, designated apocrine secretion odor binding proteins 1 and 2 (ASOB1 and ASOB2), apparent molecular masses of 45 and 26 kDa respectively. To gain a better understanding of how axillary odors are formed and the structural relationship

between 3 M2H and its carrier protein, the amino acid sequence and glycosylation pattern of ASOB2 was determined by matrix-assisted laser desorption ionization time-of-flight mass spectrometry in conjunction with specific enzymatic cleavages. ASOB2 was identified as apolipoprotein D (apoD), a member of the $\alpha 2_{\mu}$-microglobulin super family of carrier proteins also known as lipocalins. The pattern of glycosylation for axillary apoD differs from that reported for plasma apoD suggesting different sites of expression for the two proteins. Synthesis of an anti-sense, oligonucleotide probe against apoD mRNA followed by *in situ* hybridization with axillary tissue demonstrates that the message for synthesis of this protein is specific to the apocrine glands. These results suggest a remarkable similarity between human axillary secretions and non human mammalian odor sources where lipocalins have been demonstrated to carry the odoriferous signals used in pheromonal communication.

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236. Taste perception in patients with damage to the anterior insular cortex

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Anatomical (Pritchard *et al.*, 1986) and physiological (Scott *et al.*, 1986) techniques have been used to show that primary taste cortex is located within the dorsal part of the rostral insula in nonhuman primates. It also has been shown that taste, in a departure from the other major sensory systems, ascends from the tongue to cortex along ipsilateral pathways (Beckstead *et al.*, 1980). Although the central organization of the gustatory system of humans should recapitulate the pattern observed in nonhuman primates, few studies have examined this issue. In this report we describe four patients with unilateral damage to the rostral insular cortex. In each case the location and extent of neurological damage was assessed with either MRI or CT films. Control data were collected from normal age-matched subjects and 3 other patients with brain damage outside the insula. All subjects showed normal movement of the tongue and were able to correctly localize light tactile stimulation of the tongue to either the left or the right side. Gustatory stimuli (four suprathreshold concentrations of sodium chloride, sucrose, citric acid, and quinine hydrochloride) were applied to the left and right sides of the anterior tongue separately with cotton-tipped applicators. The patients were asked to identify the stimulus quality and indicate the magnitude of the stimulus (0–12, weakest to strongest) by pointing to a number line (Bartoshuk *et al.*, 1985). Only one of the four subjects in the experimental group suspected that taste sensation was weaker but all four demonstrated an impairment in discrimination of both taste intensity and taste quality on the side of the tongue ipsilateral to the cortical damage. Focal testing revealed a global hypogeusia in another patient with bilateral damage to the rostral insula. Two other subjects with damage in either the posterior insula or the ventral half of the rostral insula showed normal taste sensitivity. These data support our hypothesis that primary taste cortex in humans is located in the rostral insula and, like monkeys, is organized in an ipsilateral fashion.

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237. Dark and light cells are two distinct cell types in rat vallate taste buds: some light cells express gustducin and the Lewis^b antigen

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Mammalian taste bud cells have been classified as light, dark or intermediate cells on the basis of several ultrastructural criteria. Light cells are said to have large, round or ovoid, smooth nuclei and a relatively electron-lucent cytoplasm. Dark cells are characterized as having elongated and invaginated nuclei with electron dense cytoplasm. Intermediate cells lie between these extremes. It is not clear from such descriptions whether these cells comprise a single cell type at different stages of differentiation or whether they represent different cell lineages.

Rats were perfused with 1% paraformaldehyde/2.5% glutaraldehyde in 0.05 M sodium cacodylate buffer; tongues were removed and semi-serial thin sections were cut transverse to the longitudinal axis of the vallate taste buds. To develop objective criteria for cell classification, the nuclear and cellular morphology of 314 cells was quantified with several measures that are purported to correlate with the light/dark cell classification. These measures discriminated well among taste cells. The resulting matrix of measurements was analyzed with hierarchical cluster analysis, which suggested two distinct groups corresponding to light and dark cells, with no intermediate cells. Light cells were distinctly round in cross section, tapering to smaller diameters at both their apical and basal extents. Dark cells were smaller, irregular in nuclear and cell shape, with sheetlike projections of cytoplasm which surrounded adjacent light cells.

Taste bud sections were immunoreacted with antibodies to the gustatory G-protein, gustducin, and to the Lewis^b blood group epitope. Confocal microscopy showed that labeled cells were the elongated spindle-shaped cells without sheetlike projections (i.e. the light cells seen at the ultrastructural level). A subset of gustducin-immunoreactive (IR) cells expressed Lewis^b; all Lewis^b-IR cells were also gustducin-IR.

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238. Entry of inhaled xylene and its metabolites into the olfactory bulb of F344 rats

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A nose–brain barrier that protects the CNS from inhaled toxicants has been hypothesized. Nasal xenobiotic metabolism may be a component of this barrier and may affect the entry of pollutants into the CNS. Following a 1 h inhalation of [^{14}C]xylene, autoradiograms—at 30 min post-exposure—showed localization of total and nonvolatile radioactivity in the olfactory bulb glomeruli of rats. In the present study, methyl bromide (MeBr) or untreated F344 rats were exposed to [^{14}C]xylene for 2 h following exposure to unlabeled xylene 6 h/day for 0, 2 (untreated only) or 9 days. At the specific activity used, bilateral radioactivity was observed in the olfactory bulb glomeruli and external plexiform layer of 50% of rats following a 2 h exposure to [^{14}C]xylene. In rats first exposed for 2 days to xylene, radioactivity varied bilaterally in the olfactory nerve layer, glomeruli, and external plexiform layer after the [^{14}C]xylene exposures. Following 9 day exposures to xylene, autoradiograms showed more intense radioactive labeling of olfactory bulbs; bilateral radioactivity was uniform throughout all the cell layers. MeBr-induced loss of olfactory epithelium resulted slight traces of radioactivity in bulbs after a 2 h [^{14}C]xylene exposure. However, with pretreatment with MeBr and 9 days of xylene exposure, staining density after [^{14}C]xylene exposure was more variable and less intense than without MeBr treatment, and was localized in the external and internal plexiform layers. These rats were similar to rats exposed only to 2 days of xylene, indicating the regenerated epithelium responded like a naive epithelium exposed for 2–4 days. In conclusion, 9 day prior exposure to xylene enhances the intensity and depth of penetration into the olfactory bulbs of [^{14}C]xylene and its metabolites relative to that seen following 2 day prior exposure to xylene. Differences may be explained by initial inhibition of nasal xylene metabolism (previously reported) that disappears with continued xylene exposure.

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239. Information processing in the olfactory bulb of goldfish (*Carassius auratus*)

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In comparison to mammals, the olfactory bulbs (OB) of fishes contain a smaller number of different neurons which are less strictly layered. Action potentials (1–1.5 ms, constant in amplitude) were recorded simultaneously with single tungsten electrodes (~10 MΩ) from 1–3 relay neurons [mitral cells (MC)] and from 1–2 inhibitory interneurons [granular cells (GC)]. GC potentials were variable in amplitude and of significantly longer duration (3–5 ms) than those of MCs. Stimuli {[10^{-4}–10^{-12} M of various natural stimuli: amino acids (AA), a pheromone [17α^2,0β-dihydroxy-4-pregnen-3-one (P)], a bile acid [taurolithocholic acid (B)], and non-familiar stimuli (NFS): amyl acetate, β-ionone, 2-phenylethanol} were repetitively applied for 15 s and removed during 180 s interstimulus water application. Dose-dependent excitation or inhibition was mainly recorded during application of P and B, during application of AAs, however, in ~50% of MCs only. In ~25% inverse responses were recorded during application of AAs at high concentrations in contrast to application of lower concentrations (e.g. 10^{-4}: excitation and 10^{-6}–10^{-10}: inhibition), and in ~25% of MCs increasing effectiveness was found when decreasing AA concentrations were applied. Effects elicited by higher AA concentrations probably were caused by coactivation of less specific epithelial receptors and/or different interactions between OB neurons. Similar responses were found in ~75% of recordings from neighboring pairs of MCs, whereas in the remaining ~25% excitation of one MC resulted in activation of a GC that laterally inhibited other MCs in the pool (and vice versa). Phasic–tonic responses were the result of receptor cell adaptation. During complex responses (e.g. 3 s phasic excitation followed by 12 s total suppression of APs during a 15 s stimulus application), the delayed inhibition probably resulted from lateral inhibition due to efferent activation of adjacent GCs. Excitatory versus suppressive responses of single MCs to different natural stimuli were frequently recorded, whereas responses of single MCs to NFS were either only excitatory or only suppressive. Circumscribed local areas in the BO representing projection areas for one of the applied stimuli were not found.

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240. Olfactory threshold tuning

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Olfactory thresholds are higher when estimated by a descending method of limits (decreasing concentrations; D) relative to an ascending method of limits (increasing concentrations; A), historically thought to result from adaptation. Our experiments, in which different concentrations of amyl acetate (AA) were presented by means of squeeze bottles using different psychophysical procedures, revealed the following: (i) the difference between D and A thresholds (D/A) is not suppressed by increasing the intervals between stimulus presentations; (ii) stimulation with low concentrations of AA during inter-stimulus intervals is ineffective while high AA concentrations increase A thresholds but do not influence D thresholds; (iii) randomized stimulus concentrations produce thresholds at D levels; (iv) alternating the sequence of descending concentrations with that of ascending ones results in thresholds between D and A; (v) stimulation with concentrations close to previously determined A values, which disrupts the ascending stimulus ordering, increases the threshold while stimulation with concentrations close to D decreases it. Thus the D/A phenomenon does not seem to be caused by olfactory adaptation. More likely, the threshold is being actively tuned toward weaker stimuli during use of the ascending series in connection with stimulus expectation. This might result from the situation where the actual perceptual threshold for an odorant, which is probably dynamic, most likely is higher than the sensory threshold; and (vi) The threshold in a binary, ascending,

forced-choice procedure (odorant versus blank in successive pairs) is close to D. Thus a further condition for the D/A phenomenon appears to be the absence of a clear blank reference and the presence of the preceding ordered sequence of ascending low concentrations.

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241. Reduced level of NOS correlates with defects in olfactory response and adaptation in *anosmic*, a *Drosophila* mutant

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Nitric oxide (NO) is a second messenger molecule produced by the enzyme nitric oxide synthase (NOS) in neurons and certain other cell types. NOS activity can be visualized by staining for fixative-insensitive NADPH-diaphorase activity, to which it corresponds in many, if not all, mammalian tissues.

NOS immunoreactivity and NADPH-diaphorase activity are detected in the antenna and maxillary palp, the olfactory organs of *Drosophila*. Most of the NOS immunoreactivity and NADPH-diaphorase activity are detected in the neurons of sensilla basiconica, which are responsive to general odorants.

anosmic, a recently isolated olfactory mutant, shows reduced levels of NADPH-diaphorase activity in its olfactory organs. Levels of NOS-immunoreactivity in the *anosmic* maxillary palp are also reduced. *anosmic* flies are defective in olfactory responses, as measured in two different behavioral paradigms. Interestingly, olfactory adaptation is also defective in *anosmic*, as revealed through behavioral tests. Electrophysiological analysis of the mutant and molecular cloning of the *anosmic* gene are underway.

We have also detected NOS immunoreactivity and NADPH-diaphorase activity at different stages of *Drosophila* development. NOS immunoreactivity in all stages of development is detected as a protein of ~155 kDa in size, very close to the size of neuronal NOS. NOS immunoreactivity is detected throughout embryonic development; strong expression is detected in the embryonic central nervous system. During larval development, NADPH-diaphorase activity and NOS immunoreactivity are detected in the antennal imaginal discs, which give rise to adult olfactory organs. These results indicate that the NO messenger system most likely plays a role in the development of the *Drosophila* olfactory system as well as in olfactory function.

242. RT-PCR and *in situ* RT-PCR analysis of EGF receptor expression in the olfactory mucosa of mice

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The aim of our study was to test the hypothesis that progression of deeply quiescent progenitor cells through the initial stages of the cell division cycle is mediated by the activation of epidermal growth factor receptor (EGFR). The presence of EGFR transcripts in olfactory mucosal tissues was determined by RT-PCR and localized in progenitor cells by *in situ* RT-PCR. EGFR protein was identified by Western blot analysis. Olfactory marker protein (OMP) mRNA and protein served as positive controls. Mouse olfactory mucosal total RNA was reverse transcribed with MuLV reverse transcriptase (RT) and amplified with *Taq* DNA polymerase and specific primers to EGFR. The authenticity of PCR products was verified in Southern blot analysis with digoxigenin-labeled oligonucleotide probes for an internal sequence of EGFR. *In situ* RT-PCR was used on 6-μm-thick frozen sections. The tissue mRNAs were reverse transcribed with MuLV RT and EGFR cDNA fragments were amplified *in situ* using *Taq* polymerase. Western blotting was performed with polyclonal antibodies to EGFR. In RT-PCR, an expected 304 bp sequence of EGFR and 348 bp sequence of OMP fragments were amplified from olfactory mucosal RNA. In negative controls, omission of RT resulted no visible bands. In cellular localization studies, EGFR mRNA expression was observed in the cells located in the basal cell layer of the olfactory epithelium. Similarly in the positive control tongue epithelium, EGFR mRNA was localized in the basal cells. Doublet bands of 170 and 150 kDa of EGFR and 19 kDa of OMP proteins were observed in the Western blots of olfactory tissue proteins, whereas positive control kidney and skin tissue proteins showed only EGFR. These results demonstrate that EGFR mRNA and protein are expressed in the mouse olfactory epithelium and support the hypothesis that activation of the EGFR mediates the progression of progenitor cells towards the S phase of the cell division cycle.

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243. Effects of food rheology on food intake and energy balance

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In this pilot study we investigated the effects of an energy supplement of either a liquid or a solid with comparable macronutrient composition, on spontaneous food intake and body weight of 16 subjects. Evidence from the literature suggests that calories derived from liquids elicit a weak compensatory response. In a within-subject study design, eight women and eight men consumed a dietary carbohydrate supplement of 450 kcal a day in a solid (jelly beans, condition 1) and liquid (soda, condition 2) form during two 4 week sessions, separated by a four-week washout period. The supplement was consumed outside the laboratory as part if the subjects' normal dietary routine and no dietary advice was offered. During the entire 12 week period, biweekly dietary records were collected and measurements of weight and body composition were taken weekly. After completion of the study, subjects filled out a restrained eating questionnaire. Average daily energy intake and body weight were compared at the end of baseline, jelly bean, washout and soda conditions. Change in the average energy intake and body weight across the different conditions indicated that dietary compensation in the jelly bean condition was greater that in the soda condition, That is, free feeding energy intake decreased more when subjects supplemented

their daily diet with jelly beans as compared with the response when subjects supplemented their diets with soda. Restrained eaters showed more attenuated response compared with non-restrained eaters. These preliminary data suggest that rheological properties of a food influence the dietary response they elicit.

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244. Identification and bioactivity of an estrous pheromone of Asian elephants, *Elephas maximus*: an unexpected result

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Male Asian elephants exhibit multiple, discrete chemosensory responses and subsequent definitive pre-mating behaviors toward preovulatory urine from females. The presence of an urinary sex pheromone is indicated. Observed and quantified chemosensory responses, especially flehmen responses, have been used to determine effective fractionation and purification protocol. Recent modifications, including a final reverse phase high performance liquid chromatographic separation, have yielded a highly active fraction with a characteristic odor. First by capillary gas chromatography, and subsequently by capillary gas chromatographic/mass spectrometric analyses, only a single component was demonstrated. Analyses by mass and nuclear magnetic spectrometries of the active fraction clearly established the molecular weight as 226 and the chemical identity as a mono-unsaturated 12 carbon acetate. Confirmation of the molecular weight and the double bond location was accomplished by dimethyl disulfide derivatization followed by capillary GC-MS analysis of the derivatives. Comparison with standards demonstrated that the principal component of the elephant pheromone is (Z)-7-dodecen-1-yl acetate. The synthetic authentic compound is bioactive as repeatedly tested with male Asian elephants. Over 126 species of female insects release this compound as part of their sex pheromonal blend.

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245. Novel characteristics of human olfactory neurons

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An extensive body of research with a variety of animal systems has led to a picture of olfactory transduction in which odor molecules interact with receptors on the cilia of olfactory neurons (ON) triggering production of cAMP or IP3, opening nonselective cation channels resulting in action potentials carried along the axon to the olfactory bulb. ON from rats are characterized by responding to odors with increases in Ca_i (100% of responding ON) dependent on either cAMP or IP3-related pathways; and often respond to multiple odors (30% of responsive cells). Rat ON also respond to depolarizing levels of extracellular K^+ (high K_o) with increased Ca_i (92%), evidence of voltage-sensitive calcium channels (Restrepo *et al.*). To study the extent to which these models apply to human ON (HON), we have used the calcium-sensitive indicator Fura-2 to study changes in Ca_i in 173 HON freshly isolated from olfactory tissue biopsies taken from human volunteers. Using comparable odor mixtures and methods, these data show that HON, like rat ON, respond to odors with increases in Ca_i (68% of responding ON) mediated by either cAMP or IP3 dependent pathways. In contrast to rat ON, 1/3 of responding HON exhibited a decrease in Ca_i; none responded to more than one odor mixture or individual odor, and only 25% responded to high K_o with increased Ca_i.

These findings indicate that current models of olfactory transduction apply to HON, but also suggest the need to expand these models to accommodate characteristics of HON that appear to be quite different from those of other vertebrates.

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246. Odor learning and memory in patients with Alzheimer's disease

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It has been shown that brains of patients with Alzheimer's disease (AD) show neuritic plaques and neurofibrillary tangles most widespread in regions used for processing olfactory information and memory. In the present study, short (immediate) and long (20 min) delay odor learning, free recall, and recognition memory were assessed in a group of AD patients and age-matched normal controls. In the short delay portion, subjects were presented with a total of 16 target odors which were selected from the following four categories: Sauces and condiments, fruits, spices and herbs, and personal products. Subjects were given five learning trials in which they recalled as many odors as they could immediately following the presentation of the 16 odors. During the short delay free recall portion, subjects were asked to recall the target odors after the presentation of 16 distracter odors. Subjects were given categorical cues to aid recall of target odors during the short and long delay cued recall trials. Subjects were presented with the 16 target odors as well as 28 distracters during the yes/no recognition portion of the test. Results revealed that AD patients displayed poorer ability for learning odors than controls, and they recalled far fewer odors during the short and long delayed recall trials, even when aided by the categorical cues. AD patients also performed poorly on the odor recognition task by achieving significantly fewer hits than the controls. The results of the present study

suggest that free and cued recall for odors, learning over trials, and odor recognition is severely affected by Alzheimer's disease and that such odor memory tests may be helpful for early diagnosis of the disease.

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247. The transcription factors Olf-1 and Pax-6 in olfactory development

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The transcription factor, Olf-1 appears to play a role in the expression of several genes that function in olfactory signal transduction including G_{olf}, type III adenylyl cyclase and the cyclic nucleotide activtated channel, OcNC. Recent evidence has suggested that the Olf-1 factor is expressed in the undifferentiated globose basal cells before the expression of the terminally differentiated markers. Expression of the Olf-1 protein is not restricted to the olfactory epithelium during embryonic development in the mouse. Its expression is seen in discrete, post-proliferative neuronal precursors in retina, spinal cord and the developing brain and disappears at about the time of birth. These observations suggest that Olf-1 may have a more general role in the differentiation of post-mitotic neuronal precursors and, moreover, validates examination of the role of Olf-1 factor in olfactory differentiation. Recently, we have identified two additional Olf-1 like genes in mouse and demonstrated that at least one these genes is expressed at high levels in the olfactory neurons.

We have examined the pattern of expression of the PAX-6 transcription factor, expressed at high levels in olfactory tissue, in the developing and adult olfactory neuroepithelium. Interestingly, antibodies to Pax-6 specifically recognize horizontal basal cells and sustentacular cells within the epithelium and, therefore, defines the non-neuronal cells within the epithelium. Given the olfactory defects associated with mouse Pax-6 mutants, we are currently investigating the role of this factor in the development of the olfactory epithelium.

248. Quantitative comparison of female axillary secretions as a function of the menstrual cycle phase

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Recent results suggest that females who received axillary secretions from donor females collected during the follicular phase experienced a shortening of their menstrual cycles while females who received secretions collected during the ovulatory phase experienced a lengthening of the menstrual cycle. These results suggest that an analytical examination of axillary extracts from individuals during these two phases might reveal marked difference in axillary constituents.

Axillary pads of four donors from a single day in both the follicular and ovulatory phases were used to create the follicular phase and ovulatory phase extracts (FPE; OPE) respectively. Cycle phases were judged by basal body temperature and commercially available ovulation kits to determine the LH surge. The characteristic odors found in the acidic components of both the FPE and OPE were isolated and analyzed by GC/MS results indicate different patterns in both phases. High levels of n-C_7, C_8 and C_{10} acids as well as sub-nanogram levels of (E) 3-methyl-2 hexenoic acid (E3 M2H) are found in the FPE. E3 M2H levels increased significantly and concentrations of normal acids remained constant or decreased in the OPE.

Quantification of donors axillary flora indicated no difference in the number of micrococci (*Staphylococcus epidermidis* and *S. saprrophyticus*) or cutaneous lipophilic ditheroids (CLD) in either cycle phase. However, differences were found in the population of lipophilic cutaneous diphtheroids (LCD) with significantly greater amounts of LCDs found in the ovulatory phase. The increased levels of E3 M2H in the OPE may be due to changes in the number of LCD bacteria, which are associated with stronger smelling axillary secretions.

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249. Effect of selective deafferentation on metamorphic changes in the primary olfactory projection of *Xenopus laevis*

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In adults of the African clawed frog, *Xenopus laevis*, the main nasal cavity is divided into two parts: the principal cavity (PC), and the middle cavity (MC). PC olfactory receptor cells project to the dorsomedial part of the main olfactory bulb (dMOB); MC olfactory receptor cells project to the ventrolateral part of the main olfactory bulb (vMOB). In premetamorphic larvae, by contrast, the MC is absent and the PC projects to the entire MOB. As the MC arises during metamorphosis, MC and PC projections overlap in the vMOB; subsequently the PC projection to this region disappear.

To determine whether interactions between PC and MC axons are required for this change in the PC projection, the MC rudiment was unilaterally excised from stage 55–57 tadpoles (in which PC and MC projections overlap). The animals were raised through the end of metamorphosis (stage 65–66) and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer. After 4–6 h additional fixation, specimens were transferred to 30% sucrose in phosphate buffer and sectioned horizontally at 20 µm on a cryostat. Sections were stained with peroxidase conjugated soybean agglutinin (HRP-SBA), which differentially labels projections from the PC (weakly staining) and MC (strongly staining). The glomerular layer of the olfactory bulbs was divided into weakly and strongly staining regions, and the outline of each traced using a video-equipped microscope connected to a

microcomputer running Bioquant-OS/2 software (RandM Biometrics, Inc.).

