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ABSTRACTS

The Thirteenth International Symposium on Olfaction and Taste (ISOT XIII) and the Fourteenth European Chemoreception Research Organization Congress (ECRO XIV)

Held in Brighton, UK, July 20–24, 2000

Plenary Lectures

1. Is there a working memory for smell and taste?

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The concept of working memory assumes a temporary and limited capacity storage system that forms an interface between perception, attention and long-term memory. As such, it is assumed to underpin the performance of a range of complex cognitive activities including learning, comprehending and reasoning. In 1974, Baddeley and Hitch proposed that it should be considered as a tripartite system involving an attentional controller, the *central executive*, aided by two subsidiary systems, one concerned with auditory verbal information, the *phonological loop*, while the other, the *visuo-spatial* sketchpad, performed a similar function for visual and spatial information. It is not easy to see how modalities such as smell and taste could fit into such a model. Within recent months, however, evidence from a range of sources has led to the postulation of a fourth component, the *episodic buffer*. This is assumed to form a system capable of creating and maintaining multimodal representations based on information derived from perception or from long-term memory. The system is also assumed to be involved in conscious awareness, which is seen as a device for binding together precepts into perceived objects and scenes. The question of whether smell and taste information could be maintained in such a system will be discussed, and the implications for a working memory of smell and taste will be considered.

2. Taste and smell in the natural world

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Most chemical signals in nature are volatile, although there is increasing evidence that many animals can respond to involatile plant chemicals. Volatile signals, when mixed with other chemicals, may become more active due to synergism and/or become more persistent. Volatile scents are generally efficient, since only trace amounts are needed to produce an effect. They are often multifunctional: a defence odour may also mediate in interactions between competing herbivores. Chemical signals are important for reinforcing species-species interactions, but there are examples of 'accidental' responses to plant odours. However, some plants when attacked by insect larvae deliberately release particular volatiles in order to attract the parasitoids of those larvae. Taste has an important role in protecting plants from grazing animals, although taste responses may be quite different from those of humans. Recent studies on the plants selected for food by chimpanzees in the Budongo rainforest will be reviewed.

3. The molecular biology of glycogenin. A model carbohydrate receptor

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Glycogenin is a unique autocatalytic protein that constitutes the primer for glycogen synthesis. It has so far been characterized in mammals and yeast, and its molecular biology is becoming well understood (Alonso *et al.*, 1995, FASEB J., 9: 1126–1137; Roach and Skurat, 1997, Proj. Nunc. Acid Res. Md. Biol., 57: 289–316). The 38kDa protein acts as a receptor for α-glucose, which it bonds to itself from UDP glucose through a unique carbohydrate-protein bond, at Tyr 194. The process is intermolecular. Pairs of molecules glucosylate each other (Alonso *et al.*, 1995, J. Biol. Chem., 279: 15315–15319; Liu *et al.*, 1999, Arch. Biochem. Biophys., 363: 163–170), The process continues by the addition of further α-glucose residues, in 1,4-linkage, until the oligosaccharide chain is 5–13 residues in length (Alonso *et al.*, 1995, FASEB J., 9: 1126–1137). The varying length is probably a consequence of the inter-chain glucosylation. In this condition glycogenin has now become the primer for glycogen synthesis. Glycogen synthase extends the oligosaccharide chains and, assisted by branching enzyme, the mature 10^7 Da molecule (macroglycogen) is formed. However, there is a stable intermediate, polyglycogen, and different forms of synthases may mediate the synthesis of the two forms of glycogen. A genetic deletion of glycogenin is probably a lethal trait. Any underexpression of the glycogenin receptor could result in hyperglycemia because of the inability of muscle to act as the reservoir in which excess glucose is stored. The search for a link between insufficient glycogenin and diabetes has attracted the attention of several laboratories, including our own. It seems likely that proteins, perhaps autocatalytic, will be found to be the primers/receptors for the synthesis of storage polysaccharides and that their integrity will be found to be vital to proper cellular function. One may also consider the consequences of genetically manipulating the over-expression of such a protein. In man, it could be used to combat hyperglycemia, to enhance glycogen stores in preparation for organ transplants or to increase endurance in long-distance runners. In plants, it could be used to enhance the

storage of sugars in polymeric form, increasing their calorific value and biomass. Under-expression of a primer protein could be used to suppress polysaccharide storage. The consequences of such manipulations in altering the taste of food and of scavenging sugars merits attention.

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4. Scientific applications of machine olfaction and vice versa

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While the electronic nose was initially born out of a research programme aimed at providing an analogue to the biological olfactory pathway, there has since been little work focusing on the parallels between this technology and the biological system.

Here we will overview the synergies between these two research fields and show how each can be used to the benefit of the other. In particular, two studies will be discussed demonstrating the synergy. Firstly, by exploiting arrays of optical microbeads combined with a simple neuronal model of the early stages of the biological olfactory system it is possible to investigate efficient coding of odour stimuli in a neural code.

Secondly, using the same sensor technology, it is discussed how convergence of sensory information in an electronic nose, reminiscent of the architecture of the early olfactory pathway, can lead to sensitivity enhancement in an electronic nose.

Symposia and Free Sessions

Molecular Biology I

5. Molecular receptive range of an olfactory receptor

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Determining the receptive field for a sensory receptor has played an important role in understanding how a perception is constructed from raw stimulus input. In sensory systems, such as the visual, auditory and somatosensory, the notion of a receptive field is somewhat easier to construct since the stimulus varies along physical continuum—i.e. it can be translated into a spatial map. The chemical world of odors is less straightforward in this regard as there are no primaries or fundamentals in analogy with vision or audition. We have applied the principles of medicinal chemistry to olfactory receptors in an attempt to define a comprehensive molecular receptive field for a single odor receptor. We have utilized the I7 odor receptor in an adenovirus driven expression system and made use of the large and diverse number of chemicals available for testing activity at this receptor. These chemicals represent various 'pharmacophores', and their activity or inactivity defines the range of chemical qualities that are discriminated by this receptor. We find that the I7 receptor has an absolute requirement for an aldehyde functional group; that the receptor is activated by molecules with carbon backbones between 7 and 11, and lengths between 8 and 12 Å; and is tolerant of double bonds, methyl groups and even bulky ring structures near the tail end of the compounds. However, we also find that there are very specific steric constraints near the aldehyde moiety such that the combination of double bonds and methyl groups at C2 or C3 that brings the groups into the same plane as the aldehyde is not tolerated. Taken together, these results define a molecular receptive range and demonstrate that this receptor is highly discriminating for certain features of a ligand while very tolerant of others. This enables us to define an 'aromaphore' for this receptor.

6. A unique subfamily of olfactory receptors

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Detection and discrimination of odorous molecules is based on specific odorant receptor proteins located in the ciliary membranes of olfactory sensory neurons. The repertoire of genes encoding such receptors is extremely large, numbering as many as 1000 genes for some mammals. Most olfactory receptor neurons are expressed in sensory neurons which segregate in rather broad rostro-caudal zones in the nasal epithelium. In contrast, a small subfamily of genes (OR37), originally identified in rats, exhibits a unique distribution pattern: neurons expressing these genes are clustered in a small area of the nasal neuroepithelium. The mechanisms controlling the topographic patterns of odorant receptor expression in distinct anatomical zones are still elusive. For other multigene families it has been shown that the chromosomal organization of the genes may provide a physical basis for regulatory principles. We therefore characterized the repertoire of mouse OR37 genes and determined their genomic organization. Five mOR37 genes were identified and the mapping studies revealed that they are tightly clustered within the genome; the genes reside on chromosome 4 within only 60 kb of DNA. Towards an understanding of the mechanisms underlying their expression control, the structural motifs in the upstream regions of the genes were analyzed. Genomic sequencing of the cluster and subsequent computer analyses revealed a complex intro/exon structure for the mOR37 subtypes. 5′ from their putative initial exons, all genes present in the cluster share conserved sequence motifs which represent potential binding sites for transcription factors.

To get an insight into how the system is designed to encode information about a stimulus, the axonal projection patterns of olfactory neurons expressing distinct genes from this receptor subfamily were analyzed. A gene targeting strategy in mice allowed the coordinated translation of a receptor along with a marker protein, permitting the visualization of the cells including their axonal projections. Using either taulacZ or tauGFP as axonal markers, two different receptors could be visualized in the same individual by double labeling. Each gene was expressed in a different subset of olfactory neurons in the nasal sensory epithelium. Analyzing their axonal projections revealed that all cells expressing the same receptor project upon a common glomerulus. The different populations target distinct glomeruli which are all grouped within a restricted domain of the olfactory bulb. Analysis of a large number of bulbs revealed that the relative positions of these glomeruli are not fixed but display local permutations.

7. Molecular bases of odor discrimination

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In mammals, odorant detection is mediated by ~1000 different odorant receptors (ORs) that are expressed by olfactory neurons in the nose. We have asked how the OR family encodes the identities of thousands of chemically diverse odorants. To explore this problem, we devised a novel technique to identify ORs that recognize specific odorants (Malnic *et al.*, 1999, Cell, 96: 713–723). We first used calcium imaging to identify mouse olfactory neurons that respond to particular odorants, and then used a two-step single cell RT-PCR procedure to identify the OR genes expressed by the responsive neurons. In control experiments, we verified that each neuron expresses only one OR gene. Thus the response profile of a neuron reflects the recognition properties of the OR it expresses.

Using a series of *n*-aliphatic odorants that varied in carbon change length and functional group, only a very small percentage of neurons responded to individual odorants. Consistent with previous observations that one neuron can respond to several odorants and that different odorants activate different combinations of olfactory bulb glomeruli [reviewed elsewhere (Kauer, 1987, in Finger and Silver, eds, Neurobiology of Taste and Smell. John Wiley & Sons, New York, pp. 205–231; Hildebrand and Shepherd, 1997, Annu. Rev. Neurosci., 20: 595–631; Buck, 2000, Cell, 100: 611–618)], we found that one OR can recognize multiple odorants and that one odorant can be recognized by multiple ORs. However, different odorants, even if highly related, were recognized by different combinations of ORs. Thus the OR family is used in a combinatorial fashion to encode odor identities. In this scheme, each OR serves as one component of the multicomponent 'receptor codes' for many odorants, thereby allowing for the discrimination of an immense number of structurally diverse odorants.

Our results revealed extensive variability in the recognition properties of ORs, with different ORs recognizing odorants with different carbon chain lengths and different combinations of functional groups. In addition, individual odorants were recognized by ORs whose sequences were highly related as well as ORs with dissimilar sequences. This high level of recognition diversity provides a basis for the olfactory system's discriminatory capacity and its ability to detect odorants with varied structures.

Interestingly, we found that slight changes in odorant structure and changes in odorant concentration both yielded alterations in an odorant's 'receptor code', potentially explaining how such changes can dramatically alter odor perception in humans. Recently, we have begun to extend these studies to additional odorants with diverse chemical structures.

8. Odorant receptors and their regulation in *Drosophila*

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We have used a novel computer algorithm to identify candidate chemosensory receptors from genomic databases. The algorithm maps the predicted products of open reading frames into an *n*-dimensional protein space and uses a discriminant function to determine which predicted proteins map into a portion of the space occupied by known G protein-coupled receptors. Using this algorithm, we have identified a large family of seven transmembrane domain proteins likely to be odorant receptors in *Drosophila*.

Immunohistochemistry and *in situ* hybridization show that the genes are expressed in subsets of olfactory receptor neurons, and that protein localization is that expected of a dendritic protein. The predicted proteins are extremely divergent in sequence, raising questions about their evolution and the diversity of signaling proteins with which they interact.

A particularly interesting issue is the problem of receptor gene choice. How do individual neurons select which receptor genes to express, and how are the choices of the different neurons coordinated so as to produce a system capable of coherent coding? We have found that the process of receptor gene choice depends on at least one POU domain transcription factor, Acj6. In mutants of acj6, a subset of receptor genes is not expressed normally; correspondingly, a subset of ORNs acquires abnormal odor-specificities. We have found evidence that Acj6 binds directly to promoter regions flanking receptor genes, and have constructed a model in which receptor gene choice depends on a combinatorial code of POU transcription factors.

9. Molecular dissection of urine odor evoked response in the main olfactory bulb

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The first stage of odor discrimination involves the interaction of odorant with its cognate olfactory receptor(s). Odor processing continues when neurons expressing these receptors transmit signals to the olfactory bulb (OB), where they are received and processed before being transmitted to higher brain centers. Odor-induced neuronal activity patterns in the OB are believed to originate in the differential odor responses of olfactory receptor neurons, which synapse on mitral and tufted cell dendrites in olfactory bulb glomeruli. Olfactory receptor mRNA has been shown to be present in olfactory receptor neuron axons, making it theoretically possible to clone olfactory receptors involved in a specific odor response. In order to understand the mechanisms responsible for *in vivo* discrimination of a biologically meaningful odor stimulus we have generated an olfactory receptor library from glomeruli responsive to urine. Awake female mice were exposed to novel male urine odor. The neural activity patterns evoked within their main olfactory bulbs were mapped by measuring increases in c-fos mRNA, a marker for neuronal activity, in periglomerular cells. Coordinates for tissue microdissection and subsequent cloning were determined by defining areas with the highest urine-evoked activity. Thirteen different receptors with sequence similarity to the olfactory receptor family were identified. Antisense cRNA probes made from two of these receptor cDNAs hybridized to the areas we microdissected. These results suggest that it is possible to generate olfactory receptor libraries with known odor specificities by a combination of c-fos mRNA activity mapping and RT-PCR.

Functional expression studies of members of this library are underway to determine if these receptors respond to urine odor.

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10. OMP gene deletion results in an alteration in odorant quality perception

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Previous work suggests that odorant quality perception might be altered in mice lacking the gene for OMP. The loss of OMP results in a marked reduction of the EOG response to odorants that is coupled with a slowing of response and recovery kinetics. Thus, it could be suggested that these defects would degrade the odorant-induced spatial activity patterns that are characteristic of different odorants, as well as alter the differential temporal activation of the mucosa. These altered spatiotemporal patterns would, in turn, alter the organized and stereotyped patterning of information that occurs at the level of the mucosal projection onto the bulb. Such alterations would be important because one model of odorant quality encoding has proposed that a stimulus is encoded along multiple dimensions as a vector of *n*-dimensions. It has been suggested that the glomeruli of the olfactory bulb are an obvious candidate for the *n*-dimensions that encode odorant quality at this level. Thus, degrading or disrupting the neural activity of the bulb would be expected to alter odorant quality perception.

To test the hypothesis that odorant quality perception is altered in OMP-null animals we have trained and tested seven mice (three OMP-null and four background controls), using our five-odorant identification confusion matrix task (AOCM). Using standard operant techniques, mice were trained to differentially report (i.e. identify) the odorants ethyl acetoacetate, carvone, propanol, propyl acetate, and citral. Following criterion training, animals received forty testing sessions, using a standard 5 × 5 confusion matrix design, in order to acquire data for the comparison of odorant quality perception between these two groups. On average, control and OMP-null animals performed at equivalent levels (mean ± SD: 91.47 ± 1.82 versus 91.93 ± 0.53, respectively). These results demonstrate that despite the altered neurophysiological activity known to occur in OMP-null mice these animals can perform the identification task at levels comparable to controls. The composite matrix for each animal (both OMP-null and control) was compared with every other animal yielding a dissimilarity matrix of animal AOCM responses. An MDS analysis of the dissimilarity data yielded a three-dimensional solution with each animal (both OMP-null and control) occupying a point in MDS space. Preliminary statistical analysis (MANOVA) provides strong evidence for an effect of genotype in determining the location of an animal in the MDS space. These data suggest, therefore, that, compared with controls, odorant quality perception is altered in OMP-null animals. Moreover, they support previous neurophysiological and behavioral sensitivity measurements which indicated an alteration in neural function in the null mutant.

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11. Introduction

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If we take the date of publication of the structure of bombykol (Butenandt *et al.*, 1961, Hoppe Seyler Z. Physiol. Chem., 324: 71–83) as the birth of semiochemistry, then today, it is reaching middle age. However, this does not indicate that the area is mature or slowing down, indeed the pace of developments continues to accelerate, with advances from a host of new techniques and approaches (Kelly, 1996, Chem. Biol., 3: 595–602). This morning's session intentionally reflects many disparate aspects of semiochemistry, including agricultural applications, structural elucidation and molecular biology. On one hand, the determination of the structure of semiochemicals can now be achieved in a reasonable time (less than one research grant), given an adequate supply of materials, a suitable bioassay and some luck! However, the role of a given semiochemical and its relationship to other semiochemicals, plus visual and auditory cues and behavioural imperatives is more difficult to determine. This is shown most clearly in the use of pheromones for pest insect control, on agricultural crops, which has not fulfilled its early promise. In the first lecture, John Pickett describes recent developments in push–pull strategies for diverting pests from crops and the science which underpins it (Pickett *et al.*, 1999, Adv. Bot. Res., 30: 91–115). This approach uses low inputs and hence can form a component of a sustainable agricultural system. On the other hand, pheromones have been effective for monitoring insect pests and mating disruption of moths. The successful deployment of a pheromone based monitoring or control system requires careful exploitation of the factors described above, and all at an economically viable price! These issues are discussed in the lecture by Owen Jones, the founder and chairman of Agrisense BCS (Jones, 1998, Pest. Sci., 54; 293–296). The last four lectures are a pot pourri of topics from the cutting edge of semiochemistry.

The sex pheromones of the social Hymenoptera have been investigated in exquisite detail, but the non-social species have been neglected, although many attack the seeds of economically important crops. Basilios Mazomenos has turned his attention from olive pests (Hungerford *et al.*, 1998, Chem. Commun., 863–864) to the almond seed wasp, *Eurytoma amygdali*, and has discovered a potent male attractant.

The black truffle has delighted gourmets for centuries and has been the source of a considerable body of folklore. The ability of dogs, pigs or insects to locate truffles has also provoked much speculation. Following on earlier work with fungus volatiles (Breheret *et al.*, 1999, Mycologia, 91: 117–120), Thierry Talou has started to give the folklore a scientific explanation. In a study which encompasses plant, insect and mammalian semiochemistry, the key attractant for truffle hunting animals has been identified.

Some years ago, I proposed the first law of semiochemistry. This states that 'our knowledge of the semiochemistry of a species is inversely proportional to its size'! The examples of this law are legion: we know more about the semiochemistry of insects than of

all other organisms put together. On the microscale, yeast pheromones are well understood, whereas mammalian semiochemistry is dominated by examples from mice and rats. Very little is clearly understood about larger mammals, including important domesticated animals such as cats, dogs, cows and horses, although the pig pheromone has been used commercially for several years. Thus far, there is only one exception to the first law: the Asian elephant oestrus signal (Kelly, 1996); however, I am fairly confident that the pheromones of the blue whale, *Balaenoptera musculus*, may not be discovered for some years. More seriously, the semiochemistry of mammals has only really begun to make significant progress in the past 10–15 years, and Raimund Apfelbach has made pioneering contributions (Kirner *et al.*, 1999, Behav. Process., 48: 89–99). In the lecture he will describe a unique study, made with Russian collaborators. The dwarf hamsters *Phodopus campbelli* and *P. sungorus* have sacs at the openings of the cheek pouches, which are used to mark food stored in the pouches. The secretion from these sacs is essential for the development of the young and acts as a marker on food stores. Thus these secretions provide an excellent opportunity to investigate pheromonal materials which are essential for survival of the species. The essential role of odorant binding proteins (OBPs) in mammalian [and insect (Danty *et al.*, 1999, J. Neurosci., 19: 7468–7475)] semiochemistry has emerged over the past 10 years, as a consequence of the extraordinary developments in molecular biology. It is becoming clear that pheromonal OBPs signal their presence by release of a volatile ligand [the pheromone's pheromone (Kelly, 1996)], which attracts a conspecific and promotes sniffing and licking. This causes transfer of the pheromonal OBP to the target organ, which is usually the vomeronasal organ. Aphrodisin is an OBP of the lipocalin family of proteins, which facilitates copulatory behaviour by the male golden hamster, *Mesocricetus auratus*. Jean-Claude Pernollet has achieved heterologous expression and purification of aphrodisin, which should enable 'large-scale' production and a thorough investigation of its properties.

12. Semiochemicals: understanding insect olfactory mechanisms for exploiting semiochemical based strategies in plant and animal defence

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Contrary to widely extant views, olfactory interactions relating to the location of plant and animal hosts are based principally on highly specific olfactory neurons as opposed to generalist molecular receptor systems. This has underpinned the demonstration that host location relies on the detection of specific compounds representative of suitability in host taxa or physiological state, and more recently that morphological features at the peripheral olfactory neural system may allow accurate determination of the relative composition of semiochemical mixtures. Furthermore, it is now evidenced from examples of different insect orders that the avoidance of unsuitable hosts, again by either taxa or physiological state, also involves recognition of specific molecular structures associated with specific olfactory neurons. In addition to taxonomic differences that determine host suitability, the issue of stress causing induction of novel semiochemicals or increased release is now seen to play a prominent role. With this understanding and the ensuing identification of host and non-host semiochemicals has come the greater ability to exploit semiochemically mediated interactions in crop protection. Thus, push–pull or stimulo-deterrent diversionary strategies can be employed in which colonization of plant or animal hosts is reduced by deployment of non-host semiochemicals, while at the same time pests are aggregated onto trap plants or animals where attractant semiochemical release is maximized. Such push–pull strategies can be managed by use of slow-release semiochemical formulations, and considerable success has recently been achieved in subsistence agriculture by use of living plants to produce, directly, the push–pull effects in order to control immigration of insect pests. Although the latter is suitable for low-input sustainable agricultural systems, only by understanding the underpinning neurophysiology and associated chemistry can the approach be made sufficiently robust and sustainable for widespread development.

13. The commercial exploitation of semiochemicals

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The semiochemical industry today has total worldwide sales at the producer level of about US$60–70 million. The majority of this business is split equally between North America (USA, Canada and Mexico), ENAME (Europe, N. Africa and Middle East) and the Far East (Japan, India and China predominantly). Over 60% of the semiochemical market is made up of the sales of about a dozen companies, over half of which are based in North America. It has taken nearly 25 years for this market to grow to its current level, but it still constitutes no more than 1% of the world insecticide market ($7 billion). The sale of traps and lures for monitoring insect pests accounts for nearly half of the semiochemical market, while the bulk of the remaining sales come from mating disruption products for moth pests.

Whereas the expectations for the insect monitoring market were never very great, the market for control products based on semiochemicals was always projected to be great, but very difficult to realize in practice. Many start-up companies have entered the market historically and a number have failed, withdrawn from the market or been sold to other bio-pesticide companies. Those that are active in this market today have had to develop strategies which give the best chance of success given the limitations on the technology while at the same time allowing for the relatively slow pace of development of the market.

Three factors have been paramount in determining the rate of development of the insect control market using semiochemicals: (i) the reliability and robustness of the technology; (ii) its cost-effectiveness; and (iii) the regulatory requirements for semiochemical-based product registrations.

The industry's understanding of the technology has improved greatly over the last 10 years so that the technology's limitations can now be taken into account when applying the products in the field. Area-wide programmes have been the key to the success of the technology in crops such as cotton and apples. Much has been done to improve the synthetic processes for manufacturing thesemiochemical active ingredients, and their formulation for controlled release has moved on greatly. The regulatory authorities in most countries have also taken an enlightened view when it comes to the registration of products based on pheromones; this

has helped greatly in mitigating the registration cost element of what is essentially a species-specific product.

This paper reviews the current trends in both the 'monitoring' and the 'control' market for pheromones and other semiochemicals, and looks at possible future developments which may further influence the growth of this market.

14. Chemical communication of Eurytomidae (Hymenoptera)

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Research on chemical communications in Hymenoptera has focused mainly on the social insects and little information is available for other families. The family Eurytomidae (Hymenoptera) includes many parasitoids and some herbivorous species that attack the seeds of very economically important crops, e.g. almonds, pistacchio, prickly custard apple, alfafa. The herbivores present much interest regarding the chemical communication system employed, since many species reproduce asexually whilst others reproduce sexually. Little knowledge on the chemical communication of these herbivore species is available. Oct-1-en-3-ol, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, (*Z*)-2-hexen-1-ol, (*Z*)-3-hexen-1-yl acetate, (*E*)-β-farnesene and caryophyllene released from the host plants affect host finding and ovipositional behaviour of the two sibling alfalfa and clover seed wasps *Bruchophagus roddi* and *B. gibbus* (Light *et al.*, 1992, J. Chem. Ecol., 18: 333–352). Plant volatiles also affect the oviposition preference of the almond seed wasp *Eurytoma amygdali* (Koulousis and Katsoyannos, 1994, Ent. Exp. Appl., 73: 211–220). Evidence for chemicals cues used for their sexual communication has so far been reported for the species *Bephratelloides pomorum* (Leal *et al.*, 1997, J. Chem. Ecol., 23: 1281–1289) and *E. amygdali* (Katsoyannos *et al.*, 1992, Ent. Exp. Appl., 62: 9–16); however, the sex pheromone compounds were not identified. We report the identification of the sex pheromone compounds of *E. amygdali*. The active compounds were isolated from the crude extract by preparative fractionation of the crude hydrocarbons on a silver nitrate-impregnated silica gel column, which efficiently separates alkanes, alkenes and alkadienes. The greatest male response was elicited by alkadienes and the smallest by alkenes, with the alkane fraction being inactive. The identification of alkenes and alkadienes was based on gas chromatographic, mass spectrometric and gas-phase infrared data. Bioassays suggest that the two alkadienes, (*Z*,*Z*)-6,9-$C_{23:2}$ and (*Z*,*Z*)-6,9-$C_{25:2}$, and to a lesser extent alkenes produced by females, are potent male attractants.