The condition of the MC in the lesioned animals ranged from apparent absence, to presence of a reduced, but otherwise normal cavity. The region of the glomerular layer of the MOB strongly labeled by SBA was also reduced on the lesioned side, ranging from 5–30% of the volume of the strongly labeled region on the unlesioned side. Surprisingly, some strong SBA staining was always present, even when no morphological MC was apparent. Three-dimensional reconstruction of the olfactory bulbs showed that the PC projection on the lesioned side extended further ventrolaterally than on the unlesioned side, into the dorsal part of the region normally occupied by the MC projection. These results suggest that interactions between PC and MC axons are at least partly necessary for the normal metamorphic change in the PC projection pattern. However, in cases with marked reduction of the MC a gap was left between strongly staining and weakly staining regions. This shows that much of the normal change can occur in the absence of such interactions.

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250. Developmental changes in the dendritic architecture of sodium-best neurons in the rat nucleus of the solitary tract

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Mistretta and Labyak (1994) have recently shown that the dendrites of some neurons in the rostral nucleus of the solitary tract (NST) enlarge and become more complex (more dendritic branches) during a period of development that coincides with an increased NST response to NaCl. There is also evidence that this increase in dendritic complexity is followed by a period of remodeling that is characterized in part by a loss of dendritic branches (Bao *et al.*, 1995). We might expect, based on these findings, that the NaCl-best neurons from late postnatal animals would exhibit more dendritic branches than adult NaCl-best cells. As an initial test of this hypothesis, we have labeled individual physiologically characterized neurons in young (postnatal day 21–28) and adult (>60 days old) rats. A total of 47 neurons in young animals and 71 neurons in adult rats were successfully labeled with Neurobiotin and reconstructed in three dimensions. As a group, the neurons from young animals had a higher maximum branch order (8.2 ± 0.6 versus 6.9 ± 0.3, $t = -2.3$, $P = 0.03$) and a trend toward more branch points (22.5 ± 2.5 versus 18.3 ± 1.1, $t = -1.7$, $P = 0.1$) than the adult neurons. This contrast was most apparent when we examined the NaCl-best neurons. NaCl-best neurons in the young animals had more dendritic branch points (8.7 ± 0.7 versus 6.7 ± 0.4, $t = -2.6$, $P = 0.01$) and a greater maximum dendritic branch order (26.1 ± 4.5 versus 17.9 ± 1.8, $t = -2.0$, $P = 0.5$) than the adult NaCl-best neurons. There were no differences between the young and the adult HCl-, quinine- and sucrose-best neurons (for these measures). We consider these results to be consistent with the postulate that NaCl-best neurons exhibit an increase in the number of dendritic branches during early development, with the number of branches decreasing during subsequent maturation.

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251. Chemesthetic sensations in the mouth and throat during irritant ingestion

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Common experience indicates that sensory irritation due to consumption of hot spices is not confined to the mouth. In two experiments subjects used the labeled magnitude scale to rate the perceived intensity of irritation at the following four locations: front of the tongue, back of the tongue, roof of the mouth, and throat. In the first experiment subjects ingested 10 ml samples of an ascending concentration series of capsaicin (0.327–18.34 μM). Each sample was held in the mouth for 3 s and then swallowed. Perceived irritation was rated 20 and 90 s after ingestion. Irritation ratings increased as a function of concentration and decreased over time at all locations. Mean ratings of irritation were significantly higher for the throat than for the front or the back of the tongue ($P < 0.05$). There was no difference between ratings for the throat and the roof of the mouth.

In the second experiment an ascending concentration series of piperine (24 μM–1.33 mM) was presented in alternate sessions with capsaicin, using a slightly different psychophysical procedure. Results for capsaicin were similar to before except that mean irritation ratings for the throat differed significantly only from those for the front of the tongue ($P < 0.05$). In contrast, piperine yielded no significant effect of location.

These results confirm an earlier finding that the sensitivities to capsaicin and piperine have different spatial distributions, and reveal that the oropharynx, which is innervated by the glossopharyngeal and vagus nerves, is a significant and sometimes dominant locus for the perception of ingested irritants.

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252. OBP-1: a *Drosophila* odorant-binding protein specifically required for avoidance of high concentrations of ethanol

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As an initial approach to understand the functional mechanisms mediating olfaction in *Drosophila*, we are isolating genes encoding potential components of the olfactory system. We used the enhancer trapping technique to identify genes expressed exclusively in the olfactory organs of the fly. This approach involves randomly mobilizing P transposable elements in the *Drosophila* genome. The P elements are modified to express LacZ

when driven by endogenous enhancers acquired upon integration in the genome. Lines exhibiting a LacZ expression pattern restricted to the olfactory structures have a high likelihood of inserting in or near olfactory-specific genes.

We have identified a novel *Drosophila* member of the invertebrate odorant/pheromone-binding protein family we call OBP-1. OBP-1 is expressed in non-neuronal support cells in a small subset of olfactory hairs (sensilla). These support cells secrete OBP-1 into the fluid bathing the olfactory neurons within those sensilla. The *in vivo* function of this family of proteins is unknown, but it has been suggested that they might solubilize, concentrate or degrade odorants.

To determine the function of OBP-1 protein, we made flies specifically lacking the OBP-1 gene. These flies make no OBP-1 protein and have been examined for olfactory deficits using established and novel olfactory behavioral assays. Our results demonstrate that OBP-1 is required for the normal behavioral response to avoid high concentrations of ethanol. Responses to other odorants are unaffected by the loss of OBP-1. Because of the remarkably restricted expression pattern of OBP-1, this behavioral deficit in OBP-1 mutants must result from changes in activity of olfactory neurons within the small number of sensilla that normally express OBP-1. This data suggests that odorant binding proteins have a role in determining the chemical specificity of individual sensilla *in vivo* and that some sensilla in *Drosophila* may specifically mediate avoidance behavior. A model consistent with these results will be discussed.

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253. An algorithm for the construction of idealized current traces: analysis of InsP3-induced single channel openings in rat olfactory neurons

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Recording of single channel currents by patch clamping is a powerful technique for characterization of the kinetics of ion channels. For recordings with low levels of noise, half-amplitude algorithms provide a convenient way to construct idealized current traces for use in determining kinetic parameters. However, when channels have multiple subconductance states, or significant amounts of open channel noise, the half-amplitude algorithm makes a significant number of mistakes that must be corrected by the investigator in a subjective manner. These problems can be alleviated by using the mean-variance (MV) algorithm of Patlak (1993) where the mean current and the variance are computed within a fixed time window for each point. An MV histogram can then be constructed where different subconductance states are easily visualized as peaks. This algorithm provides a more objective determination of the number of states. However, determination of kinetic parameters is tedious because it entails computation of the volume under each peak in the MV histogram for different size windows. This is a problem particularly when the kinetics involve more than one time constant. We have designed an algorithm to construct idealized current traces. Each point is assigned to a particular state based on its location within the MV histogram. The resulting idealized trace can be used to determine time constants, sequence of transitions, open channel noise, etc. The algorithm is being implemented in a Windows program written in Borland C++.

Using the patch clamp technique we recorded $InsP_3$ induced currents in excised inside-out patches of rat olfactory neurons. Two different channels, a nonselective cation channel and a Ca^{2+}-selective channel could be identified. Kinetic properties of these channels were characterized using the MV algorithm.

This work was supported by NIH grants DC 00566 and DC01838.

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254. Ultrastructure of the taste buds in the spotted gar, *Lepisosteus oculatus* (Holostei)

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This is the first report concerned to the ultrastructure of the taste buds (TBs) of a holostean fish. Up to now, we know about the TB morphology of most actinopterygian fish, as the Chondrostei and Teleostei, but not the Holostei. In view of the phylogeny of the taste system in fish, it was of special interest to investigate the gar as a member of the latter group.

In the spotted gar, TBs occur exclusively in the squamous epithelium of the buccopharyngeal cavity. The organs are ~65 µm high and 45 µm broad and comprise ~40 slender cells that form together the TBs sensory epithelium. This is situated on a corium papilla and stands vertically in the epithelium. Using transmission electron microscopy, we observed TB cells of different electron densities that can be divided into light and dark cells. The light cells are slender and spindle-shaped and bear apically either one cone-like and large microvillus or several small and undivided microvilli. The nuclei of light cells are oval and their cytoplasm is rich in organelles such as mitochondria, microfilaments and microtubules. Apically, they may contain a pair of centrioles. The dark cells are elongated cells, too, and possess lobar processes by which they ensheath the light cells. Their elongated nuclei are often irregularly indented. The cytoplasm contains numerous bundles of intermediate filaments. Apically, they terminate with up to ten small microvilli and their apical cytoplasm may contain large and extremely electron dense vesicles. Especially the bases of dark cells are divided into lobar- to sheet-like processes which intermingle with the nerve fibers of a relatively small nerve fiber plexus. Basal cells are situated directly on the basal lamina. Their cell body is divided into arm-like processes which contain dense-cored vesicles and which intermingle with nerve fibers and the processes of light and dark cells. Afferent synapses occur between (mostly) light, dark and basal cells (presynaptic sides) and nerve fibers. Efferent synapses are rarely found between nerve fibers (presynaptic side) and light cells.

Morphologically, the *Lepisosteus* TB is organized quite differently to other fish TBs. The question whether the observed

characteristics are to be considered as phylogenetically conservative and/or as signs of specialization and/or adaption of holostean fish is still open.

255. Evidence that adenylyl cyclase mediates excitatory odorant responses to both 'cyclic AMP' and 'IP$_3$' odorants

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Vertebrate olfactory cilia contain two odorant-activated enzymes: adenylyl cyclase (AC) and phospholipase C (PLC). Electrophysiological studies on terrestrial species have shown that AC mediates excitatory transduction, but the function of PLC remains controversial. One hypothesis holds that AC and PLC both mediate excitatory responses, but for different subsets of odorants. Another hypothesis holds that AC mediates only excitatory responses, while PLC mediates only inhibitory responses. We addressed this issue by comparing the effects of the AC inhibitors 2',5'-dideoxy-3'-adenosine (ddAdo; 100 μM) and MDL-12,330A (mdl; 10 μM) on excitatory responses to odorants that preferentially stimulate AC or PLC. 'Cyclic AMP' odorants tested were: acetophenone, citralva, eugenol, geraniol, hedione, menthone and phenylylethyl alcohol, and 'IP$_3$' odorants tested were: ethylvanillin, isovaleric acid, lilial and triethylamine, all at 10 μM (Breer and Boekhoff, 1991). Odorant responses were measured in the rat by recording the odorant-induced potential (EOG) across the septal epithelium mounted in an Ussing-type chamber. All responses were negative in polarity, indicative of excitatory responses in single cells. Odorants were delivered in saline. The odorant responses in the presence of the inhibitors as a percentage of the control odorant responses were:

	'Cyclic AMP' odorants	'IP$_3$' odorants	Difference
ddAdo	61 ± 10%	61 ± 10%	$P < 0.96$
mdl	64 ± 8%	56 ± 12%	$P < 0.17$

The decreases did not differ significantly between the 'cyclic AMP' and 'IP$_3$' odorants using either AC inhibitor. This is consistent with the hypothesis that the adenylyl cyclase pathway mediates excitatory transduction for most, if not all, odorants.

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256. Synergism among ternary mixtures of fourteen sweeteners

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The purpose of the present study was to determine the degree of synergism of sweet taste existing between ternary mixtures of fourteen sweeteners. A trained panel evaluated ternary mixtures of the following fourteen sweeteners: three sugars (fructose, glucose, sucrose), two polyhydric alcohols (mannitol, sorbitol), two terpenoid glycosides (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). The ternary mixtures that were tested were limited to those in which the compounds comprising the mixture were synergistic in binary combinations, according to an earlier study by Schiffman *et al.* (1995). Each self-mixture was also tested (e.g. 2% sucrose + 2% sucrose + 2% sucrose). All sweeteners in the ternary mixtures were isointense with 2% sucrose, according to formulae developed by DuBois *et al.* (1991). Most ternary mixtures were synergistic (greater than the average of the three self-mixtures) to some degree. When comparing the synergism of the triads to their associated dyads (Schiffman *et al.*, 1995), however, it appears that a greater degree of synergism may be reached with the dyads rather than the triads.

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257. Habituation to sweet foods in Caucasian and African-American subjects

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Habituation is a relatively long-lasting decrease in oral responsiveness that results from the repeated presentation of a single taste stimulus. The purpose of this study was to evaluate the degree of habituation to sweet-tasting foods that differ in caloric density, and to determine if there are differences in the rate of habituation between Caucasians and African-Americans. Nine different commercial foods and beverages served as stimuli. Subjects rated each food once per min for a 30 min period on scales related to liking, sweetness, desire for another taste of the same sample and desire for a different taste. The stimuli and portion size for each of the 30 samples were: two candy bars [Ultra Slim-Fast® Cocoa Almond Crunch Bar (1/16 of a bar), Natural Nectar® Peanut Butter Granola Bar (1/16 of a bar)]; three beverages

[Nestea® Lemon Flavored Instant Tea with NutraSweet® (5 ml), Welch's® Grape Juice (5 ml), Pink Swimmingo Kool-Aid® (5 ml)]; two gelatin desserts [Cherry Flavored Jell-O® Gelatin (5 g), Cherry Flavored Jell-O® Gelatin with NutraSweet® (5 g)]; one enteral nutrition drink [Vanilla Ensure® Plus (5 ml)]; and one pudding [Ultra Slim-Fast® Chocolate Pudding (5 g)]. Subjects consumed the entire portion of each sample. The major finding was that products with a high nutrient density showed the greatest habituation (reduction in hedonic ratings and desire for another taste) while those with a low caloric density showed little to no habituation. Both sensory and postingestive satiety effects may play a role in the reduced hedonic ratings over time for products with a high nutrient density. There were minor differences in the degree of habituation between Caucasians and African-Americans. However, young African-Americans had a greater desire than young Caucasians for another taste in 7/9 foods. In addition, young African-Americans had a greater desire than young Caucasians for a different taste in 7/9 foods. The greater desire for sweet taste may be a factor in the elevated incidence of obesity and diabetes in African-Americans. A further finding in the study was that young African-Americans have greater perceived stress. Thus, young

African-Americans may use sweet taste to compensate for feelings of stress.

258. Effects of pre-rinsing with ±-(4-methoxyphenoxy)propionic acid (Cypha) on subsequent sweetness intensity ratings for fifteen sweeteners

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Cypha™ [±2-(4-methoxyphenoxy)propionic acid] is a substituted phenoxyalkanoic acid that has been shown to selectively inhibit certain sweeteners in mixtures (Schiffman *et al.*, 1995). The purpose of the present study was to determine the effect of pre-rinsing with Cypha™ on sweetness intensity ratings; rinses were performed either immediately prior to or 30 s prior to the tasting of sweeteners. A trained panel evaluated fifteen sweeteners varying in chemical structure after rinsing with 500 p.p.m. Cypha™. Each sweetener was tested at four concentrations isointense with 2.5, 5, 7.5 and 10% sucrose according to formulae developed by DuBois *et al.* Some sweeteners were not tested at levels equivalent to 7.5 or 10% sucrose, however, because they do not achieve this sweetness intensity level at any concentration. For the immediate evaluations, significant suppression of sweet taste occurred for several sweeteners at various levels; however, there was almost no instance of suppression for the 30 s delay data. There was significant enhancement of sweetness ratings relative to the target levels, particularly at the 30 s delay evaluation, with the greatest enhancement for monoammonium glycyrrhizinate (MAG), neohesperidin dihydrochalcone (neo-DHC) and thaumatin. These three sweeteners (MAG, neo-DHC and thaumatin) were the only compounds of 15 sweeteners tested previously by Schiffman *et al.* that were not suppressed in intensity when mixed directly with Cypha™. The effect of pre-rinsing with Cypha™ on subsequent sweetness intensity ratings was also associated with the size of the sweetener molecule with the larger molecules showing the greatest enhancement.

259. Characterization of odors from swine operations

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Odors from swine operations were evaluated by a human odor panel, by gas chromatography/mass spectrometry, and by an electronic nose. The trained odor panel evaluated the magnitude, quality, and acceptability of the odors of air samples collected from swine houses and lagoons. Members of the odor panel were students and employees at Duke University who ranged in age from 26 to 47 years. Three methods were used to collect the odors: (i) bag in drum technique; (ii) fabric swatches; and (iii) Tenax columns. For the bag in drum technique, 25 l Tedlar® bags were used to collect air samples. The Tedlar® bags were inserted into collection drums, and the drums were pressurized with a sample pump at the rate of 5 l/min to fill the bag with sample air. The air samples collected from the hog farms were delivered to the subjects using two methods. In the first method, an olfactometer was employed to dilute the air samples into six different concentrations for evaluation. In the second method, air samples from the hog farms were diluted within the Tedlar® bags to the desired concentration by adding charcoal filtered and dehumidified (odorless) air, i.e. cleaned air was pumped into the bags along with air from a hog farm at a given volume to create the desired concentration ratio. These air samples were delivered directly to the subjects by placing the Tedlar® bags in a drum and pressurizing the drum to force air out of the bags into sniffing ports. Cotton fabric swatches which adsorb odors from outside air were also used to collect samples. The cotton fabric swatches were cut into 7 cm square pieces and baked in an oven for 3 h at 95° C to eliminate any odors present in the fabric. The fabric was exposed to air on or near a hog farm at various time intervals and then placed into small bottles to be used for testing by the odor panel. Air from the farms was drawn through Tenax columns using a sample pump. The Tenax columns were analyzed by a gas chromatography (GC) system with an odor port that permits human olfactory assessment of separated VOCs as they elute from the GC column. This assessment is important to help identify the VOCs that characterize an odor. The instrumental approach splits the effluent gas of the GC column and sends a small fraction to a flame ionization detector (FID), with the remainder directed to the nose port that is sampled by a human. An artificial nose was also used to evaluate air and cotton swatch samples collected from swine operations. The results of these studies reveal that odors from swine operations contain many compounds which synthesize perceptually to create characteristic 'hog farm' odors.

260. Continuous neurogenesis in the central olfactory pathway of adult shore crabs

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The olfactory system of vertebrates shows substantial structural plasticity in that neurons are generated throughout adult life in the periphery and the CNS. In the olfactory pathway of adult arthropods, however, neurogenesis has so far only been found among local interneurons (Kenyon cells) of the secondary olfactory neuropils (mushroom bodies) in few insect species.

Here I present evidence that in the brain of the shore crab, *Carcinus maenas*, olfactory projection neurons and local interneurons of the secondary olfactory neuropils—the hemiellipsoid bodies—are continuously generated throughout adult life. Counts of somata and axons showed that in post-larval *Carcinus* the number of olfactory projection neurons increases continuously and doubles over the entire life span of the crabs. In vivo labeling of adult crabs with bromdeoxyuridine (BrdU) and subsequent immunocytochemical detection of BrdU in serially sectioned brains, revealed string-shaped groups of mitotically active somata in the lateral soma cluster (LC), which is comprised of the cell bodies of olfactory projection neurons, and in the soma cluster containing the cell bodies of hemiellipsoid body local interneurons (HBC). In both soma clusters the string of labeled somata immediately adjoined the respective neuropil and demarcated a small proliferation zone. Since the groups of labeled somata did not contain typical, large neuroblasts as they occur during embryonic and larval development of the arthropod CNS another type of neuronal precursor cell appears to be active in both soma clusters. With BrdU incubation times as short as 4 h and regardless of size, moult stage and sex of the labeled crabs, always ~30 somata in each LC and ~20 somata in each HBC were stained. These findings demonstrate continuous neurogenesis and indicate persistent integration of newly formed neurons in the olfactory pathway of the adult crab brain.

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261. The fluid dynamics involved in chemical signal transport in the antennae of the sphinx moth, *Manduca sexta*

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Sensory systems have a complex task of extracting relevant information from environmental signals. The interaction between fluid flow and morphology of the antennae acts as a physical filter and plays an important role in extracting information from signals. This interaction determines the boundary layer structure which alters the spatial and temporal distribution of the chemical signals arriving at the receptors. This study was designed to examine the microscale fluid dynamics and chemical dynamics around the antennae of the sphinx moth, *Manduca sexta*. We have measured boundary layer structure and chemical dynamics of an antennae mounted in a flow tank under different flow speeds (0.57, 3.4 and 5.3 m/s). We used the IVEC-10 and electrochemical microelectrodes to quantify chemical dynamics around the antennae. The preliminary findings indicate the majority of the chemical signal flows around the antennae rather than through it. Boundary layer structure greatly effects the spatial and temporal nature of chemical signals by acting as a smoothing filter for incoming signals. The exact nature of this physical filter depends upon the ambient flow speed and the angle of the antennae to the flow. This study illustrates the importance of form and function in chemoreception and in an organism's ability to detect environmental cues. Through the analysis of the boundary layer, the actual spatial and temporal nature of chemical signals arriving at the receptor cells for moths can be defined.

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262. Luteinizing hormone-releasing hormone neuronal migration in 48 h cultures of whole ten day old embryonic Swiss mice

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The origin of luteinizing hormone-releasing hormone (LHRH) neurons in embryonic mice occurs within a narrow time-frame (day 10; plug day = 0) and the migration of these cells follows a spatially and temporally ordered course from the medial olfactory placode, across the nasal septum (days 11.5–14) to their termination in the forebrain (day ~16). The purpose of this study was to develop a reliably reproducible procedure for growing whole mouse embryos in culture in order to study the development of LHRH neurons. Pregnant mice were killed by cervical dislocation on day 10, and the embryos removed with care taken to open and reflect (but leave attached to the embryo) the amnion, visceral yolk sac and placenta. Control embryos were placed in fixative. Experimental embryos were transferred individually to sterile warmed culture tubes with culture medium and a gas mixture of 95% CO_2 and 5% O_2. The culture tubes were placed in the integral rotisserie device of an Autoblot Mini-Hybridization oven incubator (Bellco Glass Co.) maintained at 37.5°C, rotating at 30 r.p.m. and the cultures were gassed about every 6 h. Compared to littermate controls collected at 10E the cultured embryos collected after 24 or 48 h in culture showed development of the somites, limb buds, and branchial arches appropriate for11-or 12-day-old mouse embryos. Double-label immuno- cytochemistry of 24 h cultures showed development of the medial olfactory pit and LHRH-immunoreactive (ir) cells were present within the epithelium of this part of the placode. A neural cell adhesion molecule-(N-CAM)-ir migration route was present in a normal pattern of distribution for 11 day mouse embryos. In 48 h cultures, LHRH-ir cells were now seen with N-CAM-ir axons in the nasal mesenchyme and in the ventromedial forebrain in a spatiotemporal pattern of development characteristic of 12-day- old embryonic mice.