15. Black truffle odor key compounds identification based on truffle hunting animals and human experts' olfactory evaluation

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Black truffles are the fruiting bodies of the hypogeous fungus *Tuber melanosporum* Vitt., which grows in symbiosis with certain trees, especially oaks. They are harvested with pigs or specially trained dogs, or by observing a particular fly (genus *Suillia*) in several parts of Italy, Spain and France. The typical flavor of fresh black truffle is very much appreciated by gourmets, to the extent of being called 'the black diamond' of French cuisine. Despite its economic interest, by the beginning of the last decade, few works had been performed on black truffle aroma volatiles analysis, especially for identification of key flavor compounds. Similarly, the aromatic chemicals responsible for underground truffle localization by truffle hunting animals were unknown. The present paper reports a global approach for identification of fresh black truffle odor key compounds by combining instrumental, behaviour and electrophysiology analysis among human experts from the truffle industry and truffle hunting animals (pigs, trained truffle dogs and *Suillia* species flies).

Chemical identification of key flavor compounds was performed by using gas chromatography combined with dynamic headspace concentration and coupled with mass spectrometry and olfactometry. Analyses were carried out on freshly harvested truffles and the odorous effluent from GC column was assessed by experts according to the olfactory referential 'the field of odours'. Different samples of oil-based mixtures of chemicals identified as the major black truffle volatiles and nature-identical truffle flavouring were buried in truffle field soil for the behaviour study among sows, boars and piglets, trained dogs and three species of *Suillia* flies. Complementary tests with genuine truffle, 5α-androstenol (steroïd identified in truffles) and various volatiles (odorous but not identified in truffles) were performed. The electrophysiological study is based on an electroantennographic recording of sexually mature flies' heads (males and females) in the presence of an odorized airstream from diluted parafin solutions of the single identified truffle volatiles.

More than 50 volatiles components were identified in black truffles (Talou *et al.*, 1989, in G. Charalambous, ed., Flavors and off Flavors '99, pp. 1308–1315) for which less than nine are reported by human experts to be key flavor compounds (Talou, 1992, Doctoral Thesis, INPT). Dimethyl sulfide appeared to be the key odor compound for truffle localization by truffle hunting animals (Talou *et al.*, 1990, Mycol. Res., 94; 277–278) but two other sulfurous, and three C8 compounds were reported to be attractive for truffles flies (Talou, 1992).

16. The secretions of the supplementary sacculi at the openings of cheek pouches of the dwarf hamster *Phodopus campbelli*

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Supplementary sacculi (sacs) at the openings of cheek pouches are a unique structure found only in the two dwarf hamsters *Phodopus campbelli* and *P. sungorus*. Earlier studies have demonstrated the importance of the secretion from these sacs for the survival, normal growth and development during the first month of life of *P. campbelli* (Vasilieva and Feoktistova,1993, Zool. J., 72 (6): 103–113). However, besides being of vital importance for the developing young, other functions of the strong smelling secretion are known (species and individual discrimination). It is known, for instance, that hamsters collect food items in their cheek pouches and transport them to their food stores. While in the cheek

pouches, the food items come into contact with the secretion of the supplementary sacs and become odorously marked; when revisiting the food stores, the marked food might aid as a guiding substance leading the animal to the food store. To check this hypothesis we analysed the possible role of the secretion when animals are searching for food. When exposed simultaneously to unmarked food and to food marked with this secretion, *P. campbelli* prefers scented food. However, animals do not differentiate between food marked by themselves and that marked by other family members, but they clearly differentiate between food marked with the secretion of their own species and food marked with the secretion of *P. sungorus*. The secretion from the supplementary sacs of *P. sungorus* does not elicit food searching behaviour of *P. campbelli*.

Chemical analysis (by headspace–GC/MS) of odorous substances in the secretions of several individuals shows the same qualitative composition; minor interindividual differences occur and might be the basis for individual odour recognition. The odorous substances found are: acetic acid, butyric acid, isobutyric acid, valeric acid and phenol. Subsequent behavioural tests revealed that the pure substances do not elicit searching behaviour; they only seem to be effective when offered as an odour mixture.

17. Comparison of biochemical and ligand-binding properties of natural and recombinant hamster aphrodisin

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Aphrodisin is a soluble glycoprotein of the hamster vaginal discharges, which is involved in pheromonal facilitation of the copulatory behavior in male hamsters via its detection by the vomeronasal organs. It belongs to the lipocalin family and exhibits 40% sequence identity with the rat odorant-binding proteins (OBP-1).

We identified the soluble proteins of the vaginal discharges among which aphrodisin was revealed to be a major protein. Natural aphrodisin was purified by liquid chromatography and characterized by mass spectrometry and Edman sequencing. The presence of three isoforms was proved to be due to the variable glycosylation state, while all shared a pyroglutamic acid at their N-termini. These natural post-translational modifications were located by peptide mapping.

Aphrodisin has been expressed in the yeast *Pichia pastoris*, secreted into the minimal medium using the α-factor pre-prosequence of *Saccharomyces cerevisiae* at a concentration of 230 μg/ml. It was purified by anion-exchange chromatography as two major recombinant isoforms, with a third one as a contaminant. One isoform was unglycosylated and the second was glycosylated solely at site Asn69, whereas the Asn41 was also found to be slightly glycosylated in the third minor isoform. In all isoforms, only 80% of the amino terminus were found to be blocked to Edman sequencing by a pyroglutamic acid residue. Natural and recombinant aphrodisin secondary structures were very close to each other and confirmed that they exhibited the lipocalin features. In addition, they were observed to occur as dimers in natural conditions.

Heterologous expressed aphrodisins efficiently bound odorants such as IBMP (2-isobutyl-3-methoxypyrazine) and MTB (methyl thiobutyrate) and also DMDS (dimethyl disulfide), an attractant pheromone, independently from their glycosylation state.

These observations suggest that aphrodisin could act as a pheromone carrier instead of or in addition to its own pheromonal effect, since MTB and DMDS are natural ligands present in vaginal secretions. The overproduction of recombinant aphrodisin should allow structural and mutational analysis in order to understand the relationships between structure and biological function of this intriguing protein.

The Use of Innovative Sensory Methods

18. Behavioural extensions of preference mapping: the process of synthesis

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Preference mapping techniques are becoming standard tools of analysis in the sensory and consumer science communities. However, a major disadvantage of traditional preference mapping models is that they do not take actual consumer behaviour into consideration. In application, this is reflected in the limited use of elliptical models. It is suggested to tackle unsolved methodological problems in preference mapping by taking account of the behavioural processes underlying preference formation. To this end an information processing model of preference formation is proposed. Specifically, when rating preference, we propose that consumers engage in a sequence of distinct cognitive actions: perception, processing, synthesis, evaluation and scoring.

Synthesis is the stage where consumers relate the sensory information they have perceived and processed to the product they are sampling. Although current applications of external preference mapping assume that consumers and trained judges synthesize sensory stimuli similarly, accumulating evidence in the literature suggests that this is not so. We therefore propose that analysing preference data in terms of a product space that accurately reflects how consumers see products will improve the performance of preference mapping methodology. Differences in synthesis can be accounted for by applying sets of synthesis weights reflecting differences in the relative weighting given to each sensory attribute. For example, consumers may rate all sensory attributes as equally important, or sensory judges and consumers may give different relative weights to sensory attributes.

An empirical analysis of sensory and preference data pertaining to eating apples supports the hypothesis that consumers use only a few key sensory attributes rather than synthesize a large number of attributes during preference formation. In accord with the proposed model, we have demonstrated that consumers synthesize sensory information in a way that allows them to form a simplified overview of product characteristics. To take this work further, the stage of evaluation must be considered next. Then, the model should be validated with data from other product categories. Further, there is a need to improve the way synthesis weights are identified and calculated. The performance of partial least squares regression for this purpose should be considered.

19. Psychophysical methods reveal multiple mechanisms in bitter perception

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People vary widely in their sensitivities to bitter compounds, but the intercorrelation of these sensitivities is unknown. By investigating correlations as a function of individual sensitivities to several bitter compounds representative of different chemical classes, the number and variety of potential bitterness transduction systems can be inferred. To this end, 26 individuals were asked to evaluate 11 different bitter compounds [quinine HCl, caffeine, (–)-epicatechin, tetralone, L-phenylalanine, L-tryptophan, magnesium sulfate, urea, sucrose octaacetate (SOA), denatonium benzoate, and PROP (*n*-propylthiouracil)]. To determine each individual sensitivity, a combination of sensory methodologies (drawn from the latest developments in taste psychophysics and sensory evaluation) were used.

For one portion of the procedure, subjects were asked to rate the bitterness and total intensity on the recently developed LMS scale (Green *et al.*, 1993, Chem. Senses, 18: 683–702) for 10 of the 11 compounds (all but PROP). In a separate phase, subjects were asked to rank nine of the 11 compounds (all but PROP and epicatechin) from weakest to strongest in bitterness on 15 occasions. These rankings were then analysed using *R*-index calculations (O'Mahony, 1992, J. Sens. Studies, 7; 1–47) in order to compare each compound with every other compound. Whether the members of each stimulus pair were significantly different from one another was determined from the table in Bi and O'Mahony (1995, J. Sens. Studies, 10: 341–347). For each stimulus pair that did not show a significant difference, 20 forced-choice paired comparisons were performed in subsequent sessions to further refine each individual's idiosyncratic significant rank order. Finally, subject PROP status was determined in a session in which six levels of PROP, tones and weights were assessed for intensity on the LMS scale.

Using correlation analyses, principal components analyses, and repeated-measures analyses of variance, these data were examined. At least three tight clusters (urea/tryptophan/phenylalanine; quinine/caffeine/SOA; tetralone/denatonium), none of which contained PROP, magnesium sulfate or epicatechin, were revealed. This implies the existence of at least four mechanisms in bitter perception. In addition, when subjects were grouped based upon PROP sensitivity, a significant difference in ratings but not rankings was found. This suggests that there are subjects who vary in their absolute sensitivity to bitter stimuli, but not in their relative sensitivities to these compounds.

20. Learning about chemical senses by sensory techniques to improve sensory methodologies

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Sensory science uses human chemical senses as detectors for measuring perceptible differences between stimuli. The most common stimuli evaluated are consumer goods such as food products and beverages. Sensory practices have been established according to the particularity of those detectors: a substantial inter-individual variability in terms of sensitivity and discrimination ability. Indeed, phenomenon such as adaptation, anosmia or detection threshold have greatly influenced sensory methodologies developed to recruit panel members and train them, as well as the protocols to conduct sensory measurements. Moreover, human beings acquire different olfactory experiences during their lives, which introduces cognition as another source of inter-individual variability.

Sensory science is a young discipline, and scientists still feel the need to improve the methodology and enhance the accuracy of sensory measurements. Some sensory properties, such as astringency, are still difficult to characterize because the perception mechanism is still uncertain. Using sensory techniques, some studies are being undertaken to clarify this mechanism. Investigation of basic tastes and olfactory stimuli is also undertaken to improve the procedure and measure appropriately these stimuli.

When working for a food or beverage company, sensory scientists tend to adapt sensory practices to improve the accuracy of measurements made on their products. Moreover, since sensory science is increasingly used to complement market research, sensory scientists are willing to use sensory techniques to explore consumer preferences and identify the sensory drivers of their likes or dislikes for a particular product.

Examples from the published literature and from the author's work will be presented to illustrate the usefulness of sensory techniques to learn about the chemical senses as well as to improve the methodology and to better understand our consumers..

21. Dynamic methods in sensory and flavour science

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Flavour perception is a dynamic process, which must involve the consumer as well as the chemistry and physics of the food, and must be matched by dynamic research methods. In time–intensity measurement a sensory characteristic is tracked as it changes over a period of time. Availability of volatiles depends not only on their being present in the food, but also on their being released from the food and transferred to the olfactory receptors. Simulated mouths and chewing machines have provided useful data to aid understanding of what happens when food is tasted, and systems have been devised to allow sampling of the headspace from the nose or mouth. Understanding of the interactions of flavour compounds with each other and with other components of the food, and of the fundamental physics of mass transfer, has provided a limited ability to predict the behaviour of flavour compounds in some food systems.

22. The effect of solution viscosity on the release and perception of aromas

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The addition of biopolymer thickening agents to solutions

containing taste and aroma compounds typically results in a reduction in the perceived intensity of one or both attributes. In some instances these changes are the result of direct interactions (binding) between the thickening agent and the taste/aroma compounds. However, there are some systems where no chemical interactions occur, and yet perception is still reduced.

This is thought to arise as a result of entrapment of compounds within the matrix, restricting the movement of molecules, and is highly dependent on the concentration of the thickening agent. The crucial concentration at which changes in perception begin is c^*, the concentration at which coil overlap and entanglement of the polymer start to occur.

Recent developments in instrumental techniques (the MS Nose^{TM}) can be used to measure the rapid changes in breath volatile concentration over the course of a single exhalation (temporal resolution 10 ms). Using the MS Nose, we have been able to determine the effect of biopolymer concentration on aroma compounds, as thickened solutions are consumed.

Results will be presented to show the effect of the biopolymer, hydroxypropylmethylcellulose (HPMC), on the perception of aroma compounds and the corresponding breath aroma concentration following consumption.

23. Retronasal aroma simulator (RAS), a useful sensory tool

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Mixtures of aroma compounds in the vapour phase of foods that approach the olfactory epithelium through the nostrils (orthonasal route) have different compositions than mixtures that enter from the mouth and respiratory system (retronasal route), thus imparting different perceptions. The perceptions from the retronasal route are associated more strongly with the flavour experienced during consumption. Although recent work has shown that the perception of compounds administered orthonasally can be equivalent to compounds administered retronasally, these two routes generally produce different perceptions and the retronasal route is generally believed to determine food preferences (Pierce and Halpern, 1996, Chem. Senses, 21: 529–543; Wininger and Halpern, 1999, Chem. Senses, 24: 600).

A retronasal aroma simulator (RAS) has been developed to mimic mouth conditions in terms of temperature, salivary environment and shearing, as well as the non-equilibrium gas flow conditions inherent in eating and drinking. The device is composed of a 1 l stainless steel blender container, a high torque variable speed motor, a water jacket, and a regulated gas inlet and outlet (Roberts and Acree, 1995, J. Agric. Food Chem., 43: 2179–2186). Closely correlated to the *in vivo* conditions of eating with respect to stimulus ratios (CV < 1%), the RAS has the advantages of reproducibility, sensitivity, control over physical parameters, compatibility with chromatography and (theoretically) predicts aroma better. Flavour released from food in a RAS can be extracted using solid phase microextraction (SPME) or porous polymers and analysed by gas chromatography mass spectrometry (GC-MS), gas chromatography olfactometry (GC/O) or direct sniffing by a human. Using the RAS averages the large variation in mouth dimensions and eating methods common to *in vivo* techniques but is unable to record the dynamics of individual experiences.

Threshold values derived from sniffing the RAS effluent can be used to account for some food matrix and mouth interactions. These thresholds can further be applied to determine what odourants in a mixture are potent enough to impart flavour. Used to calculate odour activity values, these thresholds remove the effects of taste sensations on aroma perceptions (Ong *et al.*, 1998, J. Agric. Food Chem., 46: 611–615; Ong and Acree, 1999, J. Agric. Food Chem., 47: 665–670). The RAS is a flexible tool applicable to many sensory investigations.

24. Aroma compound release during consumption: similarities and differences between consumers

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A food aroma stimulus could be defined as the quantity and balance of aroma compounds which are released from the food during consumption and are available for perception. The aroma stimulus of any individual consumer, which is composed during consumption of a food, is determined by physical interaction between the consumer and the food. It has been suggested that individual aroma stimuli only differ quantitatively, and that differences between consumers could be measured using an Aroma Stimulus Index (ASI), regardless of food type (Delahunty and Guilfoyle, 2000, in Proceedings of the Annual Meeting of the American Chemical Society, New Orleans, August 1999, in press). This theory also suggested that a heterogeneous population of consumers could be classified into groups with similar aroma stimuli by their ASI, which could facilitate improved food formulation.

The rate of aroma release is controlled by the mechanisms of mass transfer and partition of volatile compounds into the gaseous phase. During consumption these mechanisms are driven by the energy and mixing imparted by mastication, the solvent strength of saliva and the renewal of headspace within the buccal cavity by breathing rate and volume. On the other hand, not all of any volatile compound found in the food, or potentially generated during consumption, will be released. The physical and chemical properties of the volatile compound, e.g. its structure, shape, hydrophobicity, will determine the nature of rate limiting interactions between it and other compositional components of the food, e.g. carbohydrate, lipid, protein. In addition, the rate of aroma release will depend on whether the food is a liquid or a solid, an emulsification or homogenate, and whether specific aroma volatiles have been protected by encapsulation. To determine the validity of ASI theory, effects of consumer in-mouth physiology on release of specific aroma volatiles from food systems, representing typical composition and phase state, was evaluated. Important differences and similarities between consumers, and how these determine aroma release, were identified. This work introduces a novel approach to understanding aroma perception.

Insects

25. The search for biting sites by tsetse flies: an interplay of the senses

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By their bite, blood-feeding tsetse flies (*Glossina* spp.) can infect a host with trypanosomes—protozoa that cause sleeping sickness in human beings and a disease called nagana in cattle—if they have bitten an infected host before. As a complement to insecticide spraying, targets are being developed in Zimbabwe for the control of tsetse flies [Targets were developed within the framework of the RTTCP (Regional Tsetse and Trypanosomiasis Control Programme). Our field experiments on the effect of uric acid, as described below, were performed at the Rekomitjie Research Station in Mana Pools Natl Park, Zimbabwe, and I gratefully acknowledge the help of my host there, Dr G.A. Vale, and his co-workers.]. These resemble blue–black–blue banners mounted on frames 170 cm wide and 100 cm high. The black center field is treated with insecticide. Attracted from up to 90 m by odour baits, the flies come to the targets and usually land on the black material where they take up a dose of insecticide upon contact. With the aim of prolonging the flies' contact with the insecticide-impregnated cloth, we investigate the skin surface factors that induce the flies to bite.

Though some of the taste hairs on the legs are presumed to function in mate recognition, spike trains to a stimulus of human sweat can be recorded from receptors in two sensilla proximal to the base of the empodium at the distal end of the fifth tarsomeres (Van der Goes van Naters and Rinkes, 1993, Chem.Senses, 18: 437–444). Further investigation revealed that of 14 major solutes in human sweat tested, leucine, valine, lactic acid and uric acid elicit action potential responses while creatinine, lysine, histidine, threonine, arginine, glucose, acetic acid, urea, ammonia and sodium chloride do not. Whereas the effective amino acids (Van der Goes van Naters and Den Otter, 1998, Physiol. Entomol., 23: 278–284) elicit responses from a single cell in each hair, uric acid stimulates two or more, as does sweat itself.

In a small arena in the laboratory, flies showed more biting behaviour, i.e. they probed longer, on a warmed paper treated with 0.4 nmol/cm^2 of uric acid than on warmed control papers and this difference was removed by chemical ablation of the taste receptors. Already in 1954, Dethier (Am. J. Trop. Med. Hyg., 3: 160–171) demonstrated that warmth from the surface alone could induce tsetse flies to bite once they alighted, but the effect of uric acid is evident only in combination with heat (Van der Goes van Naters *et al.*, 1998, Physiol. Entomol., 23: 285–288), though preliminary investigations show that host odours can substitute for heat. Analysis of bouting sequences showed that uric acid does not affect the frequency of probing bouts but nearly doubles their duration. Applied to the targets in Zimbabwe, uric acid slowed the rate at which flies left the target surface within the first few seconds after landing but less so thereafter. Uric acid appears to be a good candidate for prolonging the contact times, possibly allowing a decrease of insecticide-density on the target surface without impairing the targets' effectiveness.

26. Chemical lexicon for olfactory communication in the Colorado potato beetle

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Communication in insects is extremely important for species propagation and utilization of available resources. Chemical messages are especially critical for specialized feeders such as the Colorado potato beetle (CPB), in which both immature and adult forms exist on the same host plant. Reception of chemical signals emitted by conspecifics and their hosts, and coding of textual differences in them, requires receptors that are uniquely sensitive and exquisitely tuned to individual components. Both temporal and qualitative differences in semiochemical blends that comprise the chemical lexicon of the species must be detected by peripheral sensory neurons and deciphered by the central nervous system. Recently, progress was made in understanding the chemical lexicon of the CPB as blends of chemicals emitted by potato plants were identified that were attractive to adult insects (Dickens, 1999, Agric. Forest Entomol., 1: 47–54; Dickens, 2000, J. Chem. Ecol., in press; Dickens 2000, Agric. Forest Entomol., 2, in press). Nevertheless, the occurrence of chemical communication among developmental stages of the CPB and the nature of this lexicon remained unknown. Now we have determined chemical signals emitted during larval and adult feeding on potato plants and revealed textual differences among them. Detection of chemical blends characteristic of immature and adult stages of CPB may result in attraction or avoidance of occupied host plants. The text of these messages is composed of at least nine chemicals emitted as different blends during insect feeding. Behaviours elicited by the chemical messages depend on the context in which the blends are encountered and may be modified by previous experiences. Thus, a chemical lexicon is utilized by CPB for intraspecific communication and it seems likely that different lexicons are used by other species where larval and adult forms compete for the same resource. The expanded lexicon inherent in these intraspecific 'chemical conversations' demands a corresponding enhancement of receptor types, plasticity of the central nervous system and a diversity of behaviours in response to overlapping syntax of the blends.

27. Odour-guided host finding of yellow fever mosquitoes: composition of the attractive blend and flight behaviour in attractive odour plumes

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We studied basic principles of olfaction in mosquito–host interactions using the yellow fever mosquito *Aedes aegypti*, a diurnal species that is anthropophilic and occurs primarily in the urban environment. In olfactometer bioassays we investigated which compounds of the complex blend of human emanations play a role in attraction toward hosts and which factors make some

hosts more attractive than others. We found that, besides L-(+)-lactic acid and carbon dioxide, ammonia as well as distinct short chain fatty acids contribute to the attractive effect of host emanations. The key compound and distinctive mark of the human scent is lactic acid. This compound is produced by eccrine sweat glands, which are most extensively developed in human beings. The other components, however, contribute also to the highly attractive effect of a synthetic mixture when presented in a distinct blend-specific ratio. By comparing hands of different humans in direct choice tests we verified the common experience that certain individuals are more attractive than others to mosquitoes. The addition of lactic acid to less attractive people significantly increased their attractiveness and changed the mosquitoes' preference. The importance of lactic acid was also confirmed in experiments with odour samples from different animals. These samples did not contain significant amounts of lactic acid and elicited only weak behavioural responses. The attractiveness of these animal odours, however, increased enormously when lactic acid was added. These findings emphasize the key role of lactic acid, which is only a weak attractant by itself but is an essential element in the pattern of kairomones emitted from human hosts.

28. Odorant reception in phytophagous and haematophagous insects

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A greater diversity of odorant-binding proteins (OBPs) than previously known, together with a complex expression pattern in subsets of chemosensory sensilla, support a model in which OBPs selectively transport and present odorants to membrane-bound olfactory receptors.

OBP-related proteins have been identified in several insect orders, but not in haematophagous species. In moths, to date, five binding protein classes (pheromone-binding proteins, general odorant-binding protein classes 1 and 2, antennal binding protein X and OS-D like proteins) have been described (Vogt *et al.*, 1999, Chem. Senses, 24: 481–495). This classification is based on sequence homology, but lacks functional studies. We focus on searching OBPs which are present in minor amounts and have been overlooked so far, and on correlating a given OBP with a specific function by applying odorant-binding assays and immunolocalization studies. We recently identified three different pheromone-binding proteins (PBPs) in the silkmoth species *Antheraea polyphemus* and *A. pernyi*, in agreement with three sex pheromones and three types of pheromone-receptor cells present in these species (Maida *et al.*, 2000, Eur. J. Biochem., 267: 2899–2908). Thus, the number and diversity of PBPs is increasing, in contrast to the two classes of general odorant-binding proteins, where only a single protein has been discovered in all species studied so far. This makes it likely that each pheromone uses its 'own' PBP and a different signal transduction pathway than general odorants.