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263. The endogenous lectin, galectin-1, utilizes unique laminin isoforms and β-lactosamine-containing glycolipids for axon guidance of olfactory neurons

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Galectin-1 is a divalent, lactosamine-binding lectin expressed in the vertebrate nervous system. We have previously demonstrated that galectin-1 binds and co-localizes with two ligands in the rat olfactory system: a β-lactosamine-containing glycolipid, and a putative member of the laminin family. The glycolipid, paragloboside, is expressed on the surfaces of olfactory axons originating in the olfactory epithelium (OE) and vomeronasal organ. The laminin family member, laminin-2 (merosin), is present in the extracellular matrix (ECM) of axonal pathways leading to synaptic targets in the olfactory bulb. We have shown, in cell culture, that galectin-1 can promote crosslinking of adjacent axons and axonal adhesion to the ECM. Immunocytochemical studies reveal that five laminin chains (α2, α3, β1, β2 and g1) are expressed in the olfactory system. However, α2-laminin is the only detectable heavy chain associated with axonal pathways. Using reverse transcriptase PCR, we recently cloned and sequenced a 600 bp C-terminal region of rat olfactory α2-laminin. *In situ* hybridization analysis revealed α2-laminin chain probes hybridized specifically to the lamina propria and the outer nerve layer of the olfactory bulb, and is particularly abundant within vomeronasal nerve bundles. It is also clear that galectin-1 and α2-laminin synthesis occurs in different cells, galectin-1 message being concentrated in stromal cells surrounding the olfactory and vomeronasal nerve bundles, whereas α2-laminin message appears to be expressed in glial cells within axon fascicles. These data suggest that this novel adhesion mechanism may play an important role in axon–axon and axon–matrix interactions during developing olfactory system. Immunocytochemical studies suggest that laminin-2 and laminin-4 are the major laminin isoforms expressed in the prenatal and neonatal olfactory system, however a third isoform that contains the α3 heavy chain is expressed in the basement membrane of the olfactory epithelium and may be involved in basal cell adhesion to the basal lamina.

264. Cypha™ [propionic acid, 2-(4-methoxyphenol) salt] inhibits the sweet taste of sucrose in humans but not in rats

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Cypha™ propionic acid, 2-(4-methoxyphenol) salt, is a commercially available sweet taste inhibitor used in food products. Schiffman *et al.* confirmed the effectiveness of Cypha in reducing the sweet taste response to a variety of sugars and artificial sweeteners. The present study determined if Cypha also blocked sweet taste perception in rats. This was accomplished by measuring the consummatory response of rats to sucrose solutions during short-term 'taste' tests. Nondeprived female rats were given two-bottle choice tests (10 min) with a standard 10% sucrose solution versus an alternate solution. When given the choice between 10% sucrose and 10% sucrose containing Cypha at concentrations of 0.0125–0.1% the rats showed no reliable preference. In subsequent tests they reliably preferred 10% sucrose to 8 and 6% sucrose, demonstrating the sensitivity of the behavioral test. To confirm the activity of the Cypha sample, a second experiment was conducted with humans. Using a visual analogue scale, the subjects rated the sweetness of various sucrose solutions (1–10%) and 10% sucrose solutions containing 0.0125 and 0.025% Cypha. Cypha reliably reduced the sweet taste intensity of the 10% sucrose solution. The 10% sucrose + 0.0125% Cypha solution was estimated to be isosweet to an ~2.9% sucrose solution, and the 10% sucrose + 0.025% Cypha solution was isosweet to an ~1.2% sucrose solution. Taken together, these data confirm the sweet taste inhibitory effect of Cypha in humans and demonstrate its ineffectiveness in rats. These results are consistent with other reported differences between rats and humans in their responses to other sweet taste inhibitors as well as to artificial sweeteners.

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265. Electrical responses to odor systematically vary with position along the turbinate bones in the rat

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Electro-olfactogram responses to a series of terpene compounds were recorded from the rat olfactory epithelium. The animals were anesthetized with ketamine-xylazine during surgical removal of the bone and epithelium from the right side and were overdosed when the septum was removed to expose the epithelium overlying the turbinate bones of the left side. Odors were injected into a stream of humidified air applied directly to the epithelium. Four electrodes for simultaneous recordings were placed either along the anterior border of endoturbinate 4 or in homologous positions on endoturbinates 2, 2′, 3 and 4. Recordings at all sites were normalized to the amyl acetate response at the same site. These positions allowed rough comparison with published maps of olfactory receptor gene expression zones from several laboratories. Sites corresponding to the most ventral expression zones responded best to compounds without oxygen: limonene and α-terpinene. Those in the dorsal zones responded best to ketones: carvone and menthone. Cineole evoked larger responses in intermediate zones. These data suggest a relationship between chemical structure and the odors activating most of the sensory cells in each receptor expression zone.

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266. Sweet taste in women with gestational diabetes mellitus

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Gestational diabetes mellitus (GDM) is a type of glucose intolerance which develops during pregnancy. Individuals with other types of diabetes (types I and II) are known to experience changes in sweet taste perception. This impairment may result in excessive intake of sweet foods contributing to poor dietary habits. This might explain why dietary compliance in a pregnancy complicated by diabetes is particularly difficult to achieve. Changes in sweet taste in women with GDM have never been studied and was the objective of the present study. 30 normal pregnant women and 17 pregnant women with GDM were recruited from an outpatient pre-natal clinic. The groups were matched in age, and ethnicity. Subjects evaluated a battery of test stimuli for liking and intensity using standard rating scales. These included cherry Kool-Aid prepared with 5 concentrations of sucrose (1.5–24%); a strawberry milk beverage varying in fat (0, 5 and 10%) and sucrose (0, 5 and 10%) concentration; and 5 sweet commercial foods. Preliminary results suggested there were no group differences in sweetness intensity ratings for any of the stimuli. However, the GDM group gave significantly higher liking ratings for sweetness and overall flavor in the 0 and 5% fat strawberry milk samples ($Ps \leq 0.05$–0.01). There were no group differences in liking ratings for the Kool-Aid or the commercial foods with the exception of vanilla pudding ($P < 0.05$) which was less liked by the GDM group. The lack of group differences particularly for the commercial foods may be due to response bias since the use of sweet foods is strongly discouraged in diabetes. Examination of their dietary records, information about their food cravings and the planned re-testing of the subjects in the post-partum period should help clarify this possibility.

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267. Immunocytochemical comparison of the localization of NGF and NGF-receptor (p75) in olfactory bulbs of developing normal and hypothyroid rats

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The immunocytochemical localization of the neurotrophin NGF and its low affinity receptor (p75, NGF-R) was investigated in the developing olfactory bulbs (OB) of normal and hypothyroid Sprague–Dawley rats. Thyroid deficiency was induced by adding propylthiouracil (PTU) in water (0.1% v/w) from birth which reduced body and brain growth. Our previous research has shown that >50% of the olfactory receptor neurons fail to develop (Paternostro and Meisami, 1993, 1994) and 20% of the mitral cells degenerate (Sendera and Meisami, 1993) in the OB of the developing hypothyroid rats; while the number of glomeruli is unchanged, their average size is markedly reduced (Sendera and Meisami, 1993). Immunocytochemical determination of NGF using NGF antibody M-20 (Santa Cruz Biotechnology) in the OB of normal 25- and 90-day-old rats revealed extensive localization within the cell bodies of the small cells, most likely the internal granular and periglomerular neurons. NGF was conspicuously absent from the cell bodies of the large relay neurons such as the mitral cells and tufted cells and from the neuropil of the glomeruli. The pattern of localization of NGF in the OB of hypothyroid 25- or 90-day-old rats was generally similar to the age-matched control OB even though hypothyroid OBs were markedly reduced in size and showed elevated NGF-R levels as stated below. As shown by us recently (Sendera and Meisami, 1994, 1995), immunocytochemical expression of NGF-R using MAb 192-IgG (Bohringer) in the normal OB was found to be patchy and mainly limited to the glomerular neuropil where expression intensity varied from none to moderate, depending on the glomeruli. NGF-R expression in the 25- and 90-day hypothyroid OB was markedly elevated with most glomeruli showing moderate to high levels of NGF-R (Sendera and Meisami, 1994, 1995). These results suggest that NGF-R in the normal rat OB is associated with either the terminal dendrites of neurons innervating the glomeruli or the processes of glomerular astrocytes. In short mitral and tufted cells apparently do not contain NGF and NGF-R and that NGF maybe associated mainly with the late arising and developmentally plastic local inhibitory neurons.

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268. The effects of sodium-free diet on gustatory neural responses in the geniculate ganglion

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Neural activity in the geniculate ganglion was compared between animals maintained for at least 10 days on a sodium-free diet (deplete condition) and animals maintained on the same diet to which 1% (w/w) NaCl had been added (replete condition). The rats were anesthetized and the geniculate ganglion was exposed dorsally by burring through the petrous ridge of the temporal bone. The neural activity of 65 single units (33 deplete, 32 replete) was isolated using glass insulated tungsten electrodes while stimulating the anterior tongue with a series of sapid stimuli. Sodium deprivation reduced the responsiveness of these neurons to each of the four standard stimuli: 0.3 M sucrose, 0.1 M NaCl, 0.01 M citric acid and 0.003 M quinine HCl ($P < 0.01$). Individually, however, only the response to citric acid was lowered significantly. Because neither sucrose nor QHCl produced large responses under either diet condition, this greater reduction in the relative effectiveness of citric acid increased the salience of the neural message produced by NaCl. In the replete condition, citric acid produced an average response that was 84% of the average response to NaCl. In deplete rats, acid was only 49% as effective as

salt. Thus, although dietary sodium deprivation reduces the amplitude of the NaCl response, its distinctness relative to other common sapid stimuli is increased.

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269. Reinnervation of taste buds following unilateral chorda tympani nerve cut at adulthood: effects of Na^+ restriction during reinnervation

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When the chorda tympani is sectioned, taste buds degenerate and subsequently regenerate upon reinnervation. Due to this characteristic, the gustatory system is a model system for the study of nerve–target interactions. In order to study such interactions, we have developed a technique whereby the number of geniculate ganglion cells that innervate a given taste bud can be determined by iontophoresing small amounts of fluorescent tracer into single papilla, taken up by neuronal processes, and transported to their cell soma in the geniculate ganglion. In adult rats, the number of geniculate ganglion neurons that innervate single fungiform taste buds are highly correlated with the size of the taste bud ($r = 0.92$). This linear function can serve as the standard, enabling comparisons between the normal innervation of taste buds and the effects of experimental manipulations. Since dietary Na^+ restriction has variety of effects on the gustatory system, we sought to learn whether the dietary manipulation alone and in combination with unilateral chorda tympani nerve cut at adulthood altered the normal innervation patterns. Results similar to those of sodium replete, unilateral cut rats were obtained in Na^+ restricted, cut rats. Both sides of the tongue showed altered innervation patterns. The uncut side had a greater than normal number of innervating neurons, and the cut side had a reduced innervation pattern following regeneration. Taste bud size was also affected. The mean taste bud volumes in rats placed on a Na^+ restricted diet during regeneration was significantly larger on the uncut side compared with the regenerated side. Preliminary results indicate that taste bud size in Na^+ restricted rats were smaller than the respective taste buds in rats with the control diet. These results indicate that the number of neurons innervating taste buds may determine the size of the taste bud, or vise versa, and that dietary Na^+ has an additional influence in unilateral cut adult rats.

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270. Solution properties and sweet taste of D and L sugars

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With an aim to investigating the chiral aspect of the sweet taste receptor, solution properties and taste of D, of L and of D,L sugars and other tastants have been measured.

It is well established that the chemical and physical properties of D and L sugars are identical apart from the direction in which they rotate the plane of polarized light. Recently it has been accepted that contrary to early theory, L sugars, like D sugars, taste sweet. The similarities in sweet taste of some D and L sugars are further investigated here. The solubility of sugars is known to be less for racemic mixtures than for the pure enantiomers. Solution properties of the single enantiomers and of equimolar mixtures of the two, however, show no significant differences, and are, indeed, remarkably similar. For example, the apparent molar volumes of D- and L-arabinopyranose and of the D,L mixture range from 93 to 94 $cm^3\,mol^{-1}$ for concentrations of up to 4.0 molal, which is typical of pentopyranoses and characteristic of pure sweet taste. Isentropic apparent molar compressibility values range from -2.2×10^{-3} $cm^3\,mol^{-1}\,bar^{-1}$ to -6.0×10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$ over the same concentration range. These results rule out the existence of any chiral recognition between the enantiomers.

The properties of analogous sugars with similar structures are also studied. For example, β-D-arabinopyranose is an analogue of β-D-fructopyranose, the sweetest simple sugar. α-L-Arabinopyranose is an analogue of β-D-galactopyranose, which is low insweetness. Both the arabinose enantiomers are, however, low in sweetness. This apparent anomaly seems to originate in the attachment of the hydroxymethyl group to the anomeric carbon atom.

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271. Olfactory and trigeminal sensitivity to nicotine

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Investigation of olfactory and trigeminal responses to vapor phase nicotine offers a potentially useful way to establish a link between perceived irritation and odor and the activation of specific receptor proteins in the nasal cavity. Many different types of neuronal nicotinic acetylcholine receptors (nAChRs) have been characterized. Work is currently underway in our laboratory to identify the nAChR subunits present in the nasal cavity and to measure the binding affinity of L- and D-nicotine to olfactory and respiratory epithelia. Our human psychophysical and animal electrophysiological work shows the following:

(i) In normal human subjects, the odor threshold for nicotine (0.03 p.p.m.) is at least 30-fold lower than that to acetic acid, amyl acetate, or propionic acid. In anosmic human subjects, the nasal irritation threshold for nicotine (0.6 p.p.m.) is 15–500 times lower than that for these other odorants.

(ii) Thresholds based on pigeon olfactory nerve and rat trigeminal nerve responses to nicotine are ~1 log unit higher respectively, than the odor threshold for normal human subjects

and the nasal irritation threshold for anosmic human subjects. Rat trigeminal recordings show that, although thresholds to L- and D-nicotine are similar, suprathreshold responses to D-nicotine are much weaker.

These findings are consistent with the hypothesis that nAChRs in the olfactory and respiratory epithelia mediate responses to nicotine.

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272. Genetically determined body odors in mice

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The genes of the major histocompatibility complex (MHC) appear to influence mate selection via olfaction so that inbreeding is minimized within a population. The evidence for this proposition comes mainly from observations of mate choice and the genetic makeup of offspring in freely interbreeding populations of mice, as well as numerous observations that a mouse can smell the difference between two other mice that are genetically nearly identical—differing only in the 0.1% of the genome that constitutes the MHC (i.e. a congenic pair of mice). The most detailed observations of the relationship between mate choice and the MHC have necessarily been made in laboratory investigations of congenic mice, but some evidence suggests that such a mechanism is operative in other species, from humans to guppies. The odorous signals apparently emanate from skin glands and urine or even blood serum and amniotic fluid. In mice, urine is a convenient and reliable source of strong MHC-regulated odors. We report here the results of recent gas chromatographic analyses of volatile compounds in diethyl-ether extracts of acidified urine samples from congenic C57BL/6 male mice having MHC types H-2b (B) or H-2k (K). Of the 32 distinguish able gas chromatographic (GC) peaks that were reliably quantifiable in either type B or K urine samples, eight were found to occur in significantly different quantities in the two types of urine. There were no compounds detected that were unique to either of these two MHC types. The biological relevance of these chemical results was supported by the finding that mice trained to distinguish B and K urine samples in a Y-maze could, without further training, distinguish volatile compounds emanating from the diethylether extracts used for the GC analyses. They did not distinguish volatile compounds from the urine samples after extraction. Further chemical and behavioral experiments indicate that the active compounds, or their labile precursors, in the urine are acidic.

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273. Olfactory receptors: molecular basis for a functional map in the olfactory bulb

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Sequence analysis and preliminary *in situ*, knockout, and immunocytochemistry studies have suggested that olfactory receptors may not only bind odor molecules, but also guide sensory cell axons to their targets in the olfactory bulb. Correlated mutation analysis shows that residues in the predicted transmembrane odor-binding sites are correlated to residues in the extracellular loops. If sensory cell axons are sorted and targeted based on the loop sequence(s), this mechanism could create a functional, determinant-based map of odor ligand structure in the olfactory bulb. In order to test this hypothesis we have studied correlations between olfactory receptor sequences and the positions of glomeruli which correspond to those sequences. Based on *in situ* results, glomeruli with similar (cross hybridizing) sequences are nonrandomly distributed in the olfactory bulb. To address this question more directly, we have developed rt-pcr methods which are enabling us to clone olfactory receptors from small samples of olfactory bulb. The results of these methods will be compared with functional data from 2-deoxyglucose and c-*fos* results to study the relations between olfactory receptor sequence, locations of the corresponding glomeruli, and sites of odor-induced activity in the olfactory bulb).

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274. The primacy of taste in aversion learning: was Garcia right?

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Conditioned taste aversion is often used as a prime example of a biological constraint on or preparedness for associative learning. Thus, prior experiments indicate that rats readily associate taste but not other sensory cues with illness and, further, taste cues are effective in producing one-trial learning even when the taste and onset of illness are separated by hours. That odors are relatively poor cues for aversion learning unless combined with a taste appears to provide additional evidence for the primacy of taste in aversion learning. We report results of experiments on odor aversion learning which strongly contest these views and reveal an important methodological flaw in prior studies comparing the effectiveness of taste with other cues in acquisition of illness-induced aversion learning.

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275. Suppression of primary taste and secondary taste and smell cortices during flavour processing in the human

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Anatomical and physiological investigations in monkeys suggest that the caudolateral orbitofrontal cortex is the first sight of convergence of gustatory and olfactory information. Bimodal cells in this area have been identified which are selectively tuned to respond to a matched taste and smell such as sucrose and banana odour. It has therefore been proposed that this area corresponds to the flavour cortex in monkeys.

To examine how the human processes simultaneous presentation of taste and smell information (flavour) we used positron emission tomography (PET) to evaluate differential processing of olfactory, gustatory and combined olfactory and gustatory stimuli as indicated by invoked cerebral blood flow changes during presentation of these stimuli. We devised two independent conditions of taste alone and smell alone as well as two combined conditions using identical stimuli as in the independent condition so that in one condition the odour and taste matched (i.e. soy sauce odour and salty taste) and in the other condition the odour and taste did not match (i.e. soy sauce and sour taste).

We found significant suppression of primary taste and secondary taste and smell cortices during stimuli presentation compared with independent presentation of identical stimuli. Additionally, blood flow increases in the left amygdala and left diagonal band of Broca in the unmatched condition compared with the matched condition were observed. These areas have previously been implicated in assessment of both novel and aversive stimuli.

276. Electrophysiological findings in the primary gustatory nucleus of the goldfish: response to glutamate antagonists in the vagal lobe

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The vagal lobe of the goldfish is a highly laminated structured involved in the sensory coding of taste. *In vitro* slice preparation of this dorsal medullary structure permits pharmacological access to the gustatory nerve terminals in the CNS. We sought to characterize pharmacologically the neurotransmitters that may be involved in sensory processing, beginning with excitatory amino acids.

Vagal lobe slices 300–700 μm thick were cut on a vibratome then bathed in artificial CSF with a flow rate of 2 ml/min for 30–60 min. Following this, small fascicles of the primary gustatory nerve (X, vagal nerve) were stimulated with paired single pulses (60 ms apart) at 12–40 V for 0.1 ms using bipolar electrodes, while recording with a 2 M NaCl-filled glass micropipette from local regions in the sensory layers (within layers VI–VIII) of the vagal lobe. The recording electrode was positioned within the vagal lobe so as to maximize the amplitude of the two negative-going post synaptic population responses.

The two negative-going responses were abolished at different post-application times following application of kynurenic acid (final concentration = 100 μM), a glutamate antagonist, to the bathing medium. Kynurenic acid affects the second synaptic response generally within the first five min post-application, whereas longer application of the antagonist is needed to effect the first synaptic response. Each response returns upon wash out of the kynurenic acid from the bathing medium. Absence of the responses following application of Ca^{2+}-blockers or bathing in Ca^{2+}-free medium indicate that the responses observed were indeed due to synaptic currents. The synaptic responses were completely abolished by the selective antagonists that block both NMDA and non-NMDA receptors (APV and DNQX respectively). These results indicate that both the primary synaptic response and higher order synaptic responses in the vagal lobe are sensitive to both NMDA and non-NMDA antagonists and therefore are most likely an excitatory amino acid system. Studies are continuing on the pharmacology of the excitatory amino acid receptors utilized by this system.

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277. Evidence of pore diffusion in the temporal perception of sweetness

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Sweetness of approximately equisweet levels of thaumatin, monellin, brazzein, sucrose and aspartame was continuously rated by trained judges using time–intensity (TI) methodology. The onset and decay rates of the average TI curves differed among compounds. For the decay portion of the curves, an exponential decline in intensity with time was exhibited, indicating a rate process that is first order in sweetener concentration. If the decay is controlled by the diffusion of the compound through a stationary boundary layer, the rate would be directly proportional to the diffusivity of the compound. The first order rate constants were not correlated with the estimated component diffusivities, but they did increase in the same order as the diffusivities. A model for the temporal perception intensity, based on a description that considers the extent of binding to be controlled by pore-diffusion with reversible adsorption, is proposed to describe the nature of the decay curves. In such a case, the rate of decay is dependent on the product of the component diffusivity and the desorption rate constant, rather than the diffusivity alone. Using estimates of the depth of the pore and the component diffusivity, the desorption constant for each compound has been estimated and is compared for the five sweeteners.

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278. Blocking in binary odorant mixtures: does the peripheral olfactory system act as a filter in processing sensory information?

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A central problem in olfaction regards how the olfactory system allows for consistent recognition of biologically meaningful but variable odor mixtures. Olfactory blocking experiments have indicated one possible solution to this problem by showing that components of an odor mixture that have been previously learned suppress learning of components that are subsequently added to the mixture. Recent data from vertebrates and invertebrates indicate that modulation of synaptic transmission within peripheral olfactory circuitry occurs, but the function of this modulation is unknown.

Here we present a realistic neural network model of the honeybee antennal lobe that incorporates Hebbian synapses embedded into known circuits. The model shows that the neural representation for an odorant mixture is much more similar to one of the mixture components when that component has been previously learned compared with when it was not learned—that is, it can account for olfactory blocking. The results presented here suggest that plasticity in the peripheral olfactory system can give rise to a neural representation that is robust to perturbation of the sensory pattern. The model thus defines a feature extraction task that could be an important function of early synaptic processing in the olfactory system.

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279. The perception of saltiness is eliminated by adaptation to NaCl but not by amiloride treatment

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The epithelial Na^+-channel blocker amiloride produces a marked reduction in the chorda tympani response to Na^+ and Li^+ salts in several species. Studies of the effects of amiloride on human perceptions of the taste of NaCl have yielded mixed results; some show a reduction in its perceived intensity or saltiness and others show no effect. Previous results from our laboratory have shown that amiloride produces an ~20% or less reduction in the intensity of NaCl, that it has a greater effect on the perceived intensity of Na-gluconate (as predicted from electrophysiological studies on the rat), and that the reduction in the intensity of both of these salts is due to the elimination of their sour side taste rather than their saltiness. In order to further evaluate the effects of amiloride on human perceptions of the sour taste of salts and to demonstrate that this result is not an artifact of the psychophysical method, we have conducted several addtional experiments.