In pheromone-sensitive sensilla, we have identified and immunolocalized several components of the IP_3-transduction pathway, like a G protein belonging to the $G_{q/11}$ family and a phospholipase Cβ, and found a pheromone-induced increase in protein kinase C activity (Maida *et al.*, 2000, NeuroReport, 11: 1773–1776). While in vertebrates some odorants lead to a transient increase in IP_3 and others lead to an increase in cAMP, the transduction pathway of general odorants in insects is still unknown.

The amino acid sequences of OBPs are so poorly conserved among different insect species that a search for OBP genes in the blood-sucking malaria mosquito *Anopheles gambiae* based on sequence similarity might fail or result in a limited OBP number. Thus, we purified OBP candidates from female head appendices and used the N-terminal sequence for designing synthetic peptides to be used for antisera production. The goal is to identify OBPs involved in the detection of human body smell by comparing male and female mosquitoes, applying odor binding assays and correlating the presence of a given OBP with the responsiveness of the sensillum.

29. Insects as specific and programmable odour sensors

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Compared with man-made physicochemical sensors, such as gas chromatographs and electronic noses, the olfactory apparatus of insects exhibits a much higher sensitivity and specificity for biologically significant volatiles. The advantages of gas chromatographs and electronic noses compared with insect preparations are evident; nevertheless, the application of the insect's olfactory apparatus for detection of minute amounts of biologically active volatiles still remains indispensable. Recent developments and applications are presented.

Generally, the output signals of insect olfactory receptors are recorded on three different levels: (i) the primary olfactory neurons (single sensillum recording, SSR); (ii) the peripheral olfactory system as a whole (electroantennography, EAG); and (iii) the whole insect (recording of behavioural responses). Application of one or more of these methods is dictated mainly by the accessibility of the olfactory apparatus and practical and scientific requirements.

Being relatively easy and versatile technique, EAG alone or in combination with gas chromatography (GC-EAD) is widely used for detection of pheromones and other behaviourally active volatiles. EAG and SSR techniques have long been restricted to laboratory applications. Recently, modification of the recording technique facilitated the development of portable EAG sensors, which are used to measure specific volatiles in greenhouses, wind tunnels and the open field.

A completely different sensor system based on the detection of delicate movements in response to odours has recently been tested as an alternative or addition to EAG and SSR methods. In this system the insect is freely exposed to the surrounding air or contained in a small glass or plastic capsule which is continuously flushed with air from the test source or environment. Movements of the head and antennae are detected by a miniature Doppler radar detector, the output signal of which is processed and recorded. The signal contains both information on the intensity and the time course of the response, and can be calibrated by simultaneous visual observation or video recordings of the movements. In contrast to EAG and SSR, the output of this 'actographic sensor' reflects information that is processed by the insect's brain. This enables the sensor to be programmed, because many insects can be trained to respond to specific odors.

Applications in the laboratory and in the open field will be discussed.

30. Odor coding in *Drosophila* melanogaster: physiology and genetics of receptor neurons

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The encoding of chemical information by sensory neurons is still poorly understood. We do not fully understand how odorants are translated into neural signals at the interface between olfactory receptor neurons (ORNs) and the environment. How specific is this process and what are the mechanisms of ligand–receptor interaction? Furthermore, it has proven difficult to determine the number of physiologically different ORN types needed to encode all relevant odor information. We are characterizing the response patterns of ORNs of *Drosophila melanogaster* to address these issues. This insect has a relatively low number of ORNs distributed over the antenna (~1200) and maxillary palp (~120). In addition, a family of ~100 putative odor receptor genes, the DOR genes, was recently isolated from its genome (Clyne *et al.*, 1999, Neuron, 22: 327–338; Vosshall *et al.*, 1999, Cell, 96: 725–736).

Extensive recordings from ORNs in basiconic sensilla of both appendages rendered at least 22 classes of neurons with different response spectra (de Bruyne *et al.*, 1999, J. Neurosci., 19: 4520–4532; de Bruyne *et al.*, 2000, in preparation). They respond mainly to simple esters, alcohols and ketones, but also to aromatics, E2-hexenal and CO_2. Many of these chemicals occur in fermenting fruit, a natural source of food for *Drosophila*. Dose–response curves are sigmoid, with high sensitivity to only one tested compound. ORNs differ not only in response spectra and sensitivity but also in response characteristics. Stimulation can result in inhibition or excitation with distinctly different temporal aspects. ORNs on the antenna are distributed in different functional types of sensilla that are intermingled but occur in restricted patterns. The distribution of one sensillum type resembles the expression pattern of one particular DOR gene.

We showed previously that acj6, a POU domain transcription factor, plays a role in specifying neuronal identity of ORNs in palpal sensilla (Clyne *et al.*, 1999, Neuron, 22: 339–347). Another mutation that affects responses to a subset of odorants is Scutoid (Dubin *et al.*, 1995, J. Neurobiol., 28: 214–233). It causes misexpression of the zinc-finger protein snail in a small section of the developing antenna (Fuse *et al.*, 1999, Devl Gen. Evol., 209: 573–580). We demonstrate that this is related to structural changes in a particular zone of the antenna and affects a subset of ORNs on antenna. It is likely that development of the *Drosophila* olfactory system employs multiple genetic pathways. Characterization of all ORN classes, combined with genetic and molecular techniques, may give us an unprecedented understanding of the coding properties of this fly's nose.

Memory

31. Olfactory cognition and emotion

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Research conducted in my laboratory on the role of emotion in olfactory cognition and its consequences on behavior will be discussed. To examine how odor-evoked memories differ from memories evoked by other cues, a number of cross-modal experiments were conducted where stimuli presented in different sensory modalities were tested for their ability to induce emotion and influence memory accuracy and vividness. Results showed that for both laboratory manipulations and autobiographical memory investigations, memories evoked by odors were significantly more emotional, as measured both by written reports and heart-rate changes, than memories evoked by any other sensory stimulus tested (verbal, visual, tactile, music). However, memory accuracy and vividness were never affected by the type of sensory stimulus evoking recall (Herz and Cupchik, 1995, Chem. Senses, 20: 517–528; Herz, 1998, Ann. NY Acad. Sci., 855: 670–674; Herz and Schooler, under review). To examine how emotion may be involved in the formation of odor associations, experiments were conducted in which subjects were induced into a heightened mood state or were in a normal mood during encoding of a list of words in the presence of an ambient odor. Results showed that odors become better memory cues (subjects remembered more words) if encoding with an ambient odor took place while subjects were in an emotionally heightened state (i.e. anxiety) compared with a neural mood (Herz, 1997, Am. J. Psychol., 110: 489–505). Thus, in addition to being the distinguishing feature of odor-evoked recall, emotion is a key aspect in the formation of odor-associated memories. Most recently, we have tested the effects of emotional associative learning with odors and thus whether odors can be conditioned to influence behavior. In this study, we induced the mood of failure-frustration in children with a task that was impossible to solve. All children performed the task while exposed to an ambient odor. Twenty minutes later subjects were exposed to either the same odor, a different odor or no odor, and were given a different task to complete. Results showed that exposure to the same odor led to worse performance on the test task than exposure to either a novel odor or no odor (Epple and Herz, 1999, Devl Psychobiol., 35: 103–107). This indicates that odors can become conditioned to specific emotions and that subsequent exposure to such odors influences behavior in a manner consistent with the associated emotion. In sum, these studies behaviorally demonstrate that emotion is a critical and distinguishing factor in many aspects of olfactory cognition.

32. The capacity of humans to analyse odor mixtures and taste mixtures is limited by working memory

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Studies during the past decade have shown that humans have a limited ability to identify the components of odor mixtures, the limit being 3–4 (Laing and Francis, 1989, Physiol. Behav., 62: 193–197). Neither extensive training, experience nor the type of odorant improved this limit (Livermore and Laing, 1996, J. Exp. Psychol.: Human Percept. Perform., 22: 267–277; Livermore and Laing, 1998, Percept. Psychophys., 60: 650–661). Investigation of the temporal processing of odor mixtures indicated that the major factor limiting analysis is working memory (Jinks and Laing, 1999, Cogn. Brain Res., 8: 311–325). As has been reported in

non-chemosensory studies, working memory has a very small capacity. Recently we used a selective attention technique to determine the capacity of humans to analyse taste mixtures consisting of 1–5 tastants, namely, sucrose, sodium chloride, citric acid, caffeine and inositol monophosphate. The results indicated that a dramatic reduction in successful identifications occurred with ternary mixtures and only one tastant was identified above chance level in five-component mixtures. Thus, in the analysis of odor and taste mixtures it is proposed that the limitation is memory-based, with the capacity of working memory the limiting factor. This outcome has ramifications for studies of suppression in odor mixtures and taste mixtures consisting of three or more components since failure to identify an odorant because of memory limitations could be incorrectly construed as strong suppression of a component. Another interesting implication of these studies relates to the question of what can be perceived during an eating episode when both odor and taste stimuli are released into the mouth and nose in a very short space of time. If working memory for the chemical senses operates as a unitary entity it could be expected that the limit will remain as about three identifiable components. This question will be discussed in the context of our current work on the analysis of odor–taste mixtures.

33. Unconscious, perceptual-cognitive memory in olfaction

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We shall deal with potential candidates of short-term perceptual memory mechanisms in olfaction. We shall also touch on the problem of whether or not they have to be always of a conscious nature. Two experimental phenomena are being discussed. First, it is known that, when measuring ascending and descending limit thresholds for odors, one finds that the former are lower than the latter. Our experiments have not supported the hypothesis that this was due to sensory adaptation induced by preceding strong stimuli. The essence of the above difference is probably the specific reduction of the thresholds during ascending limit measurement (threshold tuning). In this case very weak odors of gradually increasing concentrations, commencing with subthreshold ones, are presented until the subjects safely detect them according to a statistical criterion. It is worth emphasizing that those stimuli are not being compared with blanks during threshold limit measurement. When weak odors were related to blanks (zero references) in each trial, using an ascending forced choice threshold measurement technique, 'threshold tuning' disappeared and the measured detection threshold was higher than that estimated by the limit method. It is probable that, during 'threshold tuning', the neural traces of those weak odors, though still not reaching the level of conscious experience, are being compared with those of the preceding stimuli. The design of ascending limit measurement resembles tracing of very weak odors along the gradient of their gradually increasing concentration, taking place under ecological conditions. Serial comparison of those traces, which have been detected by the olfactory system without of the subjects being aware, and which had to be transitorily stored by some perceptual memory mechanism, could be the process underlying 'threshold tuning'. Secondly, the olfactory system seems to be unable to detect which nostril is being stimulated. However, when the intensity of stimuli addressed to one nostril is different from the intensity coming from the opposite nostril (e.g. the odor at the right side is always stronger than that at the left side), the majority of subjects can learn to use this perceptual-cognitive clue for lateralization (see Radil and Wysocki, abstract 258). Some subjects did improve lateralization performance during learning, before they became aware of the principle of the relationship between odor intensity and laterality, i.e. 'implicit learning of the intensity clue for lateralization' took place. Thus some sort of non-explicit (implicit) learning process, requiring a perceptual memory mechanism for storing odor intensities in subsequent trials, and certain neural information on the side of stimulation (which does not seem to be utilized under usual conditions), appear to be involved in these processes.