First, we demonstrated that LiCl has a stronger sour component than NaCl and that treatment of the tongue with 10 μM amiloride blocks the sour taste of LiCl at several concentrations; there is no effect on its perceived saltiness. Second, when the same subjects are tested under three different adapting conditions—distilled water, 0.1 M NaCl and 10 μM amiloride—only NaCl adaptation reduces the saltiness of NaCl and LiCl. NaCl adaptation reduces the salty taste of all salts and amiloride reduces the sour taste of Na^+ and Li^+ salts. Additional experiments demonstrate that the effects of amiloride on saltiness reported by others are due to the psychophysical method. When only a single quality is estimated (saltiness or sourness) perceived intensity is reduced, but when subjects are asked to estimate both qualities simultaneously there is no effect of amiloride on the salty taste of these stimuli.

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280. Inositol 1,4,5-trisphosphate receptor expression in rat olfactory tissue indicates a primary role in calcium-mediated secretion

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IP_3 receptor expression was analyzed in rat olfactory tissue. Northern analysis with an oligonucleotide that represented a unique cDNA sequence of type III IP_3 receptor indicated this gene was expressed in olfactory tissue. *In situ* hybridization with a probe to a conserved transmembrane region of type I IP_3 receptor showed labeling in the lateral nasal and septal glands. Uniform labeling was also observed within the vomeronasal organ, and IP_3 receptor expression here is consistent with chemoattractant stimulation of IP_3 turnover (Luo *et al.*, 1995), and with vomeromodulin secretion (Rama Krishna *et al.*, 1995). Clusters of silver grains were also observed in the lamina propria beneath the main olfactory epithelium. Finally, expression of IP_3 receptor was observed in the epithelial layer of the main olfactory system. Antibodies to type I IP_3 receptor showed immunoreactivity in the septal glands of the lamina propria, and in the vomeronasal glands. RNase protection and northern blot analysis both indicated that the SII+ (neuronal) form of IP_3 receptor was not expressed in rat olfactory tissue. RNase protection analysis of IP_3 receptor yielded protected fragments of identical size and number in both rat olfactory tissue and a rat pituitary neurosecretory cell line. Finally, northern analysis indicated that the G-protein subunit $G\alpha_{11}$ was expressed in rat olfactory tissue. Together, these results indicate that a major role of IP_3 within rat olfactory tissue is to spatially regulate calcium-mediated secretory processes. We hypothesize that IP_3 may control secretory granule migration and fusion with ciliary membranes of receptor neurons for transport of IP_3 receptors to the main olfactory epithelium.

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281. Magnitude matching adds power to the labeled magnitude scale

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The labeled magnitude scale (LMS) is anchored by verbal descriptors of intensity that reflect experiences in everyday life. It was derived by Green *et al.* in 1993 as an alternative to magnitude estimation (ME) and produces similar psychophysical functions. Thus ME and LMS are comparably effective in measuring relative perceived intensities within individuals. However, ME cannot be used to compare sensory perception across individuals (or groups) without the use of magnitude matching, which employs a standard from a control modality. The question we pose is whether or not LMS adjectives behave as such a standard by defining a common range of perceptual response. If they do, then the LMS represents an absolute scale of perceived intensity and should permit comparisons of raw LMS ratings across individuals (or groups). To test this, we used several characteristics of genetic taste blindness for the bitter compound 6-*n*-propylthiouracil (PROP). First, there is broad variation in the ability to taste PROP, such that some individuals taste nothing while others experience intense bitterness. Next, the saltiness of NaCl is essentially independent of PROP allowing NaCl to act as a standard against which PROP tasting can be assessed. Finally, the oral irritation produced by capsaicin varies with the perception of PROP. Subjects (n = 110) judged the intensity of PROP and NaCl with both the LMS and ME. Using the LMS alone, they also rated the burn of three concentrations of capsaicin (1, 10 and 100 p.p.m.) applied to the tip of the tongue. A variety of measures of PROP intensity were plotted against both raw and normalized (to 1 M NaCl) LMS ratings of capsaicin burn. The measures of PROP intensity were (i) raw LMS ratings of 0.0032 M PROP (near saturated); (ii) normalized LMS ratings of 0.0032 M PROP; (iii) a ratio of PROP bitterness to NaCl saltiness obtained with ME; and (iv) the same ratio obtained with LMS. The highest correlations resulted from normalized LMS ratings of PROP and capsaicin. Nonetheless, the raw LMS ratings produced a significant correlation for 100 p.p.m. capsaicin. LMS and ME PROP ratios only produced significant correlations when capsaicin ratings were normalized; LMS PROP ratios produced higher correlations than ME PROP ratios.

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282. Disappearance of fungiform papillae and taste pores in rats with unilateral chorda tympani section at 10 days postnatal

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In early postnatal rats (10 days), not only are taste buds affected when the chorda tympani is sectioned bilaterally but there is also a permanent loss of fungiform papillae. Since bilateral sectioning of the chorda tympani in adults is much less devastating, there must be a developmental component controlling taste bud and fungiform papilla maintenance by the chorda tympani nerve. We sought to learn whether unilateral sectioning of the chorda tympani in early postnatal rats also resulted in a permanent loss of taste pores and papillae, or whether the intact chorda tympani nerve on the contralateral, intact side was able to invade the unoccupied territory and maintain gustatory structures. The right chorda tympani nerve in 10-day-old Sprague–Dawley rats was sectioned in the neck as it bifurcates from the lingual branch of the trigeminal nerve. The lingual nerve was not damaged. Rats were killed and fixed at 2, 8, 25 and 50 days following surgery. The tongues were removed and subsequently prepared for gross histological examination by removing the underlying muscle layers and staining the dorsal lingual epithelium with methylene blue. After flat-mounting the epithelium between glass slides, papillae and taste pores were reconstructed and counted with a computer microscope system (Neurolucida; Microbrightfield). There was a loss of taste pores and papillae on the cut side of the tongue. Such a loss occurred between 8 and 25 days post-section. Normal numbers of taste pores and fungiform papilla were evident at 8 days post-section, whereas there were only ~15% of papillae seen at 25 days postsection, many of which had a filiform papilla-like appearance. This trend was maintained through 50 days postsection. The remaining papillae on the cut side predominated near the midline, indicating little or no sprouting of CT fibers from the contralateral, uncut side. These findings indicate, along with the bilateral-sectioned rats, that during early development, trigeminal neurons alone are insufficient to maintain fungiform papillae. Further, these data show that the amount of plasticity of intact neurons is limited even at 10 days postnatal. We are currently in the process of studying whether neural reorganization is induced on the intact side by examining the innervation patterns of taste buds in single fungiform papilla.

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283. Spawning male goldfish release a steroidal odorant which functions as a potent pheromonal odorant with inhibitory actions on conspecifics

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Goldfish, and likely many other species of fish, use metabolites

of gonadal hormones as sex pheromones. Particularly well established is the existence of a pre-ovulatory 'priming' pheromone and a post-ovulatory 'releasing' pheromone in female goldfish. Recent evidence suggests that the pre-ovulatory pheromone is comprised of a changing mixture of three steroids. Here we report that spawning male goldfish release a hormonal pheromone of their own, the androgenic steroid androstendion. This steroid is released by sexually active males in large quantities (1 μg/h) and functions as a potent and specific olfactory stimulant for conspecifics (threshold 10^{-11} M). Its actions are inhibitory: it suppresses the endocrine responsiveness of conspecific males to the female pre-ovulatory pheromone and elicits agonistic behavior. In addition, because sexual dimorphism in peripheral olfactory sensitivity is noted, we suspect that this cue might influence female behavior. Pheromonal communication using hormonal derivatives clearly, can be a two-way street.

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284. A behavioral assay of the amiloride concentration–response curve with respect to salt taste in the rat

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The epithelial sodium channel blocker, amiloride, is noted for its inhibitory effect on sodium transduction in taste receptor cells. In rats, there is behavioral evidence that amiloride changes the perceptual quality of NaCl, making it more similar to that of nonsodium salts. Although electrophysiological studies have produced amiloride dose–response functions with respect to its inhibitory effect on chorda tympani nerve responses to NaCl, much of the behavioral work has employed just single concentrations (usually 100 mM) of the drug. The purpose of our experiment was to (i) test the effects of amiloride on an operant salt discrimination task and (ii) quantitatively assess the effectiveness of a broad range of amiloride concentrations to disrupt performance. In our procedure, water-restricted animals were trained to lick a drinking spout for a small taste sample (10 licks; 3 s sample period) and to press one of two levers (within 5 s) if the taste stimulus were NaCl and the other lever if it were KCl. If the rat responded correctly it received water reinforcement (40 licks; 10 s reinforcement period); an incorrect response resulted in a 30 s time-out. Several concentrations (0.05, 0.1 and 0.2 M) of both taste stimuli, delivered in randomized blocks during sessions, were included to render intensity an irrelevant cue. A single concentration of amiloride (1, 3, 10, 30 and 100 mM) was mixed in all solutions (including water reinforcement) for a given session. At least two non-amiloride sessions were interposed between amiloride test sessions to both measure and maintain stimulus control of behavior. The total percentage of correct responses was reduced to chance by the 100 and 30 mM amiloride concentrations and progressively improved as the concentration of the sodium channel blocker was lowered. The estimated amiloride concentration that produced 1/2 asymptotic performance was 4.5 mM. Further inspection of the data revealed that responses to NaCl were significantly affected in a concentration-dependent manner by amiloride, whereas responses to KCl were not. This procedure shows promise as a behavioral assay of manipulations that are thought to have impact on the number or properties of amiloride-sensitive taste receptors.

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285. The effects of gustatory nerve transection on conditioned sugar discrimination in the rat

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The chorda tympani (CT), greater superficial petrosal (GSP), and glossopharyngeal (GL) nerves all respond to sugar solutions in the rat. The collective gustatory branches of the seventh nerve (CT + GSP) are necessary and sufficient to maintain normal unconditioned licking to sucrose and maltose in brief access trials. Although the GL is not necessary to maintain such behavioral responsiveness to these sugars, some concentration-dependent licking does survive the combined removal of the CT and GSP. This prior work focused on the *motivational* rather than the *discriminative* properties of these taste stimuli. We examined whether the behavioral discrimination of sucrose from maltose is affected by gustatory nerve section. Using a conditioned shock avoidance procedure, we trained rats to suppress licking to either sucrose or maltose and maintain licking to the other sugar. Stimulus presentation (5 s) was randomized in repeated blocks and concentration was varied (0.05, 0.1, 0.2 and 0.4 M). Only rats showing competent discrimination were chosen for surgery. Each sham operated rat (n = 3) showed little surgically induced change in the percentage overlapping area of the response distributions (collapsed across concentration) for maltose and sucrose (mean ± SE: before = 9.9 ± 3.0%; after = 17.9 ± 1.1%). Although bilateral section of the CT (n = 3) has been shown to impair salt discrimination in a similar task, it had no effect on sugar discrimination performance (before = 17.1 ± 7.6%; after = 22.5 ± 5.4%). GL section (n = 3) was without effect (before = 17.4 ± 8.4%; after = 19.3 ± 6.7%). In contrast, combined section of the CT and GSP (n = 4) produced a severe impairment in sugar discriminability (before = 16.3 ± 5.1%; after = 61.0 ± 7.7%). The deficit was more pronounced at the lower concentrations. These results confirm that rats can discriminate sucrose from maltose and that this ability is heavily dependent on taste input from the seventh nerve, especially at the lower concentrations. The taste input of the GL is unnecessary but may be sufficient to maintain some sugar discriminability at higher concentrations.

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286. Chorda tympani nerve transection and partial desalivation differentially disrupt two-lever salt discrimination performance in rats

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Previous research has shown that chorda tympani (CT), but not glossopharyngeal, nerve transection impairs discrimination of NaCl from KCl in rats. In some of those studies, rats were trained to suppress licking to one salt (S+) and maintain licking to the other; failure to suppress licking to the S+ resulted in a brief footshock. Rats that were impaired on the task had a robust avoidance bias (i.e. suppressed licking to both salts), which may have obscured the expression of some residual discriminability. We developed a new procedure that does not use a shock punishment and thus eliminated the shock-induced response bias. Water-deprived rats licked a drinking spout to obtain a 10-lick sample of either NaCl or KCl (0.05, 0.1 or 0.2 M). The rat then pressed one of two levers if the stimulus was NaCl, the other if it was KCl. A correct response was rewarded with a 40-lick ration of distilled water, whereas an incorrect response was punished with a 30 s time out. Before surgery, the proportion correct averaged 0.90 ± 0.01. The rats then received CT section (CTX), sublingual and submaxillary salivary gland extirpation (DSAL), or sham surgery (CON), $n = 5$ per group. The CTX group differed from the other two on the proportion correct postsurgically ($Ps < 0.05$; CON: 0.88 ± 0.01, DSAL: 0.80 ± 0.03, CTX: 0.68 ± 0.05), but the DSAL group did not differ from CON. Unlike the CON group, however, performance in both the DSAL and CTX groups significantly decayed after surgery relative to before ($Ps < 0.05$). This study confirms that the CT is necessary for normal salt discrimination to be maintained in rats. Because the CTX group performed significantly better than chance, the remaining gustatory nerves can maintain partial competence in this task. In addition, partial desalivation moderately impairs this discrimination, but not to the same degree as CTX. Therefore, the deficits seen after CTX are unlikely to be solely due to the partial denervation of the sublingual and submaxillary salivary glands.

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287. Specific androstenone-anosmia in patients with impaired sperm production

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Olfactory cues play a central role in mammalian reproduction. Humans, however, seem to be less dependent on odor cues. In hypogonadotropic hypogonadism (Kallman's syndrome; prevalent in males), total failure of sperm production is associated with general anosmia. Perhaps less extreme reproductive malfunctions, e.g. oligospermia, might be associated with impaired, but not absent, olfaction. In a preliminary study, olfactory performance of oligospermic patients was found not to be different from that of controls when tested with common household odorants. There is considerable variation in the ability to detect the odor of androstenone. In an Israeli sample, ~30% failed to detect the compound's odor. Androstenone was one of the scratch and sniff samples in the 1986 National Geographic Smell Survey; 71 patients with severely impaired spermatogenesis and 64 controls were tested with this instrument. Results showed that the number of androstenone-anosmics among patients was significantly higher than among controls. To reexamine these findings, 50 severely oligospermic patients and 50 fertile controls were tested in a forced choice method. Results revealed a significantly higher number of androstenone-anosmics among patients than among controls. This suggests that certain types of reproductive malfunctions seem to be associated with specific anosmia to androstenone. Further investigation is needed to assess whether female reproductive failures might also be associated with similar specific anosmia and to study the mechanisms underlying the association in males.

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288. Adaptation in olfactory receptor neurons of the moth, *Manduca sexta*

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Adaptation allows a moth olfactory receptor neuron (ORN) to detect changes of pheromone concentration over a range of ~7 log-units. Here, changes in ionic currents and ion channel activities after strong pheromone-stimulation were examined and the involvement of Ca^{2+} and cGMP in adaptation was tested.

Cultured ORNs from *Manduca sexta* respond to pheromone stimulation with a specific sequence of ionic currents: (i) transient IP_3-dependent Ca^{2+} currents; (ii) Ca^{2+}-dependent cation currents; and (iii) protein kinase C-dependent cation currents (Stengl, 1994). These currents are assumed to cause depolarizations and at least in part underlie the quick rise and the plateau of the receptor potential (RP). Extracellular recordings from adapted pheromone-sensitive trichoid sensilla showed that the transient rapid initial phase of the RP was missing, and that the RP was rising slower to a smaller amplitude.

Preliminary FURA-measurements (in collaboration with Dr B. Lindemann, Homburg) showed that after strong pheromone stimulation intracellular Ca^{2+} concentrations rise in cultured ORNs. Patch clamp recordings demonstrated that after strong pheromone stimulation pheromone-dependent Ca^{2+} currents were never observed and cation channels and delayed rectifier K^+ channels closed. Experiments showed that 6 mM extracellular Ca^{2+} blocked IP_3-dependent Ca^{2+} currents within ms and Ca^{2+}-dependent cation currents within s. Protein kinase C-dependent cation channels were blocked by cGMP-dependent mechanisms. Therefore, it is assumed that rises of intracellular concentrations of Ca^{2+} and cGMP play a role in insect olfactory adaptation.

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289. Quality coding of different blend ratios of binary mixtures by olfactory neurons in the spiny lobster

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The aim of this study was to investigate quality coding of blend ratios of binary mixtures by olfactory cells in the spiny lobster. Three odorants [adenosine-5′-monophosphate (AMP), glutamate, and taurine] and seven blend ratios of each of their binary mixtures at a total concentration of 100 μM were examined. Extracellular recordings from 48 olfactory cells revealed that the responses of many of these cells to blend ratios were similar to those induced by the more effective component. However, some other cells clearly responded either less or stronger to some blend ratios than to the more effective component. Such mixture interactions, especially suppression, were most frequent for the mixture AMP/glutamate. The neural code evoked by each stimulus was then evaluated using vector space analysis, where each stimulus was represented by a vector of n coordinates corresponding to responses of n olfactory neurons. A measure of similarity between two neural codes that incorporates both the difference in vector length (overall magnitude of response) and the angle between the two vectors (distribution of the response across the cells) was calculated for each stimulus pair and used in multidimensional scaling. This analysis revealed that any concentrations ≥50 μM of a single compound evoked a similar neural code. Despite the occurrence of some mixture interactions, ratios of the three binary mixtures evoked neural codes which were orderly placed within a continuum between those elicited by the components. The relative position of neural codes for blend ratios within a continuum between neural codes for the components varied from mixture to mixture and was mainly determined by the sensitivity threshold and the dose-response curve of the cells, and some mixture interactions. These results suggest that, despite the occurrence of some mixture interactions in some olfactory cells, the neural code for blend ratios of binary mixtures is intermediate between the neural codes for the components and is not novel relative to those for the components.

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290. Detecting tastants in the presence of other tastants: issues of masking and aging

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When one taste (masker) is sufficiently strong, it can completely mask another taste (target) of different quality. To quantify this phenomenon the detection threshold for each of four target tastants (sucrose, NaCl, citric acid, and quinine hydrochloride) was measured as a function of concentration of each of the three others serving as masker, over a masking range from zero to strong. The 12 masking functions thus generated reveal that the greater the concentration of the target to be erased, the greater must be the masker's concentration, but in ever-increasing proportion. Some tastants mask each other much more efficiently than others, however; for example it is easier to erase the sour taste of citric acid with the sweet taste of sucrose than with the bitter taste of quinine hydrochloride. All of these functions illustrate a critical fact about aging. namely that the older person's target threshold is a constant factor higher than the younger person's (2–7 times, depending on the target tastant), regardless of the degree to which it is raised by masking. Thus with increasing masking concentration the thresholds of younger and older subjects go up in parallel. This implies that thresholds (and suprathreshold magnitudes, too) in water alone are poor indicators of the seriousness of age-related weakness to the complex tastes of foods. The effects of age were relatively mild for sucrose and citric acid, moderate for NaCl, and strong for quinine hydrochloride.

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291. Desensitization to oral zingerone irritation: effects of parameters of stimulation

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In humans, repeated oral stimulation with the irritant capsaicin produces sensitization or desensitization, depending on the temporal relationship and, to a lesser extent the intensity, of the stimuli. We have previously shown that zingerone, an irritant present in ginger, shows only desensitization across repeated samples, as well as following a hiatus in stimulation. Because the time course of zingerone irritation differs from that of capsaicin, it is likely that optimal temporal and other stimulation parameters may also be different. In experiment 1 we examined the effects of stimulus intensity (0.5, 1.0 and 2.0% zingerone) and the number of successive stimuli in a series on psychophysical responses to zingerone irritation within the series and following a 5 min hiatus. Experiment 2 examined the effect of the duration of this hiatus on desensitization and recovery.

Desensitization was apparent across the initial series of stimuli in both experiments, and irrespective of stimulus intensity in experiment 1. Desensitization also occurred following the 5 min hiatus, again irrespective of intensity. Preceding the hiatus with five or ten stimuli produced the greatest post hiatus desensitization, but a decrease in rated intensity was also evident following a single stimulus. Experiment 2 showed that the optimal hiatus for demonstrating desensitization was 5 min, and that by 15 min, recovery had begun. In both experiments, individual differences in response were marked, with some subjects showing sensitization and others little change in response across repeated zingerone stimuli. The origin of these differences is unclear but were shown to be relatively stable across multiple sessions.

292. Behavioral response of rats to benzyltriethylammonium chloride (BTAC) taste and associated inositol trisphosphate response in lingual tissue

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Quaternary amines, such as BTAC, should exhibit a bitter taste because the ammonium group provides the necessary polar group while the aromatic group provides the hydrophobic component, theoretically required according to currently accepted models. Long–Evans (LE) rats generally reject 1 mM BTAC and generalize a conditioned taste aversion from BTAC to quinine. Sprague–Dawley (SD) rats are either indifferent to, or show only a mild rejection of BTAC. Within the LE strain, males consistently rejected BTAC whereas females showed greater individual differences in preference scores yielding an essentially bimodal distribution. Inositol trisphosphate (IP_3) production in response to BTAC stimulation was compared in two groups of females showing a marked versus a mild aversion to BTAC. IP_3 production in the millisecond time frame was assessed in taste and control tissue using a quench flow apparatus. Homogenates of taste and control tissue were exposed to 1 mM BTAC from 0 to 200 ms. No change in IP_3 levels were observed over this time frame for control epithelial tissue in the presence or absence of BTAC. No changes in IP_3 levels were observed in taste tissue exposed to 1 mM BTAC from the group of female LE rats showing only mild aversion to BTAC, but an increase in IP_3 levels was observed in taste tissue exposed to 1 mM BTAC from the group of female (LE) rats showing a marked aversion for BTAC. IP_3 levels also increased in response to 1 mM BTAC in taste tissue from the male LE rats that showed an aversion to BTAC. SD rats failed to show an IP_3 response to 1 mM BTAC.

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293. Physicochemical studies of Na^+ sensing in the hamster anterior tongue

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Evidence from neurophysiological and electrophysiological studies suggests that cells in the taste buds of hamster fungiform papillae transduce sodium stimuli, in part, via apical, amiloride-sensitive sodium channels. We used simultaneous *in vivo* lingual epithelial voltage clamping and chorda tympani neurophysiology to study sodium transduction in adult hamster anterior tongue. Consistent with the idea that sodium ions traverse an apical sodium channel in hamster taste cells, chorda tympani responses to 10, 50 and 100 mM NaCl showed significant voltage sensitivity. Compared with responses under zero-current clamp (equivalent to open circuit), chorda tympani responses to 10, 50 and 100 mM NaCl were elevated under negative voltage clamp, while they were suppressed under positive voltage clamp. In addition, 5 μM benzamil greatly diminished chorda tympani responses to these concentrations of NaCl. These results confirm previous findings regarding the role of amiloride-sensitive sodium channels in sodium stimulus transduction in hamster. It has been suggested that, in hamster, protons are also transduced, in part, by apical, amiloride-sensitive sodium channels. Therefore, we predicted that chorda tympani responses to HCl would, like NaCl, exhibit voltage and benzamil sensitivity. In fact, chorda tympani responses to 1 and 10 mM HCl were insensitive to imposed voltage perturbations between –60 and +60 mV relative to zero-current clamp potential. Likewise, neither 5 μM benzamil nor 50 μM amiloride had a significant impact on neural responses to either concentration of HCl. The absence of voltage and/or amiloride sensitivity in chorda tympani responses to HCl suggests that, as in rat, an apical, amiloride-sensitive proton conductance is not a major pathway for proton transduction in hamster anterior tongue taste buds.

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294. Serotonin-immunoreactive taste cells are related by cell lineage, but may derive from multiple progenitors

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Using mosaic analysis, we previously showed that multiple progenitors from local epithelium give rise to mouse taste buds. In this study, we address the question of how many progenitors contribute to individual taste buds. In addition, we examine two developmental aspects of serotonin-immunoreactive (ir) taste cells: (i) whether this small subpopulation of taste cells derives from one, or multiple progenitors; and (ii) whether serotonin-ir taste cells are related by lineage. The female, mosaic mice used in these studies carry a lacZ marker on one of their two X chromosomes. Following X inactivation, the marker is randomly silenced in approximately half of the embryonic cells. Because the X inactivation status of a cell is a heritable feature, the presence or absence of an active lacZ marker can be used in cell lineage analyses. Using this approach, we previously found that taste buds located on borders between two epithelial patches, one expressing the marker and the other patch not expressing the marker, contain both expressing and non-expressing cells. The mixing of cell types in one taste bud indicates that at least 2 progenitors contribute to the formation of a taste bud. In the present study, complete counts of labeled and unlabeled cells in mixed taste buds were used to estimate taste bud progenitor number. Double-labeled (for the lacZ marker and serotonin-ir) taste buds containing few lacZ labeled cells were examined to determine if serotonin-ir cells derive from

one, or multiple progenitors. Our results indicate that either numerous (i.e. >10) progenitors contribute to individual taste buds, or progenitors contribute asymetrically to taste buds. Further, the serotonin-ir cells in a taste bud are related by lineage, but may arise from more than one progenitor.

295. Identification and partial characterization of putative taurine receptor proteins from the olfactory organ of the spiny lobster

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To explore the initial stages of olfactory transduction, we have used biochemical techniques to characterize proteins associated with the dendritic plasma membrane from the olfactory receptor neurons of the spiny lobster *Panulirus argus*. In particular, we havestudied proteins that interact with taurine, an amino acid that is an important odorant for this system. The cross-linker bis(sulfosuccinimidyl)suberate (BS^3) was used to covalently link [^{3}H]taurine to cell surface proteins on membrane from the aesthetasc (olfactory) sensilla of the lateral filament of the antennule. A radioligand–receptor binding assay was used to show that this cross-linkage was highly specific for taurine at 0.2 mM BS^3. In inhibition studies, of all of the unlabeled odorants tested at excess concentrations (taurine, L-glutamate, adenosine-5′-monophosphate), only taurine significantly inhibited the cross-linkage of [^{3}H]taurine to the membrane. Membrane containing cross-linked proteins was solubilized, separated on SDS–PAGE, and examined with autoradiography. Bands with molecular weights of 100, 82, 62, 51 and 34 kDa were evident on the gels. However, only the 100 and 62 kDa bands were consistently labeled with [^{3}H]taurine, and this labeling was inhibited in the presence of excess unlabeled taurine but not adenosine-5′-monophosphate. The taurine-evoked behavioral search response of spiny lobsters was significantly reduced following treatment of their antennules with BS^3 + taurine as compared with animals treated with BS^3 alone, suggesting that the taurine-labeled binding proteins include taurine receptor proteins involved in the first stage of olfactory transduction.

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296. Phagocytes in the taste buds of rat circumvallate papillae after denervation

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The death of taste bud cells occurs by apoptosis. However, little is yet known about the mechanism of disposal and clearance of dead cells and associated nerves. We examined taste buds of rat circumvallate papillae after the bilateral section of glosso-pharyngeal nerve to study phagocytic cells. Electron micrographs taken at 2–40 days after the nerve section showed that flat cells underneath the degenerated taste buds phagocytized debris through the basement membrane. Pre-embedding immunohisto-chemistry using anti-vimentin antibody showed that flat cells strongly reacted with vimentin. These cells had many fine cytoplasmic processes in association with active phagocytosis at 2–9 days after surgery. Immunohistochemistry using anti-macrophages antibodies (ED1, ED2) showed that macrophages were not present underneath or within the taste buds throughout the postoperative days. Most of ED2-positive resident macrophages were located in the deep layer of connective tissue and a few were in the nerve bundle. ED1-positive cells were seen in the epithelium throughout the postoperative days, however, they were positive for anti-OX62 antibody, which recognizes dendritic cells. Schwann cells actively phagocytized degenerated nerves at 4–6 days after surgery. The result indicates that phagocytes of the taste buds are fibroblasts and Schwann cells together with macrophages participate in the clearance of degenerated nerves. In comparison with the olfactory epithelium, it is clear that macrophages participate less in phagocytosis of the taste buds system.

297. Distribution of c-fos immunoreactivity in the rat brain following stimulation of the laryngeal opening with chemical stimuli

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Currently, little information exists concerning the central neural pathways that underlie the laryngeal chemoreflex; a reflex elicited by application of chemical stimuli to the laryngeal opening. Consequently, we used c-fos immunohistochemistry to ascertain which neuronal populations might be involved in processing laryngeal chemosensory information.

Rats were anesthetized with sodium pentobarbital and a tube was placed into the trachea for application of stimulus solutions. The stimulus, a mixture of 0.5 M KCl and 0.01 N HCl dissolved in distilled water, was applied to the receptor area for 1 min then removed by rinsing with saline. This sequence was repeated every 3 min over a 30 min period. The animals were killed 90 min after the completion of the stimulus protocol and the brains sectioned and processed for the demonstration of c-fos immunoreactivity.

Relative to control groups, applications of the stimulus mixture produced changes in the level of c-fos expression in several nuclei in the rat brain. In the brainstem, changes were observed in the caudal and intermediate nucleus of the solitary tract, ventrolateral medulla and in the region of the parabrachial pons. In the pons most c-fos positive neurons were observed in the dorsal and external lateral parabrachial nucleus and Kölliker–Fuse nucleus. Very few immunoreactive nuclei were observed in the pontine taste area. At more rostral levels, changes in c-fos expression were observed in the region of the hypothalamus, central nucleus of the amygdala and bed nucleus of the stria terminalis. These data suggest that in the rat, laryngeal chemosensory information is primarily transmitted to brain regions involved in the control of respiratory reflexes rather than along pathways relaying gustatory information to the cortex.

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298. Immunocytochemistry of Gα14 in taste cells of the rat

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G proteins are thought to be involved in two cascades associated with bitter taste transduction. The first cascade utilizes phosphodiesterase, while the second cascade utilizes phospholipase C. Both gustducin and transducin are postulated to be associated with the former cascade, while Gα14, a G_q subfamily, is thought to be associated with the latter cascade. In the present study we have examined both the light and electron microscopical localization of Gα14 in taste cells, using antisera toGα514 in circumvallate taste buds of the rat. Rats were perfusion-fixed with a mixture of glutaraldehyde and paraformaldehyde. Sections (50 μm) were obtained for pre- embedding immunocytochemistry using a vibratome. The sections were reacted with rabbit anti-Gα14 primary antibody, followed by biotinylated goat anti-rabbit antibody. After treatment according to the ABC method, the sections were reacted with DAB solution and post-fixed with 1% OsO_4. The specimens were embedded in epon. After initial examination with light microscopy, sections were reembedded, and thin sections were sliced with a diamond knife.

We observed that a small subset of cells in the taste bud express Gα14-like immunoreactivity. Using transmission electron microscopy, the immunoreactive taste cells were identified as type II cell and another cell type that contains dense-cored vesicles. This second cell type may possibly represent a type III or I cell. The reaction product is most densely associated with the membranes and the apical microvilli of the taste cells. Our previous studies with gustducin antisera suggest that the type II cell is the only cell type to express gustducin-like immunoreactivity. Based on the current study we believe that more than one cell type may be involved in taste transduction mediated by G proteins.

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299. NTS neurons of developmentally sodium restricted rats show abnormal dendritic lengths before adulthood

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A variety of functional and morphological alterations are observed in rats whose dietary sodium is restricted during pre- and postnatal development. One of the most noteworthy effects is that the dendrites of second-order taste neurons in the nucleus of the solitary tract (NTS) of adult rats that were sodium-restricted throughout development expands dramatically, compared with that in normally fed rats. Though this central effect is obvious in adult rats (age 60–90 days postnatal), it is not clear whether these morphologies deviate from normal at specific times of development (e.g. as peripheral activity emerges). Thus, studying the time course of dendritic expansion in normal and sodium-restricted rats may provide insights into relationships between peripheral activity and central morphologies, as well as between other morphological characteristics (i.e. terminal field development and central taste neuron dendritic organizations).

Therefore, in this experiment dendritic organization of NTS neurons are studied in normal and sodium-restricted rats at 15, 25 and 35 days postnatal. Using a modified Golgi–Cox staining procedure along with a computer-microscope analysis system, we are able to identify significant changes in dendritic length of sodium restricted rats at 25, and even more dramatically at 35 days. No differences were observed at 15 days. These changes are coincident with changes in chorda tympani responsiveness to some taste stimuli. However, it has yet to be determined if the increased dendritic field is in response to activation of the taste system, or to changes independent of neural activity.

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300. Effect of partial deantennation in the early development of honey bees (*Apis mellifera*) on their adult antennal lobe anatomy and ability to discriminate odors

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If insects are partially deantennated early in development, they typically develop smaller and fewer antennal lobe glomeruli. We partially deantennated honeybee pupae to assess the effect of reduced sensory afferents on the development of antennal lobe structure as well as the effects the resulting structural defects had upon olfactory learning.

Animals had 50–100% of one antenna removed during pupal development. As adults, these animals were trained to associate odors with sugar rewards using the proboscis extension reflex protocol. The uncut antenna was covered to restrict the testing to the cut antenna and its corresponding antennal lobe. After training to either 1 hexanol, 1-octanol or geraniol, the animals were tested in three unrewarded trials using each of the 3 odorants. Afterwards, the brains of the treated animals were fixed in Bouins' fluid, sectioned and stained for analysis. Areas of different antennal lobe (AL) features on both the cut and uncut sides were estimated.

Partial deantennation had a significant effect on AL anatomy only if it was performed in the first 2 days after pupa formation (roughly 0–20% of adult development). Glomerular size and overall AL size were reduced, the amount correlated with how much of the antenna was removed. Animals with very reduced antennal lobes showed reduced ability to learn odors and a greater propensity to generalize among odors when compared with control animals that were either deantennated later in development or had their antennae partially covered, both having normal ALs.

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301. Trigeminal and olfactory activation by R- and S-nicotine in humans

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As demonstrated earlier different concentrations of nicotine evoke odorous, burning and stinging sensations. In this study we determined the thresholds (triple forced choice) for these sensations in the concentration range between 0 and 180 ng/ml R- and S-nicotine vapor. In order to quantify the trigeminal activation we recorded negative mucosa potentials (NMPs) from the respiratory nasal mucosa during stimulation with four concentrations of R- and S-nicotine vapor (40, 80, 120 and 160 ng/ml). In an additional experiment we recorded event-related potentials after stimulation with 30 and 150 ng/ml of R- and S-nicotine vapor. The stimulus duration was 250 ms in all experiments. During the experiments subjects rated the intensity of the odorous, burning and stinging sensations using a visual analog rating scale.

The ANOVA revealed significant higher thresholds for the sensation burning and stinging of R- compared with S-nicotine whereas no significant differences could be found for the threshold of the odorous sensations. Statistical significant differences could be found for the subjective intensity estimates of the sensation burning and stinging. No significant differences could be observed in the event related potentials. This might be due to the similar olfactory stimulus intensities of R- and S-nicotine stimuli. The ANOVA of the NMP data revealed significant higher NMP-amplitudes of S- compared with R-nicotine.

Thus our results point to a stereospecific activation of trigeminal primary afferents in the nasal cavity. The low threshold for the burning sensations (S-nicotine: 33.58 ± 25.69 ng/ml) demonstrates that the trigeminal system is able to contribute to the gestalt of the nicotine perception even at low concentrations.

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302. EOGs recorded from the frog olfactory epithelium after stimulation with R- and S-nicotine

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The aim of this study was to investigate the role of the olfactory system for the discriminability of the stereoisomers of nicotine. The EOG was recorded after stimulation with different concentrations of undistilled S-, distilled S- and distilled R-nicotine vapor separately in three groups of frogs (*Xenopus laevis*). The responses to all types of nicotine used in the experiments increased with increasing stimulus concentration. The responses to undistilled S-nicotine were significantly lower compared with responses to distilled S- and R-nicotine, whereas no significant differences could be found when the purified stereoisomers of nicotine (distilled S-, distilled R-nicotine) were compared. Measurements of time course and peak concentration employing a UV-detection method demonstrated that the differences between distilled and undistilled S-nicotine could not be explained by different nicotine concentrations. The fact that no differences between the pure nicotine stereoisomers could be found and that experiments in humans revealed similar detection thresholds for both stereoisomers points to a similar receptor affinity of R- and S-nicotine within the olfactory system.

The differences between distilled and undistilled S-nicotine could be explained by a formation of a compound with a higher receptor affinity but a lower intrinsic activity acting as partial agonist, reducing the response to S-nicotine by blocking the binding sites. Another explanation could be a reduction of the olfactory response by trigeminal activation at the peripheral level. Preliminary results in humans employing chemosensory evoked potentials demonstrated interactions between both sensory systems but the precise location of the interaction is not yet determined.

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303. Kin recognition: discrimination of odors and functional responses by golden hamsters

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Two different approaches were used to study the discrimination and categorization of odors of kin and non-kin by golden hamsters. First, a habituation procedure was used to assess odor discrimination: subjects were habituated to flank gland odors from one donor and then tested, first with the flank gland odor from a sibling of the first donor and then with the flank gland odor from an unrelated donor. Significant differences in the time spent investigating the odors on the last habituation trial and the first test trial indicate discrimination of the individual odors of the sibling donors. Significant differences in the first and second test trial indicate that the odors of the non-sibling donors were discriminated. When the sibling donors were the same sex as the subject, the two donors were discriminated (males: $t = 4.99$, $P < 0.001$; females: $t = 6.00$, $P > 0.001$), and were discriminated as different from an unrelated donor (males: $t = 2.72$, $P < 0.02$; females: $t = 2.65$, $P > 0.02$). When the subject was not related to the donors, the siblings were not discriminated (males: $t = 1.52$, $P = 0.16$; females: $t = 0.98$, $P = 0.35$), but were discriminated as different from an unrelated donor (males: $t = 6.13$, $P < 0.001$; females: $t = 6.17$, $P < 0.001$).

Second, scent marking responses to odors of kin and non-kin were studied. Females flank marked significantly less in a 10 min trial to the flank odors of their sisters than unrelated females ($t = 3.71$, $P < 0.003$), and also marked less to their brothers than to unrelated males ($t = 4.46$, $P < 0.001$). Males marked significantly less toward their brothers than unrelated males ($t = 2.35$, $P < 0.039$). Since flank marking indicates level of agonistic motivation,

these results suggest less aggressiveness aroused by odors of kin compared with odors of unrelated hamsters.

Data from cross-fostering experiments and the possible adaptive value of differential responses to kin and non-kin will be Discussed.

304. Characterization of the sweetness of the sweetness-suppressing effect of polypeptide gurmarin and ent-gurmarin

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The sweetness-suppressing gurmarin isolated from the leaves of *Gymnema sylvestre* consists of 35 amino acid residues including three intramolecular disulfide bonds. As part of our synthetic studies of sweet proteins, we undertook the total chemical synthesis of gurmarin and the D-enantiomer (all D-amino acid gurmarin: ent-gurmarin). Enantiomers have identical, but mirror image, physical properties but are incapable of interacting with chiral substances for the mirror enantiomer. Natural sugars have the D-configuration. The tastes of pure L-sugars have been reported to be the same as each of their respective D-sugar isomers. Therefore, it may be worthwhile to examine the sweetness-suppressing properties of the synthentic gurmarin and the synthetic ent-gurmarin. From these reasons, we studied the electrophysiological experiments on taste responses of the rat chorda tympani nerve, the synthetic gurmarin and ent-gurmarin are a potent inhibitor of sucrose, D-glucose and L-glucose tastes, but had no effect on the NaCl response in the rat. The synthetic gurmarin (10 μg/ml) or ent-gurmarin (10 μg/ml) by itself has no effect on the neural activity. It is suggested that the mode of action of synthetic gurmarin and ent-gurmarin on the membrane of the receptor may be similar and involves the hydrophobic interaction with the membrane, but without specific interaction with chiral receptors.

305. The anatomical levels at which lysine is recognized in rats given a lysine-deficient diet

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When a normal diet is offered *ad libitum*, levels of L-lysine (Lys) in plasma and brain remain constant. Rats given a Lys-deficient diet show a decline in plasma and brain Lys, and anorexia develops. Subsequently, when offered various solutions each of which contains a single amino acid, they selectively ingested and over-consumed their required amount of Lys. Using functional MRI and operant behavioral studies, it has been demonstrated that Lys concentration and intake are monitored in the lateral hypothalamic area (LHA) and the ventromedial hypothalamus (VMH). Additional electrophysiological studies have shown that neuronal plasticity is apparent in the LHA in response to Lys ingestion and to Lys levels in the brain. A similar plasticity was observed in the nucleus tractus solitarius. However, the sensitivities of the chorda tympani and glossopharyngeal nerves to Lys were not altered as a result of Lys deficiency. In the present study the anatomical level at which Lys ingestion is controlled was investigated in rats fed a Lys-deficient diet. Young male Wistar rats were offered a normal diet and a choice of eight solutions, each containing a different amino acid (including Lys), as drinking water. Lys preference was not observed. Next, a Lys-deficient diet was offered concurrently with intraperitoneal supplementation of Lys using an osmotic mini pump. In spite of their receiving adequate amounts of Lys intraperitoneally, these animals showed a selective preference for Lys. In this group of animals, the sensitivity of the hepatic vagal afferent nerve was selectively enhanced to Lys. These data suggest that recognition of Lys deficiency begins in the oral cavity and subsequently is confirmed in the alimentary tract during digestion, with the vagus nerve playing an important role in relaying the degree of Lys deficiency to the LHA and probably the VMH. These brain areas then control quantitative Lys ingestion to maintain Lys homeostasis.

306. Possible involvement of arginine and nitric oxide in the chemical mediation of symbiotic relationships between photosynthetic dinoflagellate algae and anthozoans

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Zooxanthellae are photosynthesizing dinoflagellate algae of the genus *Symbiodinium*. These algae can live either in a free-living state or in symbiotic relationships with marine metazoans, notably cnidarians of the class anthozoa, such as anemones and corals. Such symbioses are at least in part chemically mediated. When living in a symbiotic relationship, the free amino acid pools of zooxanthellae are dominated by arginine, and the cells have a high arginine transport capacity, whereas free-living zooxanthellae have very little intracellular arginine, and a much-reduced capacity for the uptake of this amino acid. These findings led us to examine zooxanthellae for the presence of nitric oxide synthase (NOS), the enzyme that converts arginine to citrulline and nitric oxide (NO); this latter molecule is a potent intra- and intercellular signal molecule. Interestingly, NO is known to play roles in chemically-

mediated behavior of metazoans ranging from the simple (cnidaria) to the complex (mollusca).

We assayed for NOS activity by monitoring the ability of algal homogenates to convert arginine to citrulline, according to the method of Bredt and Snyder. We found that zooxanthellae maintained in culture have essentially no NOS activity, whereas zooxanthellae freshly isolated from a symbiotic relationship with the anemone *Aiptasia pallida* have substantial NOS activity. After 2 days of incubating cultured zooxanthellae in a mixture of free amino acids that mimics the amino acid environment that algae would experience in a symbiotic relationship, NOS activity is comparable to that found in zooxanthellae freshly isolated from *Aiptasia*. These results lead us to conclude the following: (i) zooxanthellae living in a symbiotic relationship have the potential to use NO to communicate chemically with their host; and (ii) the NOS of *Symbiodinium* appears to be an inducible, rather than a constitutive, form of this enzyme.

307. Differential effects of aluminum on amino acid receptors: a basis for olfactory neuropathology?

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Whole-cell voltage-clamp electrophysiology was used to examine the effects of aluminum (Al^{3+}) on several types and subtypes of amino acid receptors on rat mitral/tufted (M/T) cells in primary culture. Al^{3+} is the most abundant metal in the environment, and therefore exposure to Al^{3+} cannot be avoided. Several studies have demonstrated that exposure to intranasal Al^{3+} results in uptake into the brain and distribution along olfactory pathways. Elevated concentrations of Al^{3+} in the CNS have been associated with multiple cognitive impairments and implicated in Alzheimer's disease. Because synaptic pathways that use amino acid transmitters are often involved in neuropathological processes, the effects of Al^{3+} on amino acid receptors were examined. Under voltage-clamp at –60 mV, amino acid receptor agonists evoked inward membrane currents in M/T cells. During the middle of the evoked current, 100 μM Al^{3+} was co-applied with the agonist. Al^{3+} had no effect on membrane currents evoked by either 500 μM glutamate, 100 μM kainate, 100 μM NMDA, or 100 μM glycine. In contrast, 100 μM Al^{3+} potentiated the GABA mediated current, often by several hundred percent, and 10 μM Al^{3+} potentiated the current by ~60%. However, in most M/T neurons, at concentrations ≥300 μM Al^{3+} blocked the GABA-evoked current. These results may indicate that GABA receptors express two binding sites for Al^{3+}: a high-affinity site that potentiates, and a low-affinity site that inhibits. At intermediate concentrations, Al^{3+} appeared to have no effect on the current, suggesting a balance between the actions of the two sites. Neither effect of Al^{3+} was voltage-dependent, suggesting an allosteric binding site(s).

Zinc and copper are endogenous to the olfactory bulb and can alter the function of amino acid receptors. Carnosine is a dipeptide that may act as a neuromodulator, and is contained in and released by olfactory sensory neurons. Whereas carnosine can reduce or eliminate the modulatory effects of zinc and copper on amino acid receptors, apparently through chelation, 100 μM carnosine dramatically potentiated the effects of Al^{3+} on GABA mediated currents. These results may suggest a mechanism whereby Al^{3+} could contribute to olfactory neuropathology.