34. Retroactive and proactive inhibition in implicit odor memory

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Strong proactive and weak retroactive inhibition have been invoked as an explanation for the longevity of sensory odor memory as measured by explicit and non-verbal methods such as odor recognition (Lawless and Engen, 1977, J. Exp. Psychol. Human Learn. Mem., 3: 52–59; Herz and Engen, 1996, Psychom. Bull. Rev., 3: 300–313). It has been shown that such explicit methods of retrieval do profit from verbal identification of the odors concerned (Rabin and Cain, 1984, J. Exp. Psychol. Learn. Mem. Cogn., 10: 316–325). In a recent investigation on implicit odor memory (Degel and Köster, 1999, Chem. Senses, 24: 317–325) it has been shown that verbal identification interferes with implicit memory processes. This and some other characteristics indicate that memory processes are not the same in implicit and explicit memory. Therefore, the effects of retroactive and proactive inhibition on implicit memory have been checked in an extensive study with two odors (lavender and orange) and a non-odorous control, and with 307 subjects that in a double-blind procedure were unknowingly exposed to one of 12 different conditions in which they worked under either two different odor–context combinations or an odor–context and a non-odor–context combination. They were later tested on their memory for these expositions in an implicit way. By comparison of the results of these groups the different inhibition effects could be measured. The results showed that indeed in implicit memory the proactive inhibition effects are stronger than the retroactive effects. Nevertheless, retroactive effects are not absent as some authors have supposed on the basis of explicit odor memory experiments. Odor identification plays again an important role in these effects. In the discussion attention will also be given to the relative external validity of implicit and explicit odor memory experiments for real life situations. Here also, the effects of odor identification on olfactory memory will be reviewed.

35. Odor perception is effected by memory for past experiences

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Many studies suggest that perceptual learning plays an important role in olfaction. A cross-cultural study has demonstrated the everyday experience influences on judgement of pleasantness, familiarity and even perceived intensity of odors (Ayabe-Kanamura *et al.*, 1998, Chem. Senses, 23: 31–38; Distel *et al.*, 1999, Chem. Senses, 24: 191–199). These results indicate the influence of memory through experiences on odor perception. In the present study, we investigated the effect of memory under an experimental condition in everyday life on odor perception, such as detection thresholds, intensity and hedonic judgements.

The evaluations of ~12 Japanese women aged 30–40 years to the odor of aniseed oil were compared before and after an experience period in which they had anise tea everyday for a month. They judged perceived intensity, familiarity and pleasantness of anise odor. As a control condition, other odors were also tested before and after an experience period of anise tea. Furthermore, the detection thresholds of anethol, which is a main component of anise odor, were measured. As a control condition, the detection thresholds of anethol were measured in 12 other women who had not drunk anise tea.

Before regularly drinking anise tea, no participant could identify the odor of anise and know anise itself. At the time, they judged the odor of anise to be weaker and less familiar, and showed neutral hedonicity. After 1 month of experiencing anise tea, they became able to correctly identify the odor and then judged it to be stronger, more familiar and pleasant. Their detection thresholds of anethol were not affected by the experience of anise tea in this study. These results showed that odor perception with relatively higher cognitive processing might be easily affected by memory for past experiences. However, the more sensory aspect, e.g. threshold, may be robust for the influence of memory for experiences.

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36. Odor learning and memory in aging and dementia

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Very little information is available regarding odor learning and memory in aging populations. We investigated a broad range of memory functions, including recall, recognition and identification, in both normal aging and age-related neurodegenerative disease.

In a life-span study, healthy elderly were compared with healthy young adults on both the California Verbal Learning Test and an analogous odor test, the California Odor Learning Test. The results showed that both immediate and delayed recall of odors, both free and semantically cued, as well as the ability to learn across trials, is impaired in normal aging, perhaps more so for olfaction than for audition. Poor use of semantic-clustering strategies and poor identification impact performance in the elderly was also shown. To investigate odor memory in Alzheimer's disease (AD), we compared patients with probable AD; with people positive for the Apolipoprotein E-4 allele (APOE4), and thus, genetically at risk for the disease; as well as with normal elderly controls. The results suggest that AD patients demonstrate impairment in (i) recall and learning of the odor task across trials (essentially no learning), irrespective of whether the task was immediate or delayed or whether it was free or cued; (ii) recognition memory; and (iii) identification. In those at risk for AD because of the APOE4 allele there was a marked impairment in the ability to learn across trials, relative to controls. However, the magnitude of this impairment was less than that seen in AD patients, and in contrast to AD patients, those with the APOE4 allele did show some evidence of learning over trials.

The results suggest a decline in several aspects of memory in normal aging; dramatic loss of odor memory function in AD; and in people who are at genetic risk for AD, impairment in odor learning and free recall that is of a lesser degree, but similar to, the impairment seen in AD. The results suggest that odor memory measures are sensitive to both normal aging and to incipient dementia in patients with neurodegenerative disease.

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Smell and Taste in Context—arranged by the Consumer and Sensory Research Group of SCI, London, UK

37. The interaction of odour with visual cues on brain activity

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The sense of smell is known to be emotional and evocative. In our attempts to understand better the processes involved in smelling, we have been particularly interested in non-verbal methods. One of these approaches is the brain scanning technique of following patterns of electrical activity on the cortex. A variety of experimental paradigms have been used to achieve this. We have recently been using EEG recordings to measure the effect of one sensory stimulus on another. This has exciting potential in the measurement of responses to fragrances because the olfactory stimulus is rarely perceived as an isolated modality. For instance, the fragrance of a shower gel is perceived within the context of the visual, auditory, tactile and somaesthetic experiences of showering. The total experience can change as the context of the olfactory stimulation changes.

We have used the specific technique of event-related potentials (ERP) to measure the priming effects of odour on the assessment of visual stimuli. We have been particularly interested in the role of imagery. The results address the issue of whether it is possible to distinguish between combinations of odours and pictures which do or do not fit well together.

38. The meaning of fragrances and flavours to consumers

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When consumers purchase cosmetics and, indeed, foods and drinks, they are influenced by a whole range of factors over and above the direct sensory and functional aspects on which such products are usually assessed. Imagery, attitudinal, aspirational and emotive factors come into play, often dictating in the end whether such products are purchased.

As the only way we can interact with any product is via the senses (visual, olfactory, gustatory and tactile), sensory properties must be the root stimulus for these more nebulous aspects of perception. There must be links in people's minds between the sensory perceptions and these more emotive factors. If we can understand these links, then cosmetics, foods and drinks can be designed to maximize the positive aspects of the subtler emotive factors as well as the more obvious sensory aspects.

The paper explores some of the more emotive factors associated with perfume use and shows how these relate to sensory stimuli. Examples are given of how such information can be used to design a fragrance for specific occasions or better to satisfy personal aspirations.

39. Consumers' preferences for sucrose stimulation in the context of 'sugar' concepts

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Sweetness is not just a sensory quality. It is also a social construct. The taste of sugar results from stimulation by sucrose but the gustatory afferent transmission to the brain also conveys meaning. It evokes the perception that there is sugar in the cup of tea or coffee. The stimulation from the various sugars in ripe fruit or fruit juice is expected. In both these cases, the concept of 'sugar' implies to many people the presence also of 'calories'. To different people in different contexts, gustatory perception of the presence of a lot of sugar (i.e. very sweet) is attractive not only because of an inborn sensual pleasure but also because hunger is satisfied or physical or mental energy is refreshed. To others, a strong sweet taste means risk to the teeth or the waistline. If, however, the sweet taste is perceived as coming from a low-calorie sweetener, neither that satisfaction nor the health risk is expected.

This is not mere literary speculation: they are findings of cognitive psychology that can be supported by cortical ERPs. Discrimination scaling of an individual's data in a preference test can distinguish among sensory processing, descriptive concepts and experienced images and pleasures. For example, sucrose concentration in a fruit drink was varied independently of switching between the labels 'sugar' and 'low-calorie sweetener'. Some of those consumers who liked lots of sugar in taste and for hunger treated the ideas of sweetness and calories as identical. Others treated sweetness and supposedly low-calorie sweetener as quite separate influences on their preference, including some who liked high sugar levels but zero or negative calories.

40. Taste and flavour in product development

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The evolution of product development in the food industry over the last 50 years can be split into three phases. During the first, lasting until the mid-1960s, the sensory characteristics of new products were determined by managers or company 'experts', with little input from target consumers. Although by the 1970s many companies had moved away from this approach, there are still companies developing products this way and paying only lip-service to taking customer views into account when defining the tastes and aromas of products.

During the second phase, the focus moved to satisfying customer needs and wants. Although this was something that good business people had always understood, it was now being taught in marketing courses and applied more widely. As a consequence, product development moved to centre stage in many companies' marketing departments. New marketing research methods were developed, aimed at better understanding consumer needs and at segmenting consumers into groups with similar patterns of preference.

This focus on new product development was very successful in growing markets. However, by the late 1980s, as expansion in demand slowed and competition increased, a third phase began and continues today. Now it is no longer enough simply to take into account consumer input in product development. The objective must be to be better than the competition at doing it. This means constant improvement of both product and service quality, thereby increasing the perceived value of the brand and hence retaining satisfied customers.

One consequence of this evolution is that product developers now pay much closer attention to results and ideas from academic research. This is particularly so when research leads to better understanding of the physiology of pleasing characteristics of foods and of the sensory drivers of long-term preference.

Practical examples of the evolution in product development described above and of some new developments serve to illustrate the effects of context and expectation on flavour preferences in product perception.

Molecular Biology II

41. Cloning, functional expression and characterization of human odorant receptors

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Olfactory receptors constitute a large family of G protein coupled receptors that are expressed in the olfactory receptor neurons in the nose. They are believed to be activated by odor molecules acting as ligands, as the first step of signal transduction in the olfactory pathway leading to the detection and discrimination of odors and the perception of smell. To address the problem of olfactory perception at a molecular level, we have recently cloned, functionally expressed and characterized the first human odorant receptor, OR 17–40, one member of the cluster of odorant receptor genes located at chromosome 17.

Application of a mixture of hundered different odorants elicited

a transient increase in intracellular calcium at human embryonic kidney (HEK) 293 cells which were transfected with a plasmid containing the receptor encoding DNA and a membrane import sequence. By subdividing the odorant mixture in smaller groups we could identify a single component which represented the only effective substance: helional. Testing some structurally closely related molecules we found one other compound which also could activate the receptor: heliotropyl acetone. All other compounds tested were ineffective as agonists.

Using the same experimental procedure, we functionally expressed two further human odorant receptors, OR17-p110 and OR17-p44, coded by genes located in the same cluster on chromosome 17. The sequence of the odorant receptors showed 88% and 91% of homology to OR17–40, respectively. Application of a mixture of different odorants led to the identification of specific ligands also for each of these two receptors. We found cyclamenaldehyde activating the OR17–4, whereas 2-methyl undecanal (aldehyde C12 MNA) was an agonist at the OR17–228. Comparing the amino acid sequences of the homologous odorant receptors in both, conserved and variable regions, with spatial and structural information of ligands could help improving molecular understanding of odorant receptor specificity and odorant recognition in humans.

42. A P-element induced mutation identifies a sweet taste receptor gene in *Drosophila*

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Sweet taste is believed to be mediated by specific receptors. Sugar-binding proteins and various G protein coupled receptor genes have been reported to be expressed in the gustatory tissues, but the functional characterization of these candidate proteins as sweet taste receptors remains to be shown. In *Drosophila*, a spontaneous dominant mutation of a taste gene *Tre* is known to reduce taste sensitivity to a disaccharide trehalose (Tanimura *et al.*, 1982, J. Comp. Physiol., 147: 433–437; Tanimura *et al.*, 1988, Genetics, 119: 399–406; Ozaki *et al.*, 1995, Neurogenetics, 10: 42–43). In order to obtain molecular information of the gene, we carried out P-element-mediated mutagenesis and characterized the gene and the mutations molecularly and physiologically.

Since *Tre* has been mapped between 5A10 to 5B1–2 on the salivary X chromosome, several single P-inserts near the location were used to induce small deletions through the imprecise excision/repair mechanism by crossing them to a transposase source. Progenies were then subjected to a two-choice feeding test (Tanimura *et al.*, 1982). One of the insertions produced progenies that showed significantly low sensitivity to trehalose. A total of 22 independent mutations were thus recovered from ~6000 F2s screened. A complementation test showed that they are in fact allelic to *Tre*.

The genomic DNAs of the mutants were then analyzed and the mutations were identified as deletions in the flanking genome near the P insertion site. The deletions were found to uncover a G protein-coupled receptor gene that belongs to the group 1 family, which includes rhodopsin/odorant receptor/bitter taste receptors. The gene organization, the deduced amino residue sequences, the expression of mRNA of wildtype flies, spontaneous and P-excised mutants were analyzed and compared. From the database two phylogenetically related genes homologous to *Tre* were also identified in *Drosophila*. Behavioral and physiological changes were investigated using the null mutants. The electrophysiological analysis of the labellar chemosensory hairs showed a specific decrease of the sugar sensitivity of the sensory neurons. We therefore concluded that *Tre* encodes a gustatory receptor for sweet taste perception in *Drosophila*.