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308. Catfish perceive binary mixtures of L-amino acids as their more stimulatory components

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Channel catfish, *Ictalurus punctatus*, were conditioned to search for food more intensely after stimulation with equimolar mixtures of Ala + Arg and Leu + norvaline (Nval) than after presentation of amino acids not contained in the mixture. Swimming intensity was quantified by counting the number of turns >90° of the fish within 90 s of stimulus presentation and by videotracking (Vidmex-V, Columbus Instruments, Ohio). The animals swam with equal intensity to both conditioned mixtures and to Ala and Nval, the more stimulatory components of the respective mixtures determined electrophysiologically (EOG). The intensity of the swim was significantly less (Wilcoxon test) after stimulation with the less stimulatory components, Arg and Leu. The hypothesis that catfish perceive a binary mixture as its more stimulatory component was tested by conditioning bullhead catfish, *Ameiurus nebulosus*, to two different mixtures of Leu and Nval. Concentrations of each of these stimuli were adjusted based on EOG recordings. The more stimulatory component in each of the mixtures was used at ten times greater and the less stimulatory component at ten times lower concentration that their equipotent values. When either Nval or Leu was the more stimulatory component of its respective mixture, neither was discriminated from the mixture; however, in both cases the less stimulatory component was discriminated from the mixture.

In other experiments, bullhead catfish, were conditioned to the single amino acid, Leu, and their responses were compared with binary mixtures of Leu with each of 10 other amino acids. All binary mixtures containing Leu as the more stimulatory component (10 times the equipotent concentration) were not discriminated from the conditioned stimulus, Leu; however, those binary mixtures containing Leu as the less stimulatory component (10 times less than equipotent concentration) were discriminated from Leu. To determine if the present hypothesis includes amino acids that are poorly stimulatory (EOG) at low concentrations, bullhead catfish were conditioned to Pro and tested with binary mixtures of Pro+Nval. A Pro concentration 30 000 times greater than Nval was determined (behavior and EOG) to be approximately equipotent to Nval. Binary mixtures containing concentrations of Pro 300 000 times that of Nval were not discriminated and those containing <30 000 more Pro than Nval were discriminated from the conditioned stimulus, Pro. This is consistent with the hypothesis that binary mixtures of amino acids are initially perceived as the more stimulatory component within the mixture.

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309. Reduction of calmodulin mRNA and protein correlates with inhibition of chemoresponse by antisense oligonucleotides

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Paramecia are attracted to chemical stimuli including glutamate, acetate and ammonium. These stimuli all hyperpolarize the cells, but by three different signal transduction pathways. The hyperpolarization for at least one of the pathways is likely to be through the activation of the plasma membrane calcium pump. We have interfered with this pathway by using antisense oligonucleotides (ODNs) to down-regulate calmodulin, an activator of the pump. Cells electroporated by antisense or sense ODNs are selected for swimming behavior that reflects the reduction of a calmodulin regulated Na conductance.

These cells are then tested for swimming speed response to chemical stimuli: hyperpolarization by the stimuli results in increased swimming speed. Cells electroporated with calmodulin antisense ODNs show a very specific impairment in this chemoresponse behavior induced by acetate, a stimulus that initiates signal transduction pathway involving the calcium pump, and no impairment in ammonium, a stimulus that does not involve the pump. Results with glutamate were equivocal since even the sense ODN electroporation rendered them unable to increase swimming speed.

Here we present evidence that the mechanism by which the antisense ODNs affect chemoresponse is, indeed, by reducing calmodulin levels in the cells. Semi-quantitative RT-PCR demonstrates the antisense ODN treated cells have less calmodulin mRNA than sense ODN treated cells. We developed a very sensitive ELISA using a polyclonal antibody against Paramecium calmodulin expressed in bacteria. Results with this ELISA demonstrate that the calmodulin protein levels are reduced by an average of 26%.

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310. A critical period for imprinting of social odors in two dwarf hamster species, *Phodopus sungorus* and *Phodopus campbelli*

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It is generally accepted, that early experience has a profound impact on forming a variety of physiological and behavioral functions. For instance the exposure to odors during early developmental stages affects the behavior of the adult individual later in life and generally leads to an attraction to this odors. As shown previously in the Djungarina hamster (*Phodopus campbelli* Thomas 1905) and in the Siberian hamster (*P. sungorus* Pallas, 1773), the exposure to closely related species from birth until day 30 leads to a reversal of the normally exhibited preference for conspecific individuals and conspecific odors. The study presented here was conducted in search of a possible sensitive phase during the early postnatal period which would influence the preference of odors used for social attachment in adulthood.

Male pups of *P. sungorus* and *P. campbelli* were experimentally reared by parents of the other species (cross fostering, CR). The young males of the ages of 1, 11 and 21 days were added to litters of 10 days. One group from the day of birth until day 10 (CR 1–10) a second group from day 11 to day 20 (CR 11–20), a third group from day 21 to day 30 (CR 21–30; normally reared (NR) and CR 1–30 pups served as controls. At the age of two months CR 1–10 and CR 21–30 males did not show any preference in a choice test experiment. The responses towards con- and heterospecifics and their odors differed significantly from those of NR, CR 11–20 and CR 1–30 males. At that age CR 11–20 and CR 1–30 males of both species demonstrated strong preferences for heterospecific odors ($P < 0.01$) while NR males preferred their natural species. These results suggest that the period from day 11–20 is critical for forming social odor preferences which apparently determine the later mate choice, and that even short period of exposures to a closely related species can modify species attachment.

311. Ontogeny of specialized regions in the main olfactory system of *Xenopus*: an NADPH–diaphorase histochemical study

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In adult *Xenopus*, the main olfactory organ consists of two subdivisions, a 'lateral diverticle' (LD, aquatic nose) and a 'medial diverticle' (MD, aerial nose). During metamorphosis, these develop from the common nasal pit. To study the ontogeny of the olfactory organ's regional specializations, we histochemically examined NADPH-d reactivity (ND-r) in several larval stages and in adult frogs.

In all tadpoles studied, from stage 36/38 (shortly after hatching) to the end of metamorphosis (stage 66), a portion (estimate: 30–50%) of the receptor cells displays ND-r. At stage 46/48, some ND-r cells can be detected dorsal to the vomero-nasal organ (VO) in the, as yet, undivided mucosa, but appear as undifferentiated. At stage 52/53, in this part of the epithelium, a proliferation zone is formed, which gives rise to LD. Due to its less pronounced ND-r in the layer of supporting cells, this zone can be distinguished from the rest of the mucosa. Simultaneously, the adjacent rostro-medial part of the main mucosa develops the oral cupola of MD. At stage 57/58, the 'ventrale Grenzfalte' forms and begins to separate LD and MD. In subsequent larval stages and during early postmetamorphic life, in the main part of MD, the number of labeled receptor cells decreases to zero. In the oral cupola, however, receptor cells continue to express ND-r.

From stage 52/53 on, a new type of ND-r cells can be detected. Because of their soma shape we refer to these neurons as 'globular cells'. They are scattered in the epithelium in proximity to the basal lamina of LD and of that portion of MD, which forms the oral cupola. Globular cells are unipolar or pseudounipolar. Throughout postmetamorphic life, globular cells are abundant in LD and in the oral cupola of MD.

Our study reveals NADPH-diaphorase histochemistry as a

powerful indicator of regional differentiation of olfactory mucosa cells during pre- and postmetamorphotic development.

This study was supported by Graduate College fellowship.

312. Alterations in physical conditions and their effect upon pheromone plume structure as measured by a male moth electroantennogram

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Plumes of pheromone created by a calling female moth are not homogeneous clouds. Instead, eddies and turbulence within the moving air mass cause the plume to become temporally disrupted such that strands of pheromone (filaments) are interspersed with pockets of clean air. The intermittent signal thus created is important to males as upwind progress towards the source ceases upon replacement of the plume with a uniform, homogeneous cloud of pheromone. Recently it was discovered that male moths in casting flight (a response to loss of an odor plume) can respond to contact with a single strand of pheromone by making a toward-source upwind surge. In pulsed plumes, generated at a threshold frequency for upwind flight, these single-filament responses appeared to be iterated repetitively, thus accounting for the moths upwind progress.

Clearly, the physical domain affecting the temporal pattern of pheromone filaments is important to the upwind-orienting male, and the plume structure is subject to change as a function of changes in the physical conditions. In the current study we utilized an electroantennogram (EAG) preparation to measure filament frequencies in plumes created by the release of pheromone from rubber septa in a wind tunnel. Windspeed, distance between pheromone source and EAG, and dosage of pheromone on the septa were varied in order to determine their effect upon plume structure. Cross-sectional measurements of plume structure were performed by moving the EAG preparation through a two-dimensional grid. Importantly, we were also able to record EAGs in response to plumes emitted by excised female pheromone glands and live, calling females facilitating a comparison of the temporal structure of plumes emanating from natural and artificial surfaces.

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313. Comparing the functional and anatomical features of odorant-defined glomeruli in two closely-related insect species

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The pheromone communication systems of the moths *Heliothis virescens* and *Helicoverpa* (formerly *Heliothis*) *zea* share many common features. The females of both species release a blend of volatiles as a sex attractant, and despite the chemical similarity in pheromone components used, there is little or no cross-specific attraction. Both species utilize Z11–16:aldehyde as the major blend component, but specificity of male response is conferred by the presence of a second essential component, Z9–14:Ald in *H. virescens* and Z9–16:Ald in *H. zea*. Interestingly, in *H. zea*, Z9–16:Ald can be substituted with a smaller amount of Z9–14:Ald without significantly affecting the behavioral outcome. However, if the ratio of Z11–16:Ald to Z9–14:Ald approaches that present in the *H. virescens* blend, *H. zea* males will no longer fly upwind to the source. Hence, Z9–14:Ald may act as an attractant or inhibitor depending upon its amount relative to Z11–16:Ald.

Pheromone-selective receptor neurons project to a sexually-dimorphic area of the male antennal lobe, the macroglomerular complex (MGC). Three-dimensional reconstructions of the MGC revealed clear morphological differences in the shape and number of glomeruli present in each species. *H. virescens* males have four distinct glomeruli compared with only three in *H. zea*. Intracellular analysis of MGC projection (output) interneurons revealed that the most abundant cell type communicating information to higher brain centers in both species is most sensitive to Z11–16:Ald, and these cells showed distinct arborizations in the largest of the MGC glomeruli. The shape and spatial location of this glomerulus, however, are different in the two species. Another output pathway in *H. virescens* may be functionally homologous to the Z9–14:Ald/Z9–16:Ald pathway previously described in *H. zea*, but again, the glomerulus encoding this information occupies a different position in the two species. These data point to the danger in generalizing olfactory maps and coding strategies across even closely-related species, and emphasize the need to understand the behavioral context within which the olfactory system must operate.

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314. The time course of transganglionic degeneration in the nucleus of the solitary tract following taste nerve transection in the rat

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Gustatory nerves have a remarkable capacity for regeneration and functional recovery following lesion. However, less is known of the central consequences of gustatory nerve damage. We examined the time course of transganglionic degeneration in the rostral portion of the nucleus of the solitary tract (NST) following combined lesions of two taste nerves, the chorda tympani (CT) and the lingual-tonsillar branch of IX (LT-IX), which innervate taste buds on the anterior and posterior tongue respectively. In 28 rats a 2–5 mm section was excised from both nerves, distal to the ganglion, on the right side only. Animals then survived for 2–52 days before perfusion. Controls included the intact contralateral side in each experimental rat and three additional rats that underwent sham surgeries. Preliminary fungiform taste bud counts indicated reduced numbers ipsilaterally and degenerate morphology of

persisting buds at survival times of 12 days and longer. Brains were sectioned at 40 μm, processed simultaneously with the amino-cupric silver method, and the amount of degeneration was quantified under darkfield illumination. Degeneration product was observed centrally, ipsilateral to the nerve cuts, consistent with the demonstrated projections of these nerves into the rostral NST. The amount of degeneration was greatest in 22 day survival animals and was progressively less at shorter and longer survival times; little or no degeneration was observed in the NST of 2, 5 or 52 day survival animals. Thus peripheral CT and LT-IX transection results in transganglionic degeneration of central projection fibers which may alter synaptic organization in the NST and provide the opportunity for plasticity during regeneration.

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315. Induced sensitivity to androstenone: behavioral and biochemical observations

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Inbred strains of mice that provide a model for specific anosmia to androstenone (AND) are available in the forms of NZB/B1NJ (NZB; insensitive to AND) and CBA/J (CBA; sensitive to AND). We previously estimated behavioral sensitivity of NZB and CBA mice to AND. NZB mice could detect 0.1% (w/v) AND in mineral oil, but not 0.05% AND. CBA mice could detect AND at a concentration of 2000-fold. After 2 weeks of exposure (16 h/day) all mice increased sensitivity to AND regardless of their initial level of sensitivity. After 1 more week, sensitivity of NZB mice was 64- to 128-fold lower than original estimates and those for CBA mice were 200- to 400-fold lower. In more recent work, AND exposure did not affect sensitivity of either NZB or CBA mice to amyl acetate (AA). Exposures of NZB and CBA mice to AA did not affect AND thresholds, while sensitivity to AA was slightly increased (2-fold). Alkaline phosphatase activity (APA) of immobilized proteins in olfactory and vomeronasal epithelia in AND-exposed and non-exposed NZB and CBA mice was compared. Exposures to AND resulted in an increase in APA in the olfactory epithelium of CBA mice. The effect was more profound for longer AND exposures. Vomeronasal epithelium did not respond to AND stimulation before exposure, but did so after exposure. Non-exposed NZB olfactory epithelium showed inhibition of APA in response to AND stimulation. AND exposures resulted in activation of APA in response to AND stimulation. Vomeronasal epithelium of NZB mice did not respond to AND stimulation either before or after AND exposure.

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316. GABA- and histaminergic interneurons form two distinct inhibitory pathways in the lobster olfactory lobe

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We have reported previously that GABA and histamine (HA) are inhibitory transmitters in the lobster olfactory lobe (OL), the first synaptic level in the olfactory pathway (AChemS XVI and XVII). Here, we directly compare the functional and anatomical properties of the GABA- and HA-ergic inhibitory pathways within the OL. The GABA receptor antagonist picrotoxin or the HA receptor antagonist cimetidine was perfused into the OL while recording the response of OL projection neurons to electrical stimulation of the olfactory nerve. As reported previously, both compounds increased projection neuron sensitivity, as shown by a decreased threshold of the spiking phase of the electrically-evoked response. However, the two compounds had opposite effects on the long-lasting hyperpolarizing phase of the response. Cimetidine (3 mM) reduced or abolished the hyperpolarization, while picrotoxin (30 μM) enhanced and prolonged it. Neither compound affected the latency or duration of the spiking phase. Antisera to both GABA and HA intensely stained somata of local interneurons, but not those of projection neurons. Both antisera also labeled all regions of all OL glomeruli, but HA-like immunoreactivity was distinctly higher in the subcap region, while GABA-like immunoreactivity was highest in the cap region. These and additional differences in the labeling patterns indicate that GABA- and HA-like immunoreactivity occurs in separate populations of interneurons. Thus, their common features notwithstanding, the GABA- and HA-ergic interneurons appear to form two functionally and pharmacologically distinct inhibitory pathways that contribute to olfactory processing in the lobster OL, a feature which may facilitate understanding how olfactory input is processed at the first synaptic level.

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317. Human responses to propionic acid vapor: respiratory changes

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Previous research from our laboratories has indicated that changes in breathing could serve as useful correlates of odor (O) and nasal irritation (NI). We tested 31 normal and four anosmic subjects with four propionic acid concentrations (0.2, 1.1, 9.7 and 85 p.p.m., v/v) which ranged from peri-threshold for normal subjects to clearly supra-threshold for anosmic subjects. Respiratory data were collected just before (PRE period), and during (STIMULUS period), 40 clean air and 160 odor trials over 4 weekly test sessions. O and NI intensity were rated after each trial. Inhalation flow rate (InFR), inhalation duration (InDur) and inhalation volume (InVol) were expressed, on each trial, as the percentage change

from the PRE to the STIMULUS period. Effects on breathing were assessed by comparing the responses to each propionic acid concentration with those to clean air. The key findings were: (i) with anosmic subjects, declines in InFR, InDur and InVol were observed with only the 85 p.p.m. (highest) concentration. (The NI threshold in these subjects was 10 p.p.m.); (ii) In normal subjects, InDur declines were observed at only 85 p.p.m. Concentration-related declines in InFR and InVol began with 1.1 p.p.m., although the latter showed less separation between normal and anosmic subjects at this concentration. (In normal subjects, the O and NI thresholds 0.3 and 0.9 p.p.m. respectively.)

We conclude that: (i) InFR may serve as a sensitive and graded index of the intensity of odor perception; (ii) InDur may be a useful index for measuring trigeminal sensitivity in normal subjects; (iii) in anosmic subjects, all three measures may be useful non-verbal measures of detection; and (iv) in normal subjects, InVol may serve as an index of NI (which appears to be a joint outcome of olfactory and trigeminal stimulation).

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318. Chemosignal transduction in vomeronasal organ of garter snake: Ca^{2+}-dependent regulation of vomeronasal adenylate cyclase

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We previously reported that calcium blocked forskolin induced elevation of cAMP in vomeronasal (VN) membrane preparations. We suggested that the cytosolic concentration of calcium, $[Ca^{2+}]_i$, mobilized by IP_3 negatively regulates the activity of adenylate cyclase in the VN system. To test whether the activity of adenylate cyclase in the intact VN sensory epithelium could be modulated by mobilizing the internally sequestered calcium, we have used three structurally unrelated inhibitors of Ca^{2+}-ATPase: thapsigargin (TG), butylhydroquinone (BHQ) and cyclopiazonic acid (CPA). These three inhibitors are know to cause calcium release from endoplasmic reticulum by inhibiting the Ca^{2+} ATPase, the Ca^{2+} pump. In the absence of extracellular calcium, all three compounds showed an enhancement of the level of cAMP induced by forskolin. In contrast, in the presence of extracellular calcium, they all caused the forskolin elevated level of cAMP to decrease. These results suggest that these compounds caused Ca^{2+} release from IP_3-sensitive Ca^{2+} stores and initiated a calcium induced calcium influx. In addition, ionomycin, a calcium ionophore, could effectively block the effect of forskolin suggesting that this ionophore in the absence of extracellular calcium could effectively deplete the Ca^{2+} stores. Its effect was not due to its action on forskolin, as it showed no effect on the forskolin-stimulated level of cAMP in the isolated VN membranes. This compound appears to be more powerful in mobilizing the internally stored calcium than the three Ca^{2+}-ATPase inhibitors.

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319. Nitric oxide synthase in the insular cortex of the Syrian golden hamster

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We have previously reported on the types and distribution of NADPH diaphorase [NADPHd; an indicator of nitric oxide synthase (NOS)] activity in the insular cortex of the Syrian golden hamster (Wehby and London, 1995). We now report on the isozymes of NOS present in the insular cortex, and on how the NOS immunohistochemistry distribution compares with that seen with NADPHd histochemistry. Hamsters were perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were postfixed in the same fixative for 2 h and then sectioned at 35 μm, with either a Vibratome or a freezing microtome. Brain slices were incubated with antibodies to human NOS (rabbit polyclonal anti-nNOS and anti-iNOS; mouse monoclonal anti-eNOS; Transduction Laboratories) for 24 h at 4°C, then incubated with the appropriate biotinylated goat anti-IgG. Antibodies were visualized with an ABC reaction (Vector) using DAB histochemistry. In the insular cortex, nNOS-like immunoreactivity was seen in scattered neuronal somas and these were located primarily in layers V and VI. This distribution is very similar to that of the 'solid' cells seen with NADPHd histochemistry (Wehby and London, 1995). These solid cells are large, apparently aspiny multipolar or bipolar neurons (Wehby and London, 1995). In two animals that were processed for both nNOS-like immunoreactivity and NADPHd histochemistry, every neuronal soma that appeared labeled for nNOS was also labeled for 'solid' NADPHd activity. In contrast, nNOS immunoreactivity was not seen in the 'punctate' NADPHd-reactive cells, nor in the NADPHd-reactive fibers. eNOS-like immunoreactivity was seen only in endothelial cells throughout the insular cortex, and this eNOS staining pattern did not correspond to that of any NADPHd staining pattern. There was no specific labeling by anti-iNOS. Thus, (i) the 'solid' label seen with NADPHd histochemistry signals the presence of the nNOS (but not eNOS or iNOS) isozyme; and (ii) the fibers and 'punctate' cells labeled with NADPHd histochemistry were not labeled by antibodies to either nNOS, eNOS or iNOS isozymes. This second result is somewhat surprising, and we continue to investigate this apparent discrepancy between the histo- and immunohistochemistry results.

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320. Mitral cell loss increases turnover of olfactory receptor cells

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Olfactory receptor cells are continuously replaced throughout the

lifetime of vertebrates, including mammals. Basal cells divide and give rise to new receptor cells which project their axons to the olfactory bulb, where they make contact with mitral/tufted cells and periglomerular cells. After bulbectomy the rate of cell division is increased in the olfactory epithelium for at least 50 days after surgery. The increased rate of proliferation is related to premature death of receptor cells, presumably because they lack a trophic substance provided by the bulb. We asked whether the mitral cells are the source of this putative trophic substance. In 18 day old rats (P18), mitral cells were selectively damaged unilaterally by cutting their axons in the lateral olfactory tract. Before killing at P40, P66 and P105, rats were injected with [^{3}H]thymidine or bromodeoxyuridine to label dividing cells. We determined the proliferative rate of neurons in olfactory epithelium by counting a cumulative 80 mm linear span of olfactory epithelium on the ipsi- and contralateral sides of operated animals, and control unoperated animals of the same age. Tractotomy resulted in a 26% reduction in the number of mitral cells on the operated side. Electron microscopic examination of surviving mitral/tufted cells indicated a reduction in size and reduction in amount of rough endoplasmic reticulum. The olfactory epithelium on the operated side in all three experimental groups showed a 21% increase in the number of cells incorporating the DNA label, compared with that on the unoperated side. Values from the unoperated side did not differ from those in unoperated controls. The results indicate that the loss or damage of mitral cells influences the proliferative rate of olfactory receptor cells, possibly by reducing their average life span.

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321. Rapid odorant recognition in an artificial chemosensory device based on the olfactory system

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Data from a variety of studies suggest that odorant information is processed in a distributed fashion in the olfactory pathway. To explore this hypothesis further, we are developing an artificial chemosensory device that incorporates elements of a distributed processing system. Primary chemosensing input is provided by an array of sensors, each of which consists of a fluorescent indicator dye immobilized in a polymer matrix on the distal (sensing) end of an optical fiber. The dye changes fluorescence when exposed to organic vapors; individual sensors in an array respond differentially, depending on the type of polymer used. Controlled pulses of organic vapors elicited unique spatio-temporal patterns of fluorescence change across the fibers in an array, a characteristic similar to that seen in olfactory receptor neurons. Using data from such a fiber array, artificial neural networks were trained to identify single vapors and their relative concentrations. Neural networks using temporal information performed well in identifying either the 'names' (e.g. 'amyl acetate') or 'characteristics' (e.g. 'high relative molecular weight acetate ester') of the stimulus compounds. The results indicate that temporal information may be important for robust vapor identification in this artificial system. Physiological studies also suggest that temporal information may be important for odorant information processing in the olfactory system. We have therefore used the fiber sensor array data as the receptor input to our computational model of the olfactory bulb (White *et al.*, 1992). Using the sensor data as input, the computer model generates spike activity patterns in individual simulated mitral cells that are similar to those seen in physiological recordings. Different spiking patterns are distributed across the array of mitral cells, producing an ensemble spatio-temporal spiking pattern that varies with vapor identity and concentration. The model thus may be used as a component in biologically-derived networks for discriminating input pattern differences.

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322. Salamander olfactory bulb responses to a set of nine odorant stimuli

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The distributed processing hypothesis of olfactory function states that odorant information is encoded by the activity of many neurons at each level of the olfactory pathway. As a step toward investigating the relationship between odorant structure and ensemble activity in the olfactory bulb, voltage-sensitive dye (VSD) recordings from the tiger salamander were made using a set of nine odorant stimuli. Responses were compared on the basis of polarity, magnitude, latency, and duration. As previously reported, butyl alcohol elicited primarily a hyperpolarizing signal; all other odorants tested elicited depolarizing signals. Butyl acetate was used as a standard for response comparisons. Acetophenone and benzaldehyde produced signals that were about one-half the peak amplitude of the butyl acetate response, with acetophenone having a shorter latency than benzaldehyde. Camphor elicited a response similar in amplitude to butyl acetate, but reached its peak earlier. The large depolarizing response to camphor seems paradoxical in the context of behavioral observations where tiger salamanders cannot be trained to discriminate camphor from air (Dorries *et al.*, 1994). These VSD results are consistent, however, with prior single unit recordings, where camphor elicited a range of responses in olfactory bulb mitral cells (Kauer, 1974). Two stereoisomer pairs were tested and the members of each pair produced response patterns that were similar to one another, but still discernible. The pinene stereoisomers elicited signals with larger amplitudes than butyl acetate and with shorter latencies. The response latency to (+)pinene was slightly shorter and the signal lasted longer than that to (–)pinene. The carvone stereoisomers elicited signals that were smaller in amplitude than butyl acetate. (+)Carvone had a shorter latency and the overall signal was slightly larger than for (–)carvone. Because many receptor–ligand interactions appear to be highly stereo-specific, the olfactory responses to stereoisomers such as these may be particularly useful for investigating the mechanisms by which odorant structure affects olfactory activity.

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323. Olfactory interference in serial verbal memory

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Lyman and McDaniels found that olfactory stimuli elaborated (visually or verbally), are better remembered than odors or names presented alone. What happens to verbal material when it is elaborated by odors? Day and Metzger suggested that memory is enhanced when verbal stimuli are presented simultaneously to matching olfactory stimuli. This is in keeping with Pavio's concept of dual coding, which predicts that items encoded through two different sensory channels will be better recalled than those encoded by a single channel. However, it is possible that interference could arise when two channels encounter conflicting information. What happens when the words are paired with incongruous odorants?

Subjects viewed one of two lists of words, one of which contained the names of odors, while the other list contained names of objects not associated with smells. Each word was accompanied by the simultaneous presentation of an odor. Subjects were told to remember the words, and that the odors were incidental.

Analysis indicated that although the two word lists had been equated on a number of variables, they were still unequal in their level of difficulty; odor words were more easily remembered even when neither list was paired with an odor. Neither the non-odor words, nor the odor words, were remembered better when paired with an odorant as opposed to a blank. Evidence of olfactory interference in verbal memory was apparent, in that odor words were remembered most poorly when presented with the incongruous odors.

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324. Calcitonin gene-related peptide immunoreactivity in sensory-denervated taste papillae of the hamster

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Calcitonin gene-related peptide (CGRP) is heavily expressed in gustatory papillae of all mammals evaluated to date; it has been implicated in trophic functions. This study evaluates CGRP expression in innervated and denervated fungiform papillae of the hamster. Chorda/lingual nerves of 11 hamsters were severed and devitalized unilaterally. After 3–15 weeks the animals were killed and their tongues evaluated bilaterally with immunohistochemistry. CGRP in control papillae stained a group of cells centrally placed in the taste bud and dense bundles of nerve fibers that ascend the papillary core and terminate in the perigemmal epithelium surrounding the taste bud. Experimental papillae exhibit drastically reduced CGRP. Taste bud staining is reduced, often to a single fusiform cell. Perigemmal CGRP fibers are virtually eliminated as are most fibers. A few CGRP fibers in the core, especially on blood vessels, remain. Possible autonomic sources for the remaining fibers that survive denervation were sought. In three animals, the superior cervical ganglion (SCG) was removed on the side of sensory denervation. The animals exhibited Horner's syndrome but no loss of persistent fibers. Moreover, SCG neurons were non-immunoreactive for CGRP. In contrast, sub mandibular ganglion cells were CGRP+. Therefore, the sparse CGRP+ fibers in sensory denervated tongues are likely parasympathetic and may be trophically related to fungiform taste buds.

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325. Early development of the olfactory placode and subsequent differentiation of olfactory neurons and GnRH cells in the zebrafish

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Among vertebrate sensory systems the olfactory organ is unique; it generates not only the primary olfactory sensory neurons, but also a population of neuroendocrine cells containing gonadotropin releasing hormone (GnRH) that migrate into the central nervous system (CNS). Little is known about the cellular events underlying the development of the olfactory organ and the contributions of distinct cells types to the olfactory system. Because of the known contribution of cranial neural crest to structural elements of the face and the close spatial relationship between the developing placode and migrating cranial neural crest, it is possible that cranial neural crest contribute to the olfactory system prior to morphological differentiation of the olfactory placode.

To identify the cells contributing to the differentiating olfactory placode, we have labeled single cells with a lineage marker, a lysinated rhodamine dextran dye, and followed the fates of the progeny of each labeled cell. We have labeled single cells both within the area where the olfactory placode will form, and outside the placodal region. The cells outside the placodal region include presumptive cranial neural crest cells based on their morphology and position. Our preliminary data show, surprisingly, that presumptive cranial neural crest contributes to neuronal populations in the olfactory organ.

Little is known about the subsequent differentiation of olfactory neurons and GnRH cells that arise in the olfactory organ. To determine whether there is a single precursor giving rise to both the olfactory sensory neurons and the GnRH cells, we have labeled single cells in the olfactory placode at a time when the olfactory placode is first evident with Nomarski optics. At this time, single precursors labeled in the olfactory placode generate clones containing olfactory neurons, and these olfactory neurons remain spatially restricted within the olfactory organ at two days. We have yet to label precursors giving rise to GnRH cells. Thus, we

have shown that olfactory neurons arise from precursor cells in the olfactory placode and that cranial crest contributes to at least some of these precursors.

326. Alliesthesia in pure tastes, pure food odors and taste–odor mixtures

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Chemosensory stimuli are less pleasant when subjects are sated compared with hungry. This phenomenon is called alliesthesia. The evidence for odor alliesthesia is not as clear as the evidence for taste alliesthesia. We examined whether the hedonics of pure taste stimuli and pure odor stimuli are influenced by internal state. In experiment 1, undergraduates rated intensity and hedonics of a prototypical sweet (sucrose), bitter (sucrose octaacetate) and salty (NaCl) taste as well as three food odors (chocolate, popcorn and raspberry) while sated and hungry. Taste intensity ratings were higher in the sated condition for sucrose and sodium chloride. Odor intensity ratings were significantly higher in the sated condition for popcorn odor, but not raspberry or chocolate. Taste hedonic ratings were significantly lower in the sated condition for sucrose. Odor hedonic ratings were significantly lower in the sated condition for all odors. Experiment 1 replicates taste alliesthesia and, more importantly, clearly demonstrates alliesthesia for food odors. We went on to compare alliesthesia for the odor component of a taste-odor mixture and the 'flavor gestalt' (the taste–odor combination). In experiment 2, three odors (chocolate, popcorn and raspberry) were presented with a sweet taste using the two-module delivery system. Subjects rated intensity and hedonics of the odor in the presence of a taste and the 'flavor gestalt' while sated and hungry. Intensity ratings of the 'flavor gestalt' were higher in the sated condition only for popcorn odor. Hedonic ratings for both the pure odor and the 'flavor gestalt' were lower in the sated condition for raspberry and popcorn odors. Therefore, a general reduction in hedonic ratings accompanied satiety, but no distinction could be made between hedonic ratings of odor alone and hedonic ratings of a taste–odor mixture.

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327. The projection from the rNST to the mPBN in rat is bilateral as demonstrated by a retrograde and anterograde tract tracing study

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The rat rostral nucleus of the solitary tract (rNST) contains second-order gustatory neurons, some of which project to higher brain centers via an obligatory synapse in the medial parabrachial nucleus (mPBN). In rat and hamster, this projection has been described as predominantly ipsilateral. The objectives of the current study were to determine the number and location of rNST neurons which project to the mPBN using a retrograde tracer and to demonstrate a bilateral projection by visualizing rNST terminal fields using an anterograde tracer. Projection neurons in the rNST were visualized by stereotaxically injecting the fluorescent tracer DiI (Molecular Probes) unilaterally into the mPBN in male Wistar rats (n = 8). DiI-labeled rNST neurons were always found both ipsilateral and contralateral to the pontine injection site. The contralateral rNST contained from 22 to 40% of the total number of labeled rNST neurons, with an average of 25%. Axons and terminal fields of rNST neurons were labeled by injecting biotinylated dextran (10000 mol. wt, Molecular Probes) unilaterally into the rNST (n = 4), and visualizing the dextran with an avidin-biotin-peroxidase procedure. Terminal fields were always found in the mPBN both ipsilateral and contralateral to the rNST injection site. The intensity of labeling was higher ipsilaterally, but a mirror-image staining pattern was consistently present contralaterally. Labeled axons were visible contralaterally just rostral to the rNST, suggesting that some axons cross the midline in the medulla. These results indicate that there is a substantial (25%) contralateral component of the projection from the rNST to the mPBN. This suggests that convergence of gustatory information from the two sides of the brain may occur within the pons. These findings may have important implications as to where and how bilateral gustatory information is processed and integrated.

328. Unilateral olfactory deprivation modifies bi-naral interactions in piriform cortex

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The anterior piriform cortex (aPCX) receives input from the ipsilateral nare via the ipsilateral olfactory bulb and lateral olfactory tract (LOT). The most direct input from the contralateral nare reaches the aPCX via the anterior commissure projection from the contralateral anterior olfactory nucleus (AON). These bilateral inputs are segregated in aPCX layer I, with ipsilateral LOT terminals in superficial layer Ia and commissural terminals in layer Ib. The anterior commissure develops postnatally in the rat, and therefore, might be expected to be particularly sensitive to postnatal experience. The present report examined the effects of unilateral olfactory deprivation on the relative strength of ipsilateral and crossed inputs to the aPCX.

Wistar rats had a single nare sealed on postnatal day 1 under cold anesthesia, then returned to their home cage and left undisturbed. On postnatal day 25–35, animals were anesthetized with urethane for electrophysiological tests. Evoked potentials were recorded in layer I of the aPCX to stimulation of the ipsilateral olfactory bulb and the contralateral AON. Recordings were made in both the deprived hemisphere and the control hemisphere of each animal, and included responses to single pulses of varying intensities and paired-pulses.

In control animals the classic monosynaptic response to ipsilateral MOB input to the aPCX was recorded, consisting of a negative wave representing depolarization of aPCX apical dendrites by LOT terminals. Commissural input from the contralateral AON evoked a more complex evoked potential. However, an early wave recorded as a positivity in deep layers of the aPCX reversed to a negative wave in layer I. This early response was similar to that evoked by LOT stimulation, although much smaller in amplitude and longer in latency. In deprived animals,

unilateral olfactory deprivation produced a relative decrease in the strength of deprived inputs and a relative increase in the strength of non-deprived inputs to the aPCX. These preliminary results suggest an activity-dependent, competitive mechanism controlling bi-naral interactions in the aPCX.

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329. Taste cell specific transcriptional regulation of the α-gustducin gene in transgenic mice

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α-Gustducin is a taste specific G-protein which is homologous to both rod and cone α-transducin, the photoreceptor G-proteins. These considerations have led us to propose that gustducin's role in taste transduction is similar to that of the transducins in the visual system. Recent studies of α-gustducin deficient mice demonstrate that α-gustducin is involved in both bitter and sweet signal transduction. To determine the requirements for taste cell- specific gene expression, the murine α-gustducin gene was cloned and analyzed to identify transcriptional regulatory sequences which confer taste specific expression of a heterologous marker gene, β-galactosidase (lacZ) in transgenic mice. An 8.4 kb region derived from the 5'-end of the α-gustducin gene contains sequence elements which are necessary and sufficient for high levels of taste cell-specific lacZ expression in transgenic mice. We are in the process of dissecting the 8.4 kb region to determine the number, location and sequence identity of these taste cell-specific enhancers. We have used the 8.4 kb genomic region of murine α-gustducin to direct taste cell-specific expression of rat a-gustducin cDNAs in transgenic α-gustducin deficient mice. This experiment tests whether the 8.4 kb region driving cDNA expression can functionally rescue the taste phenotype associated with α-gustducin deficiency.

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330. Androstenone antagonism

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Androstenone (AND) and a racemic mixture of the isomers 4(R)-(4',4'-dimethylcyclohexyl)-2(R)-methylcyclohexanone and 4(S)-(4',4'-dimethylcyclohexyl)-2(S)-methylcyclohexanone (DMC-MC) are functional analogs: earlier work showed that people who could smell both compounds were unable to discriminate them. Also, exposure to either AND or DMCMC resulted in mutual cross-adaptation. Furthermore, many individuals were anosmic to both compounds. These findings demonstrated a tight perceptual linkage between AND and DMCMC, suggesting that these urinous (to many people) compounds might stimulate the same olfactory receptors. During a large-scale screening, however, interesting exceptions emerged. Of 185 individuals tested 19 were anosmic only to AND (n = 11) or to DMCMC (n = 8), but not to both. In additional tests, these individuals provided baseline intensity estimates of AND (0.1% in mineral oil), DMCMC (0.1% in mineral oil) or a blank. Ratings of the odor to which individuals were anosmic were not different from ratings of blanks. Individuals then either 'adapted' to a blank or to the odorant to which they were anosmic (functionally another blank to these individuals) during one of two 5 min exposure/test periods. Every 15 s during this period they rated the intensity of the odorous compound (either AND or DMCMC). For individuals who were anosmic to AND, the ratings of DMCMC odor intensity were significantly suppressed when AND served as the 'adapting' stimulus, relative to the results obtained when the blank served as the 'adapting' stimulus. The results for those who were anosmic to DMCMC also demonstrated 'cross-adaptation' by the odorless DMCMC to AND. Hence, it is likely that a non-detectable AND-like compound (either AND itself or DMCMC) acts as an olfactory antagonist and inhibits the perception of an otherwise active ligand.

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331. Molecular cloning of two guanine nucleotide binding proteins from American lobster olfactory organ

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In lobsters, both cAMP and IP3 are employed as second messengers for olfactory signal transduction. We have isolated two cDNA clones for guanine nucleotide binding protein (G-protein) alpha subunits from a cDNA library made from the olfactory organ of the American lobster. The deduced protein sequences of the clones indicate that one belongs to the G_s subfamily, and the other, to the G_q subfamily. The putative G_q of American lobster has a high percentage of amino acid identity with known G_qs: 84.6% with *Drosophila*, 80.7% with leach, 75.0% with squid and 70% with rat, but only 35% with rat G_s. The putative G_s, on the other hand, has high percentage of identity with other G_ss: 79% with snail and *Drosophila*, 73% with octopus and 71% with rat. The two lobster sequences are 35% identical. We are working to test the hypotheses that the G_q clone mediates the IP3 transduction pathway and the G_s clone mediates the cAMP pathway in lobster olfactory receptor neurons.

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332. Colocalization of cAMP chemoreceptor and Ca-ATPase in paramecium

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Paramecia are attracted to cyclic AMP, which hyper-polarizes the cells. A receptor protein of 48 kDa has been purified and

polyclonal antibodies against this protein selectively block the chemoresponse to cAMP. Cyclic AMP appears to stimulate one of three transduction pathways in *Paramecium*, one that hyperpolarizes the cell and induces swimming that leads to attraction behavior through the activation of a calcium pump. It is of interest, therefore, to determine the subcellular localization of the receptor and the pump using immunofluorescence and confocal microscopy. Polyclonal and monoclonal antibodies against the receptor protein are available. The plasma membrane pump has not been purified, but its gene has been cloned. Therefore, a peptide from the deduced amino acid sequence of the calmodulin binding domain was generated and polyclonal antibodies were produced against this peptide. Electro-blots of an expressed peptide of 10 kDa from the cloned pump gene show that the C terminus of the pump does, indeed, bind calmodulin and is recognized by the antibody against the peptide. The receptor is distributed along the cell surface in a distinct pattern that corresponds with the middle of the surface unit called the kinetosome. Optical sections through the cell demonstrate the receptor is found at the surface only. The immunofluorescence from the anti-pump antibody shows a superimposable pattern including absence of fluorescence along the cilia. Therefore, the receptor and pump appear to co-localize, although the pump seems to be more limited in its distribution in each kinetosome compared with the receptor.

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333. Odor identification changes after recovery from nerve transection

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Two groups of animals were studied to determine if the ability to identify an odor is retained after recovery from nerve transection or if it must be relearned. Animals received either a bilateral olfactory nerve transection (BTX) or a sham surgical procedure. Animals were first trained to discriminate between two odorants using food pellets as positive reinforcement. After reaching criterion levels of response (90% correct) surgery was performed. During recovery, animals were tested without food reinforcement, on day 4 and again on days 40 and 43. On day 46 testing with food reinforcement was resumed. After surgery (day 4) BTX animals performed at chance levels (50% correct) confirming that the nerve transection had been effective, shams continued to perform at criterion levels. After 36 days without testing (day 40) sham animals performed at criterion levels demonstrating that extinction of the behavior had not occurred, BTX animals however were not able to perform the odor discrimination. After day 46 when food reinforcement was resumed BTX animals were able to quickly relearn the odor discrimination. In a previous study we reported that odor discrimination was restored in BTX animals by day 40. In that study animals received continuous testing with reinforcement throughout the 40 day recovery period and it is likely that they relearned the odor discrimination soon after nerve fibers reestablished connections to the olfactory bulb. These results demonstrate that following recovery from nerve transection animals are capable of making odor discriminations but that they must relearn the identity of odorants. It has been hypothesized that odor identification requires recognition of unique odor patterns within the olfactory bulb. This study suggests that if sensory patterns are changed after recovery from nerve injury relearning may be required to restore function.

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334. Localization of tenascin in the olfactory organ of the larval sea lamprey, *Petromyzon marinus*

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Sea lampreys, which represent an ancient evolutionary line of vertebrates, provide a comparison to the more commonly studied developing mammalian olfactory system. In this study, we have examined the localization of tenascin, an oligomeric glycoprotein that is expressed along migration pathways and axonal routes.

Pre-adsorption controls established tenascin specificity. In the olfactory epithelium, intense tenascin immunoreactivity was localized in the mucociliary complex and in the supranuclear region. The accessory olfactory organ also contained labeling. Following retrograde degeneration of the olfactory nerve, tenascin staining remained strong. Tenascin immunoreactivity was occasionally observed on the lateral and medial edges of the olfactory nerve.

These results suggest that tenascin may function as a guidance cue during postnatal neurogenesis: for dendrites to elongate apically toward the mucociliary complex; and for axons to follow the olfactory nerve.

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335. Role of amyloid precursor protein mRNA isoforms in neuronal differentiation: olfactory receptor neurons as an *in vivo* model

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Hallmarks of Alzheimer's disease brain are neurofibrillary tangles and senile plaques. A major component of these plaques is Aβ4 or amyloid protein, derived from amyloid precursor protein (APP). Although the three major alternatively-spliced forms of APP, i.e. 695, 751 and 770 have been implicated in neuronal differentiation and repair, the normal role of APP is not yet understood. Olfactory receptor neurons (ORNs) in nasal mucosa are replaced throughout life at a slow rate. Turnover can be accelerated by destroying the synaptic target, olfactory bulb. Thus these neurons provide an excellent model for studying the role of APP in neurogenesis and repair *in vivo*. We have previously reported the

expression and cellular localization of APP mRNA and their corresponding protein isoforms to rat ORNs. Here we use quantitative RT-PCR to study the alterations in APP mRNA isoforms during accelerated neuronal degeneration and subsequent regeneration triggered by unilateral bulbectomy. ORNs are dramatically reduced at 3 days post-bulbectomy, as indicated by a 74% decrease (n = 5) in OMP mRNA level and a significantly decreased immunostaining of OMP positive neurons. The loss of neurons was accompanied by a significant decrease in APP 695 (74%, n = 5), 751 (68%, n = 3) and 770 (72%, n = 3) mRNA isoforms at 3 days, although levels of GAP43 mRNA remained unaffected. APP 695 and OMP mRNA levels remained low at 5 days while 751 and 770 isoforms returned to normal. This implies that APP 695 is mainly localized in mature neurons that degenerate following bulbectomy. The earlier recovery of APP 751 and 770 isoforms suggests that they are made either in neuronal precursor cells or in the immature neurons which appear in initial stages of neuronal regeneration. Experiments are in progress to study the levels and localization of these isoforms during later stages of recovery.

336. Development and growth of the olfactory organ of lower actinopterygian fish

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Our investigations on zebrafish have shown that the olfactory placode arises from a subepidermal monolayer from which all cell types of the sensory epithelium derive. This seems to be in contrast to the clawed frog *Xenopus* in which two different cell layers are reported to give rise to the receptor neurons and the supporting cells. In several characters, amphibians show the more primitive state of the Osteognathostomata than teleosts. Therefore, we extended our light and electron microscopic investigations to bichirs and sturgeons, which belong to more basic groups of actinopterygians than zebrafish.

Forty-eight hours after fertilization (AF), the placode of the bichir *Polypterus senegalus* seems to be connected to the brain. The olfactory pits open in embryos of the bichir ~65 h AF, and in the sturgeon *Acipenser baeri* 4 days AF. At this stage, dendritic endings of the ciliated receptor neurons are found at the surface of the sensory epithelium, whereas endings of the microvillous receptor cell type appear later. Some developing receptor neurons show both cilia and microvilli on the apical surface of their dendrites. The origin, differentiation and growth of these receptor cell types is of special interest in our investigations. The primary opening of the olfactory pit elongates and is then constricted by lateral processes, which grow from each side and fuse to form a bridge separating incurrent and excurrent nares. This is considered the primitive mode of formation of the two external olfactory openings in fish.