43. Molecular and genetic identification of a sweet taste receptor gene for trehalose in *Drosophila*

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Taste receptors are thought to belong to the superfamily of G protein-coupled receptors (GPCRs). Several candidate taste receptor genes encoding GPCRs have been reported, but their function remains unsolved. In *Drosophila*, taste sensilla are present on the labellum, tarsi and wing margins. In a typical L-typed chemosensillum on the labellum there are four taste sensory cells each of which responds to either water, salt or sugar. Previous studies showed that there are at least three separate receptor sites for sugars in the sugar receptor cell of *Drosophila* (Tanimura *et al.*, 1982, J. Comp. Physiol., 147: 433–437). The *Tre* gene was first identified through studies on natural variants. Since the *Tre* gene controls taste sensitivity to trehalose without affecting the responses to other sugars, the gene product of *Tre* should function in sugar receptor cells. The *Tre* gene was proposed to be a structural gene for trehalose through studies on the gene dosage effect (Tanimura *et al.*, 1988, Genetics, 119: 399–406).

In an effort to clone the *Tre* gene, we used a differential screening strategy by taking advantage of the *pox-neuro* (*poxn*) gene which is involved in the developmental decision pathway between mechanosensory and chemosensory cell fates. In an adult-viable allele of the *poxn* mutant, all external chemosensilla are either transformed into mechanosensilla or are deleted. Based on the cytological location of the *Tre* gene, we used one P1 clone as a starting material. Utilizing the *poxn* mutant, we performed a differential screen of the P1 clone which led to the isolation of a gene, *Tre1*, encoding the trehalose taste receptor (Ishimoto *et al.*, 2000, Science, in press). We conclude that the *Tre1* gene functions as a taste receptor for trehalose based on the following results. First, disruption of the *Tre1* gene lowered the trehalose sensitivity of sugar receptor cells leaving sensitivity to other sugars intact. Second, overexpression of the *Tre1* gene restored the taste sensitivity to trehalose in the *Tre1* deletion mutant. Third, the *Tre1* gene was specifically expressed in taste cells. Finally the predicted amino acid sequence of the *Tre1* gene encodes a novel GPCR. If we assume that TRE1 is the sole receptor for trehalose, the null mutant of *Tre1* should show no response to trehalose. We found the null mutant of *Tre1* still respond to higher concentration of trehalose and this response would be mediated by another unidentified receptor for trehalose. Interestingly, by searching genomic sequences of *Drosophila* we have found several genes whose predicted proteins have homology to TRE1. Assuming that multiple sugar receptors are involved in taste reception, the next problem is

to determine how each receptor functions in combination with other receptors.

44. Intracellular signaling in lobster olfactory receptor cells

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Studies of olfactory transduction in lobster olfactory receptor neurons (ORNs) suggest that general principles of olfactory information processing can transcend mechanistic differences among species, and that species-specific differences can help understand these organizational principles. As in vertebrate ORNs, excitation in lobster ORNs appears to involve two stages, a primary G protein signaling cascade and secondary activation of a channel carrying the main receptor current. In lobster ORNs, excitation is mediated by phosphatidylinositol (PI) signaling, the target of which is an inositol 1,4,5-tris-phosphate receptor associated with the ciliary membrane. The main current-carrying channel in lobster ORNs appears to be a non-specific cation channel that can be gated by intracellular Na^+. How the Na^+-gated channel is triggered is still uncertain. Recent work, however, indicates that membrane-associated PIs can activate the channel and regulate its sensitivity to intracellular Na^+ in a manner that would allow regulating the magnitude and possibly the kinetics of the signal to the brain. Some, but not all, lobster ORNs also express a second G protein signaling cascade mediated by cyclic nucleotides. In these cells, cyclic nucleotide signaling downregulates excitation by targeting a K^+ selective channel that effectively inhibits the cell. The extent to which this pathway down-regulates excitation presumably is itself subject to control since adenylyl cyclase activity depends on the level of intracellular Ca^{2+}, providing a substrate for biochemical crosstalk with the PI signaling pathway. This bipolar input confers the potential for the receptor cell to not only regulate but also integrate the signal to the brain since different odors activate the two signaling pathways in a given cell. Such complexity in intracellular signaling can be viewed as enhancing the diversity of the pattern olfactory receptor cells send to the brain, with concomitant enhancement in the discriminatory power of the system. The extent to which these or other organizational features generalize among species, however, may be related to the specific informational demands the particular species places on its olfactory input.

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45. Peripheral odor coding in vertebrates. new data in the rat and comparison with the frog

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In the rat olfactory mucosa, recent single-unit recordings have provided original physiological data on odor response properties of olfactory receptor neurons (ORNs) in mammals. ORNs were found to be well responsive. Their major response type was excitation and 80% of them responded, at least, to one of the 16 pure chemical compounds delivered. Individually, ORNs were few selective.

ORN temporal response patterns did not characterize stimulus identity but mirrored stimulus concentration. Individually, ORNs specified stimulus intensity through continuous and progressive frequency increases of their initial spike discharges that occurred with decreasing latencies.

ORN response thresholds were distributed on the whole concentration range available with a tendency to be gathered toward the lowest and highest concentrations. As in the frog, the number of excited ORNs increased as a function of stimulus concentration, but over a concentration range that was shifted towards higher concentrations. This apparent lower sensitivity of rat ORNs may be interpreted as an increase of olfactory receptor (OR) specificity. It may reveal two functioning modes: At low and medium odor intensities, highly specific ORs would be mainly set in motion, while at high intensities, lower specific ORs would participate to olfactory coding processes.

An important result of our study is that single rat ORNs displayed qualitative response spectra comprising very different types of molecules, as was the case in the frog. Assuming that the selectivity of ORNs reflects, at least in part, the specificity of their molecular olfactory receptors, our physiological data would imply that a single ORN is likely endowed either with several types of molecular ORs or with one type capable to bind very different molecular structures.

In conclusion, olfactory reception is likely ensured through very similar mechanisms over terrestrial vertebrates. From amphibians to mammals, phylogenetic evolution has likely resulted in an increase of OR specificity. This evolution did not call into question the combinatorial coding of odors, which offers the advantage to be a non restrictive code, and thus has preserved the multiplicity of coding possibilities.

46. Mature olfactory receptor neurons express the gap junction subunit connexin 43

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Gap junctions, which consist of subunits called connexins, represent an important mode of intercellular communication in the nervous system. In the visual system, gap junctions between cone photoreceptors are thought to decrease background noise at the expense of spatial acuity (Tsukamoto *et al.*, 1992, Vision Res., 32: 1809–1815). Although antibody labeling for connexin 43 (Cx43) appears to stain the sustenacular cells in the olfactory epithelium (Miragall *et al.*, 1992, J. Comp. Neurol., 325: 359–378), dye coupling studies in salamander suggest the presence of gap junctions in olfactory receptor neurons (Schwartz Levey *et al.*, 1992, Neurosci. Lett., 140: 265–269). In this study, we investigated gene expression and protein distribution of Cx43 in the adult mouse olfactory epithelium. We observed a spatially heterogeneous distribution of Cx43 mRNA expression in the olfactory sensory epithelium; highest levels of Cx43 mRNA occurred in anterolateral and ventrolateral regions of the olfactory epithelium. These regions displayed intense punctuate immunofluorescence labeling for antibody directed against Cx43. Unilateral bulbectomy

decreased this punctuate reactivity, suggesting that some immunoreactivity was associated with the olfactory receptor neurons. Using a transgenic mouse model containing a LacZ reporter driven by a proximal region of the Cx43 promoter (Lo *et al.*, 1997, Devl Genet., 20: 119–132), we found that Cx43 was expressed not only in sustenacular cells, but also in mature olfactory receptor neurons. Expression of Cx43 in mature olfactory receptor neurons suggests gap junctions may play a role that extends beyond the coordination of cell proliferation. We hypothesize that as in the visual system, communication between sensory neurons may play an important role in peripheral olfactory processes.

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47. Co-expression of a calcium signaling component in vertebrate taste bud cells

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In all vertebrates, the sensation of taste is mediated by specialized cells in taste buds. Some taste stimuli received by G protein-coupled receptors are transduced through intracellular signaling pathways initiated by the activation of G proteins. Previous physiological studies have indicated that taste transduction utilizes two or more signaling pathways. The Ca^{2+} signaling cascade of phospholipase C (PLC) followed by IP_3-dependent Ca^{2+} release from intracellular stores has been reported to be activated by many bitter tastants such as denatonium benzoate and by some non-sugar sweeteners such as saccharin and SC-45647 in mammals. In catfish, it is known that several amino acids, including L-alanine, as well as denatonium, induce an increase in the IP_3 concentration in taste buds.

In order to investigate the molecular mechanism of Ca^{2+} signaling pathways common to the vertebrate gustatory systems, we analyzed the expression of their molecular components. We first identified a PLC subtype expressed in the taste buds of pond loach (*Misgurnus anguillicaudatus*), designated DPLC2, which is closely related to mammalian PLC2 shown recently to be expressed in rat taste buds (Rossler *et al.*, 1998, Eur. J. Cell Biol., 77: 253–261). The taste bud-specific expression of PLC2 in a fish species as well as rat strongly suggests that PLC2 mediates the tastant-induced second messenger response in taste buds, which is common to vertebrates. Next, we examined the correlation of gene expression of the candidate components leading to PLC2 activation in rat circumvallate papillae, including G proteins, Gi2 (Kusakabe *et al.*, 2000, Chem. Senses, in press) and gustducin (McLaughlin *et al.*, 1992, Nature, 357: 563–569) and a G protein-coupled receptor, TR2 (Hoon *et al.*, 1999, Cell, 96: 541–551). As a result, it was shown that the mRNAs for PLC2 and Gi2 co-exist in the same cells, and PLC2- and Gi2-positive cells include both gustducin-positive cells and TR2-positive cells. However, no correlation was found between the expressions of TR2 and gustducin as reported previously. Our results thus indicate that a taste transduction pathway comprising TR2, Gi2 and PLC2 occurs in a subset of taste cells.

Cognition

48. Does the olfactory brain account for mood disorders?

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Chemosensory distortions in depressive patients have been frequently reported. The aim of the first study is to investigate olfactory sensitivity and odor evaluation in depressive patients using pure olfactory odors. The aim of the second study is to examine odor processing in depressive patients by means of chemosensory event-related potentials (CSERP).

In Study I, 24 in-patients with major depressive disorder (MDD) were investigated during their acute depressive phase. 18 of them participated a second time after successful treatment. An age and sex-matched group of healthy subjects served as a control. Olfactory sensitivity for eugenol and phenyl-ethyl alcohol was determined using a threshold detection staircase procedure. Moreover, 10 odors were evaluated for their pleasantness and intensity. In Study II, 23 patients with major depression (MD) and a non-depressive control group were examined and both groups were divided into high and low anxiety subjects. CSERPs were recorded using the constant-flow method and, in addition, ERPs in response to colors and emotional slides were obtained to control for modality and emotion specific effects. The subjects' task was to discriminate the colors (red/ yellow) and odors (phenyl-ethyl alcohol/isobutyl aldehyde) by their quality and to judge the valence of the emotional slides. The EEG was recorded from 32 scalp locations.

In Study I, olfactory sensitivity was strongly reduced in MDD patients ($P = 0.005$). Additional correlative analyses revealed that the lowered sensitivity could be predicted by high depression and anxiety scores. However, there was no evidence for a normalization of olfactory functioning after successful medical treatment. The subjective odor evaluations were not markedly changed. In Study II, the early and late positivities within the CSERP were reduced in MD patients. When colored or emotional slides were presented, the late positivities only were smaller in MD patients. The results concerning the degree of anxiety reveal that a high level of state anxiety alters the late positivities in a modality non-specific manner, whereas a high level of trait anxiety alters odor and emotional stimulus processing but not color perception.

The results reveal that olfactory performance in MDD patients is reduced on an early perceptional level of stimulus processing. Considering possible neurophysiological conditions for this effect, a deviant functionality in the main olfactory bulb is assumed and could also explain limbic activity changes.

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49. Sensory and affective integration in taste perception

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Taste-responsive neurons have been recorded from the amygdala of

non-human animals. These cells are thought to be important in determining stimulus palatability and are at least partially sensitive to concentration (Scott *et al.*, 1993, J. Neurophysiol., 69: 1810–1820; Nishijo, 1998, J. Neurophysiol., 79: 21–36). We have performed a series of gustatory experiments to elucidate the role of the anteromedial temporal lobe (AMTL) in human taste perception. Two types of experiments will be discussed; psychophysical studies in patients with unilateral resection of the AMTL for the treatment of intractable epilepsy; and positron emission tomography (PET) studies of gustatory function in healthy subjects and in a subject with bilateral lesions in the AMTL.