337. Na^+-gated cation channel from lobster olfactory receptor cells

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We further characterized a novel Na^+-activated channel in cell-free patches from the soma of cultured lobster olfactory receptor cells (Zhainazarov and Ache, 1995). The open probability versus [Na^+] relationship could be fit by the Hill equation with a coefficient of 3.5, indicating the binding of more than one Na^+. At 210 mM (saturating) Na^+, the open probability was <1.0 [0.5 ± 0.2 (mean ± SD); n = 5]. At 10–30 mM Na^+, channel openings were infrequent and bursty. Increasing [Na^+] increased the frequency of transitions until at 180 mM Na^+ one observed long, continuous flickering transitions. [Na^+] did not affect the duration of brief transitions within bursts, suggesting that the intraburst flicker was not associated with binding of Na^+. At 30 mM Na^+, the closed time histogram could be fitted with a triple exponential function with time constants of 0.6 ± 0.3, 17.1 ± 0.2 and 729 ± 1 ms (mean ± SE). At 210 mM Na^+ the closed time histogram could still be fit by a triple exponential, three-exponential function, but the longer two components shortened considerably. The open time histograms could be fitted by a single-exponential function [4.6 ± 0.1 (mean ± SE) ms, 30 mM Na^+] that was not affected by [Na^+] [6.5 ± 0.1 (mean ± SE) ms, 210 mM Na^+]. Permeability ratios calculated from the Goldman–Hodgkin–Katz equation from the reversal potentials for alkali monovalent cations in biionic conditions were: Li (1.11) > Na (1.00) > K (0.54) > Rb (0.36) > Cs (0.20). Divalents (10 mM) reduced the slope conductance at –60 mV to different extents: Mg (–2.32 pA; 178.6 pS) > Ba (–1.56 pA; 23.0 pS) > Ca (–0.84 pA; 17.9 pS) > Sr (–0.79 pA; 15.2 pS) > Mn (–0.30 pA; 12.0 pS). A P_{Ca}/P_{Na} of 2.1 could be estimated from the extrapolated value the reversal potential with extracellular 10 mM Ca^{2+} and 200 mM Na^+. Preliminary evidence localizes this channel to the transduction zone in the cells *in situ*, but its role in transduction, if any, remains to be determined.

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338. Olfactory sensitivity and behavioral reactions of lake char to bile acids released by conspecifics

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We previously reported that the lake char, *Salvelinus namaycush*, olfactory system is capable of detecting and discriminating various types of bile acids through specific receptors. To understand the biological significance of this chemosensitivity, we further investigated: (i) the bile acids produced and released by lake char; (ii) the olfactory potency of these bile acid components; and (iii)

the behavioral reactions of fish to bile acids. Bile acids in the bile, feces, urine and fish holding water from fasting juvenile and adult fish were analyzed using ion-pairing HPLC combined with immobolized post-column reaction. Cholic acid (CA) and chenodeoxycholic acid (CD), conjugated with taurine, were the two major compounds found in all samples. Small quantities (<10%) of bile acids were sulfated at 3α hydroxy position. In prespawning male urine, we found large quantities of CA, CD and an unidentified, unamidated cholanoate sulfate. The bile acid concentration in prespawning male urine was 200 times that found in urine of juveniles or prespawning females. Changes in bile acid concentration (up to 100 times) were observed in holding water of prespawning females collected through 3 successive days. Electroolfactogram recordings showed that the olfactory potency of the samples were due to these bile acid components. The bile acids identified in the samples were among the most effective olfactory stimulants of 37 authentic bile acids tested. Lake char showed preference reactions to natural bile at thresholds of 1 nl/l and to taurocholic acid at 10^{-8}–10^{-7} M in a Y-maze trough. Thus, lake char can distinguish and behaviorally respond to bile acids produced and released by their conspecifics. The release of large amounts of bile acids with distinct compositions in prespawning lake char suggests that bile acids may function as chemical signals in spawning.

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339. Characterization of sodium transport in fungiform-, foliate- and vallate-containing epithelia from hamster and rat

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Electrophysiological studies at the cellular level in our laboratory have shown that in rat, there are significant differences in the mechanisms of Na transport into the three main classes of taste receptor cells (TRCs). Approximately two-thirds of fungiform (Fu) and one-third of foliate (Fo) TRCs contain amiloride-sensitive sodium channels (ASSCs) which account for roughly one-half of the electrogenic Na transport in these cells. Vallate (Va) TRCs transport Na electrogenically as well, but lack functional ASSCs. Therefore, there appears to be significant Na transport which does not involve the ASSC in mammalian TRCs. To examine Na transport in greater detail in the three main classes of taste buds, we have recorded transepithelial Na currents from epithelia containing either Fu, Fo or Va taste buds isolated from both rat and hamster. Epithelia were isolated and mounted in an Ussing chamber (area: 0.126 cm^2; hemi-volume: 0.75 ml) and transepithelial current and resistance were recorded by a dual channel voltage clamp. Two broad generalizations may be made from these results. First, similar to our findings in isolated cells, Na transport in rat taste tissue differed among the taste bud types. Na current density was ~60% greater in Fu-containing epithelia than in Fo- or Va-containing epithelia. Second, in all three taste bud-containing epithelia, Na transport was greater in the rat than in the corresponding epithelia in the hamster. The tight junction blocker, $LaCl_3$ (6 mM, mucosally), inhibited about one-third of Na transport in all epithelia from both species, suggesting a portion of Na transport is via paracellular pathways. Amiloride (0.5 mM, mucosally) was effective in inhibiting Na transport in rat Fu and Fo epithelia, but had no effect on Va epithelia. In hamster, however, amiloride inhibited a portion of Na transport in all three epithelial types. Serosal amiloride had no significant effect on Na transport, suggesting that amiloride-sensitive Na channels may be apically restricted.

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340. Capsaicin and protons activate cultured trigeminal neurons through different but possibly overlapping mechanisms

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Acidic pH (6.0) and capsaicin can induce mild irritation, pain and hyperalgesia as well as causing the release of the neuropeptides CGRP and substance P. Based on similarities between acid- and CAP-induced currents, inhibition of responses to both stimuli, by both ruthenium red capsazepine as well as potentiation of CAP-induced currents by protons, a common receptor mechanism has been proposed. More recently, dissimilarities in H^+ and CAP induced currents have been reported as well as evidence that H^+ fails to cross adapt CAP-induced increases in intracellular Ca^{2+} (Ca_i). Together this suggests that H^+ and CAP act through several different mechanisms. In order to further elucidate the nature of trigeminal sensitivities to CAP and protons, we examined changes in intracellular calcium in cultured trigeminal neurons using fluorescence imaging of Fura-2-loaded cells. Responses (increases in Ca_i) to CAP were found to be independent of acid sensitivity in 31% of neurons, 4% of neurons were sensitive only to H^+ and 17% of neurons responded to both. Increases of intracellular calcium induced by H^+ and CAP were both dependent upon extracellular Ca^{2+}. Dependence on extracellular Na^+ differed between CAP and H^+: increases in Ca_i due to H^+ were Na^+-dependent (in 34% of neurons) while Na^+-free conditions caused enhanced responses to CAP in most neurons. When presented simultaneously, H^+ and CAP exhibit synergistic interactions in ~58% of neurons. However, the relationship between the presence of this synergism and the presence of CAP and H^+ sensitivities on individual neurons is complicated enough to provide further support for mediation of H^+ and CAP activation by more than one mechanism.

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341. Adenovirus-mediated gene transfer in olfactory neurons

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Understanding the function of proteins frequently depends on having systems in which they can be expressed and manipulated. In the olfactory system these might include the various proteins

involved in the transduction cascade, in axon guidance, and in cellular processes of growth and regeneration. However, the odor receptor, in particular, while cloned and sequenced, has not proven to be easily expressed in a system that would allow an understanding of the detailed interactions between receptors and specific odors.

We have developed the recombinant adenovirus as a means of expressing exogenous proteins in olfactory neurons. A replication-incompetent adenovirus (type 5, Ad5) carrying the reporter gene *lacZ* which codes for the enzyme beta-galactosidase was applied in solution to the olfactory epithelia of rats under anesthesia at a titer of 10^{10} p.f.u./ml. β-gal expression was observed after one day post infection and was maximal at 3–7 days post infection. Expression could be detected for at least 21 days post infection. Expression patterns were heterogeneous, ranging from a few percent to 50% of the cells in different regions of both turbinate and septal epithelium. Staining was stronger in the olfactory versus respiratory epithelia. In olfactory epithelium staining was almost entirely restricted to mature olfactory neurons; little or no infection was evident in sustentacular or basal cells. β-gal staining was also observed in the olfactory axons so that nerve bundles could be traced to their targets in the glomerular layer of the olfactory bulb. Intense staining of some glomeruli was evident as long as 21 days post infection. Another foreign protein, human p75, was similarly expressed in rat olfactory neurons using another adenovirus construct. In neither case was there any evidence of inflammation or cytotoxicity due to viral infection.

These results demonstrate that it is possible to use recombinant Ad5 to express proteins in olfactory neurons. This technique could be used to express foreign proteins or to increase expression levels of olfactory specific proteins, including the odor receptor, putative guidance and growth molecules, or elements of the transduction cascade.

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342. Cloning of olfactory receptor genes from the mudpuppy, *Necturus maculosus*

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Extensive cloning efforts in recent years have expanded the original rat olfactory receptor gene family to a superfamily including homologous sequences from rat, mouse, dog, chicken, human, catfish, *Xenopus laevis* and honeybee. Sequence analysis clearly defines two distinct groups within the superfamily. One group was originally cloned from catfish, and contains a few receptors from *Xenopus*. The second group contains all the sequences from mouse, rat, chicken, dog, human, honeybee and a large proportion of those from *Xenopus*. Based on the assumption that these receptors serve as odor receptors, and because aquatic and terrestrial animals have access to structurally very different odorants, it has been suggested that the fish-like receptor group may detect water-borne odors while the rat-like receptor group may detect hydrophobic, air-borne odors. Recently, this hypothesis has found strong support (Freitag *et al.*, 1995).

We are currently using degenerate primers and RT-PCR to search for putative odor receptors in the olfactory epithelium of *Necturus maculosus*. We have cloned and obtained partial sequences for seven new members of the olfactory receptor gene superfamily. Sequence analysis shows that these clones share 33–55% identity at the amino acid level with the rat olfactory receptor gene family, and 21–33% identity with catfish olfactory receptors. Because mudpuppies are fully aquatic, this finding is surprising, as the odorants encountered by mudpuppies should be very similar to those of fish. Salamanders are closely related to frogs and phylogenetically between fish and mammals. Further comparative analysis of full length sequences may provide insights into the possible function for this group of receptors.

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343. The role of perireceptor events for odor reception

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Airborne odorants adsorbed on insect antennae enter the olfactory hairs via pore tubules and have to cross the sensillum lymph in order to activate the receptive dendrite for a short, limited time. The sequence and timescale of these perireceptor events contribute to the receptor cell response and will be discussed for the silkmoth *Antheraea polyphemus* in the present paper.

In the sensillum lymph two proteins interacting with odorants are present: soluble odorant binding proteins (OBPs) in mM concentrations and degrading enzymes like sensillum lymph specific esterase and aldehyde oxidase in very low concentrations, i.e. in a ratio of at least 10000:1 according to protein staining of OBP and enzyme. Due to the high pheromone binding protein (PBP) concentration and the moderate K_d, >99% of all incoming hydrophobic pheromones will be bound by the PBP and transported through the aqueous medium. Binding studies of pheromone to the PBP in sensory hair homogenates showed, that the radiolabeled pheromone was bound at the beginning mainly by the reduced PBP population where at least two of the six cysteines have free SH-groups. Increasing the incubation time resulted in a shift of pheromone binding from the reduced to the oxidized PBP form where all six cysteines are involved in disulfide bonds. Since this redox shift was not observed with purified PBP forms, it seems likely that PBP oxidation goes along with receptor cell activation. This oxidation might lead to deactivation of the pheromone–PBP–complex, whereby the pheromone degrading esterase present in individuals in highly variable amounts might serve for the final, slow pheromone sequestration.

Analyzing the OBPs with respect to their isoelectric point, revealed an increasing number of OBPs in a given insect species. It is not clear, whether our model of receptor cell mediated PBP oxidation will be of general importance for all activating odorants leading to rapid deactivation or represents a specialization of pheromone perception.

344. Dopamine (D_2) receptor activation blocks sensory evoked excitation of rat mitral cells *in vitro*

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Many juxtaglomerular (JG) interneurons in the rat main olfactory bulb contain dopamine (DA). DA-D2 receptors are localized in the glomerular layer (GL) and olfactory nerve layer (ONL). Olfactory epithelium destruction eliminates D2 receptors in both the ONL and GL, suggesting that they are present on ON terminals. Consistent with this, olfactory receptor neurons contain abundant D2 receptor mRNA transcripts. These findings suggest that DA released from JG neurons presynaptically regulates ON terminals. We recently showed that glutamate released from ON terminals excites mitral cells via non-NMDA and NMDA receptors in the rat. Activation of non-NMDA (AMPA/kainate) receptors evokes rapid, brief (10–40 ms) excitation of mitral cells; NMDA receptors mediate a delayed, long-lasting (50–500 ms) excitation.

The goal of the present study was to determine if glutamate transmission at the ON→MC synapse is modulated by D_2 receptors. Olfactory bulbs from anesthetized rats (50–125 g) were cut into 500 μm-thick horizontal slices and submerged in a recording chamber at 30°C. Single (O.4 Hz), low intensity (4–40 μA) ONL shocks were applied while recording extracellularly from single mitral cells.

Bath application of DA (100 μM, $n = 9$) significantly decreased the spontaneous rate (>50%) and the magnitude of both non-NMDA (75%) and NMDA (80%) responses evoked by ONL stimulation. The selective D_2 agonist, quinelorane (300 μM, $n = 8$) also decreased the spontaneous rate (46%, $P < 0.05$) and the magnitude of both non-NMDA (74%) and NMDA (31%) responses. ON-evoked responses recovered 5–15 min after washout of DA and quinelorane. The D_2 antagonist, eticlopride (10 μM, $n = 9$) increased spontaneous activity (70%) and increased both non-NMDA (65%) and NMDA (59%) responses.

These results demonstrate that activation of D_2 receptors on ON terminals potently inhibits spontaneous and ON-evoked discharge of mitral cells. We conclude that DA release from JG neurons presynaptically inhibits glutamate release from ON terminals via activation of D_2 receptors.

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345. Chemotaxis links the marine microbial loop to atmospheric sulfur production

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Dimethylsulfide (DMS) gas comprises 90% of biogenic sulfur emissions from oceans and is an important agent in climate regulation. DMS arises primarily via lyase degradation of dimethyl-sulfoniopropionate (DMSP), an osmolyte produced in high concentrations by globally abundant marine phytoplankton. DMSP lyase producing bacteria (*Alcaligenes* strain M3A) significantly reduce their tumbling frequency and are attracted to DMSP at levels found near senescing phytoplankton cells (10^{-8}–10^{-6} M). In contrast, genetically identical bacteria without the induced lyase are not attracted to DMSP. Combined with lyase activity, microbial chemotaxis to DMSP increases the rate of DMS production and, therefore, should play a critical role in biogeochemical sulfur cycling between dissolved organic matter in seawater and earth's atmosphere.

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346. Olfactory receptor neurons do not require contact with the olfactory bulb to develop normal chemical responsiveness

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We investigated whether contact with the olfactory bulb is necessary for regenerating olfactory receptor neurons (ORNs) to attain normal odorant specificity and sensitivity. Electrophysiological [electro-olfactogram (EOG) and multiunit neural] recordings were obtained in response to amino acid (L-isomers of arg, lys, ser, ala, met, leu and glu), bile [taurolithocholic acid sulfate salt (TLCS)] and pheromonal [17α,20β-dihydroxy-4-pregnen-3-one (17α)] odorants from immature (10–12 months) goldfish maintained at 20°C. The experimental goldfish included seven intact controls, six with bilateral transections of the olfactory nerve and twelve with bi- lateral bulbectomies. EOG recordings were obtained with calomel electrodes from the entire olfactory organ, whereas the multiunit neural activity was obtained from discrete regions of the sensory epithelium by surface recordings with glass insulated metal microelectrodes tip-plated with platinum. Specimens of olfactory lamellae of selected experimental fish and recovery times up to seven weeks post surgery were also observed with SEM and TEM. Within one to two weeks subsequent to the surgery and during degeneration of the olfactory epithelium, responses were no longer obtainable to TLCS and 17α, while responses to high concentrations (>0.1 mM) of the more stimulatory amino acids (arg, lys, ser) remained. During the fourth week, olfactory knobs with either cilia or microvilli were more evident, but the density of the receptor neurons was less than observed in control lamellae. At 4 weeks, EOG responses to amino acid stimuli recovered to control levels (thresholds ~1–10 μM), while responses to TLCS and 17α were minimal. At seven weeks, epithelia from axotomized, but not bulbectomized, animals were comparable to controls, and neural responses to the stimuli were still in the process of recovering; however, EOG responses of the axotomized and bulbectomized fish to all the stimuli at seven weeks were

comparable to those from control fish with respect to odorant sensitivity and specificity.

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347. Long-lasting adaptation of the odor response of olfactory receptor neurons depends on the CO/cGMP second messenger system

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While much information about the excitation process in olfactory transduction has been gathered over the past years, little is known about the molecular mechanisms that lead to sensory adaptation in olfactory receptor neurons (ORNs). By applying repeated odor stimuli and using perforated patch recordings under voltage-clamp from isolated salamander ORNs we have elicited long-lasting adaptation (LLA) of the odor response. LLA is clearly coupled to the stimulus strength (with respect to the $K_{1/2}$ value of the odor response) and is characterized by a strong reduction in peak current amplitude to a given stimulus. This reduced odor responsiveness can continue for several min after the end of an appropriate stimulus (in the absence of odor molecules) but recovers completely so that the effect can be triggered several times during recording from a given ORN. One consequence of LLA is that the $K_{1/2}$ values of dose-response curves of odor-induced currents are shifted to the right. In addition, maximum responses at saturating odor concentrations are strongly reduced. Another series of experiments demonstrated that Ca^{2+} entry is required for this form of adaptation and that LLA can be abolished by reducing the external Ca^{2+} concentration to ≤ 1 μM.

Because the properties LLA closely matched the effects of exogenous carbon monoxide (CO) or cGMP on odor responses we tested the consequences of a variety of pharmacological blockers of the cGMP second messenger system. Long-lasting adaptation could be uncoupled from excitation and entirely be prevented in the presence of agents that act as blockers of the CO-producing enzyme heme oxygenase-2, such as zinc protoporphyrins. In contrast, specific blockers of nitric oxide synthase had no effect on LLA. A series of control experiments ruled out that the effect of the CO pathway blockers was due to unspecific effects at the level of soluble guanylyl cyclase or at the CNG channels.

These data provide evidence that endogenous CO mediates a long-lasting form of odor response adaptation. We conclude that CO can be released in an individual ORN in response to odor stimuli of a given strength. This effect results in cGMP formation, followed by tonic activation of the CNG channels and Ca^{2+} entry leading to sensory adaptation.

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348. Expression of *Pax-6* and neurogenic genes in the *Xenopus* olfactory system during metamorphosis

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Determination of neural fate is decided by a variety of genes that act in specific cells and under temporally-regulated patterns. Among the genes known to participate in neural determination are the neurogenic genes: *Notch*, which codes for a transmembrane cell-surface receptor protein, and its putative ligands, *Delta* and *Serrate/Jagged*. Some genes that code for transcription factors, such as *Pax-6*, appear to participate in patterning the developing nervous system.

Research in our laboratory has demonstrated expression of *Notch*, *Delta*, *Serrate/Jagged*, and *Pax-6* in the olfactory placode and developing brain of *Xenopus laevis*. The current investigation has examined expression patterns of neurogenic genes and *Pax-6* in the olfactory system during metamorphosis. During metamorphosis, two water-sensing epithelia, notably the vomeronasal organ and the principal cavity, are joined by a third water-sensing epithelium, the middle cavity. The principal cavity, meanwhile, changes its sensory medium from water to air. Using digoxygenin-labeled RNA probes, we applied *in situ* hybridization to characterize this phenomenon on sections taken from *Xenopus* nose and brain during metamorphosis. We found *Pax-6* expression in the basal layer of the middle cavity, the superficial layer of the vomeronasal epithelium, and in a subpopulation of neurons and ventricular cells in the olfactory bulb. We found high levels of *Notch* expression in the basal cell layer of the olfactory epithelium of the middle cavity and vomeronasal organ, and in ependymal cells of the ventricles. The principal cavity, however, exhibits only small patches of *Notch* expression in basal sensory regions and moderate expression in adjacent respiratory regions. Experiments are underway to determine if *Delta* and *Serrate/Jagged* also are expressed at these late stages of development.

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349. Rapid alternate measurement of membrane potential and intracellular calcium in cell ensembles

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Multiparameter optical measurements have played a key role in understanding the behavior of multicellular systems. We have studied the spectral properties of the voltage-sensitive dye di-8-ANEPPS and the Ca^{2+}-sensitive dye fura-2 in azolectin liposomes and in isolated taste buds from mouse. We find that the fluorescence excitation spectrum of di-8-ANEPPS and fura-2 are largely non-overlapping allowing alternate ratio measurements of

membrane potential and $[Ca^{2+}]_i$ in the same preparation using excitation wavelengths of 340 and 360 nm for fura-2, and 440 and 500 nm for di-8-ANEPPS. There is a small spillover of di-8-ANEPPS fluorescence into the excitation ranges used for fura-2 (340 and 360 nm, 12.5 nm bandwidth). However, voltage-induced changes in fluorescence of di-8-ANEPPS excited at the fura-2 wavelengths are small. In addition, di-8-ANEPPS fluorescence is localized to the membrane while fura-2 fluorescence is localized to the cytoplasm Because of this, under the appropriate conditions the effect of spillover of di-8-ANEPPS fluorescence in the $[Ca^{2+}]_i$ estimate is <1%. Moreover, we find that in double-labeled cells the ratio of fluorescence emitted when excited at 360 nm divided by fluorescence emitted when excited at 440 nm (F_{360}/F_{440}) provides an empirical parameter to quantify the extent of the error due to di-8-ANEPPS spillover. We have applied this method to study the response of taste cells in multiple cells within isolated taste buds. We show that membrane potential and $[Ca^{2+}]_i$ can be measured alternately in isolated taste buds from mouse. Stimulation with glutamate and glutamate analogues indicates that taste cells express both metabotropic and ionotropic receptors. Alternate optical measurement of membrane potential and $[Ca^{2+}]_i$ is a technique that should be applicable to a variety of multicellular preparations.

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