We have shown that AMTL resection may result in: increased recognition thresholds but not detection thresholds for a sour taste, deficits in suprathreshold taste intensity estimation, and increased sensitivity to the intensity of a bitter taste in patients compared with matched control groups. Using PET we have demonstrated activation of the left amygdala in response to novel/unpleasant compared with familiar/pleasant chemosensory stimulation, and activation of the right AMTL in response to tasting sour compared with water. Finally, we evaluated gustatory function pre and postoperatively in a patient (J.L.) with bilateral amygdaloid lesions, which on the left extended to include most of the insula. Preoperatively J.L. had normal detection and slightly elevated recognition taste thresholds. A PET study revealed activity in residual left amygdaloid tissue in response to stimulation with an unpleasant compared with a pleasant taste. Following surgical resection of the residual left AMTL (as treatment for epileptic seizures), taste detection remained within normal limits, but J.L. was no longer able to identify taste stimuli. Importantly, she could discriminate tastes, and verbally she could report that chocolate tastes sweet and grapefruit juice tastes sour. In effect, J.L. had acquired gustatory agnosia.

In summary, we have observed hedonic and perceptual changes following damage to the AMTL region in humans, suggesting that affective and sensory processing of taste stimuli are highly integrated. We propose that normal taste quality recognition and taste intensity perception emerge as the function of integrated processing between sensory taste cells, perhaps located in the primary taste region, and hedonic taste cells in the amygdala.

50. Odor quality perception in humans, monkeys, and honey bees: different systems, but common principles

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Animals of most species are capable of discriminating between a variety of odors. In order to understand the mechanisms underlying odor discrimination it is necessary to establish which properties of an odor molecule are functional in determining the degree of interaction with a given receptor, and thus in determining its perceived odor quality. One useful means to assess possible correlations between odor quality and molecular properties is to test the discriminability of structurally related odorants.

In a first series of studies, we tested the ability of human subjects to distinguish between members of five homologous series of aliphatic substances (1-alcohols, *n*-aldehydes, 2-ketones, acetic esters and *n*-carboxylic acids) and compared their performance with that of squirrel monkeys and honey bees. With all substance classes, and in all three species, we found a significant negative correlation between discrimination performance and structural similarity of odorants in terms of differences in carbon chain length. Further, in all three species we found both position and type of oxygen moiety to affect discriminability in a substance class-specific manner.

In a second series of studies, we tested the ability of human subjects to distinguish between ten pairs of enantiomers, and again we compared their performance with that of squirrel monkeys and honey bees. We found that all three species were able to significantly discriminate between the enantiomers of alpha-pinene, limonene and carvone, whereas they failed to distinguish between the (+)- and (–)-forms of alpha-terpineol, camphor, rose oxide and 2-butanol, thus showing very similar patterns of discrimination performance. Taken together, the results of these studies provide evidence of striking parallels in olfactory discrimination abilities between primates and honey bees.

Thus, our findings support the assumptions that mammals and insects may share common principles of odor quality perception, irrespective of their completely differing repertoires of olfactory receptors, and that in both taxa enantioselective molecular odor receptors may only exist for some but not all volatile enantiomers.

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51. Ecological categorization for smell: structures and principles of cognitive organization

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Our purpose is to present the main results of an interdisciplinary research (mainly, linguistics, psychology, psychophysiology) illustrating an ecological and cognitive approach to smell that we developed within the theoretical framework of categorization initialized by Rosch and colleagues.

Most research on 'natural' categorization has been grounding the relevance of the concepts of prototypes, typicality, basic level categories and basic level terms mainly for visual objects. Our first step was an attempt (and a failure) to apply conceptualizations as well as methodologies used by Rosch or Berlin and Kay for colors or other visual objects, to olfaction: there are no 'basic odor names' (at least not in English or French), there are no a priori categories of smells well defined in natural sciences and we ignore the principles of categorization along which categories of smells may be structured (where to find similarity for smell?).

We progressed to questioning what is a smell at every level of scientific investigation (chemical, physiological, psychological as well as linguistic) without any a priori requirement of a neat mapping of each description onto another. Various experiments allowed us to specify what is smell as a psychological phenomenon, that is, a meaningful event relevant to the subject's experience. We further explore what are the constraints (bottom up—and low-

level—constraints, as described by chemistry and neurophysiology, versus top-down—and high-level—constraints, as depending on memory, knowledge and linguistic structures, and described in psychology and linguistics). A special focus is given to the relations between language and cognition in olfaction both in one individual's memory, and in collective representations and discourses.

52. Is there a hedonic dimension of odors?

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Affection and cognition appear strongly intermingled in olfactory perception: it is difficult to separate odor quality and intensity (the cognitive processing) from its affective value. Our aim is to underscore the specificities of the hedonic processing of odors.

Psychophysical intensity evaluation, which is considered as a low level of processing, appears strongly contaminated by the hedonic dimension. Thus we designed experiments to investigate the relationships between the hedonic judgement and the intensity judgement by psychophysics and neuroimagery. We showed that intensity and hedonic judgement of the same odors can be dissociated, and we designed a hedonic test for clinical use.

The same experiment in normal controls using a PET scan indicated that intensity and hedonic judgements activate common (temporal and frontal) but also different cerebral regions, the latter implying an activation of the hypothalamus.

If the hedonic judgement is the same basic and pragmatic categorization process as the intensity judgement, then response times should be identical for both. Measurement on response times to the questions 'Is this odor more pleasant/intense/dangerous than the previous one?' showed that the shortest response times corresponded to the intensity judgement, and the longest to the pleasantness judgement. Thus it seems difficult to assume that hedonic judgement is the earliest stage of olfactory processing, and that it does not differ from the intensity judgement

A number of studies dealing with the olfactory space by multidimensional methods reveal the major importance of a hedonic axis in the psychological description of odors. These findings seem to support the widely held idea that odors vary in pleasantness along a continuum between 'very unpleasant' to 'very pleasant'. We present data from categorization tasks and from linguistic inquiries which allows one to question this assumption.

According to previous data indicating an asymmetry in the cortical processing of unpleasant versus pleasant odors, we tested the hypothesis that the processing differs between malodors and pleasant or neutral ones. Response times confirmed the differences between intensity and pleasantness judgements, and showed that malodors are processed quicker than neutral or pleasant ones.

These results allow one to consider that evolution may have favored the swiftness of affective processing of odors involved in behavioral responses of avoidance or fear.

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Taste Receptors and Transduction, Molecular Biology

53. A large family of candidate taste receptors in *Drosophila*

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Using a bioinformatics approach, we have identified a large and diverse family of seven-transmembrane-domain proteins from the *Drosophila* genome database (Clyne *et al.*, 2000, Science, 287: 1830–1834). Eighteen of 19 genes examined were expressed in the labellum, the major gustatory organ of the fly. Expression was not observed in a variety of other tissues, but was found in the labral sense organ, a taste organ that lines the pharynx. The genes were not expressed in the labellum of a mutant, *pox-neuro*, in which taste neurons are eliminated.

We estimate that the family contains on the order of 75 proteins. The genes are widely dispersed in the genome, but at the same time, many are found in clusters. An unusual form of alternative splicing occurs in at least two chromosomal locations. Region 39D contains four large exons, each containing sequences specifying six predicted transmembrane domains, followed by three small exons that together specify a putative seventh transmembrane domain and the COOH-terminus. Each of the four large exons is spliced to the smaller ones, thereby generating four products that are widely divergent in transmembrane domains 1–6, but identical in transmembrane domain 7 and the COOH terminus. A similar splicing pattern exists at cytogenetic region 23A, which encodes two proteins widely divergent in transmembrane domains 1–6 but identical in the remainder of the proteins. The entire family of proteins is similar in the sense that members are extraordinarily divergent in all portions except the seventh transmembrane domain and COOH terminus.

The large size of the family may reflect both the structural heterogeneity of compounds that flies taste and, perhaps, a diversity of signaling components with which different family members interact.

54. A family of candidate taste receptors in human and mouse

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Numerous studies have suggested that the detection of bitter and sweet tastants by taste receptor cells in the mouth is likely to involve G protein-coupled receptors (GPCRs) (Kinnamon, 2000, Neuron, 25: 507–510). Although two putative G protein-coupled bitter/sweet taste receptors were identified previously (Hoon *et al.*, 1999, Cell, 96: 541–551), the chemical diversity of bitter and sweet compounds suggested that there might be a larger number of receptors. To search for taste receptors (TRs), we devised a strategy based on four ideas: first, TRs would be encoded by a family of related genes; secondly, some TR genes would be found at genetic loci associated with the ability to taste specific compounds in mouse or human; thirdly, TRs would be GPCRs that have limited sequence similarity to other members of the GPCR superfamily; and fourthly, TR genes could be found by using the resources of the Human Genome Project to look for GPCR-encoding genes in

genomic regions that had been implicated in taste perception. Using this approach, we identified a family of candidate taste receptors (the TRBs) that are members of the GPCR superfamily and are specifically expressed by taste receptor cells (Matsunami *et al.*, 2000, Nature, 404: 601–604). The same family was reported by another group (Adler *et al.*, 2000, Cell, 100: 693–702; Chandrashekar *et al.*, 2000, Cell, 100, 703–711). A cluster of genes encoding human TRBs is located adjacent to a Prp gene that in mouse is tightly linked to a genetic locus (SOA) previously shown to be involved in the ability to detect the bitter compound sucrose octaacetate (Capeless *et al.*, 1992, Behav. Genet., 6: 655–663). Another TRB gene is found on a human contig assigned to chromosome 5p15, the location of a genetic locus (PROP) known to be involved in the detection of 6-*n*-propyl-2-thiouracil in humans (Reed *et al.*, 1999, Am. J. Hum. Genet., 64: 1478–1480).

55. A truncated metabotropic glutamate receptor functions as a taste receptor

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Taste transduction for stimuli such as sugars, some bitter compounds and some amino acids is thought to be mediated via G protein coupled receptors (GPCRs). Over the last few years, several GPCRs have been cloned from taste tissue. A critical step in establishing the role of such cloned receptors in taste transduction is the demonstration that their functional properties are similar to key features of taste in animals. Monosodium L-glutamate (LD-MSG), a natural component of many foods, is an important gustatory stimulus believed to signal dietary protein. We have recently cloned a novel variant of the metabotropic glutamate receptor 4 (mGluR4), a GPCR from taste tissue (Chaudhari *et al.*, 2000, Nature Neurosci., 3: 113–119). *In situ* hybridization shows that the gene is expressed in a subset of circumvallate and foliate taste buds in the rat (Chaudhari *et al.*, 1996, J. Neurosci., 16: 3817–3826). The tastebud-expressed mGluR4 contains a truncated extracellular N-terminus and is dramatically altered in the putative binding site for glutamate. We have functionally expressed this receptor in CHO cells, and demonstrated that it does nevertheless respond to glutamate. The receptor couples negatively to a cAMP cascade and displays an unusual concentration-response relationship for L-glutamate. Importantly, the receptor is also activated by L-AP4, a compound that mimics the taste of MSG in rats and in humans (Chaudhari *et al.*, 1996; Kurihara and Kashiwayanagi, 1998, Ann. N.Y. Acad. Sci., 855: 393–397). We have termed the novel receptor taste-mGluR4. The similarities of its properties to key features of MSG taste suggests that taste-mGluR4 is a taste receptor for glutamate.

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56. Heterotrimeric gustducin couples taste receptors to multiple taste transduction pathways

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We have used molecular and biochemical means to identify and characterize the components of taste transduction. The α-subunit of gustducin, a heterotrimeric transducin-like G protein selectively expressed in taste receptor cells, was originally cloned by degenerate PCR. Using single cell PCR and differential hybridization we determined that G protein subunits Gβ1 and Gβ2553 colocalized with α-gustducin in taste cells. We also identified a new G protein γ subunit (Gγ13) that colocalized absolutely with α-gustducin in taste receptor cells. We have solubilized and fractionated bovine taste receptors that activate gustducin/transducin heterotrimers, and we are proceeding with microsequencing of candidate taste receptor bands. We have also isolated a novel phosphodiesterase (PDE) from bovine taste tissue that can be activated by the α-subunits of gustducin and transducin.

The presence in taste cells of gustducin, transducin, a gustducin/transducin-regulated PDE and bitter compound-responsive receptors that activate gustducin/transducin suggests that these G proteins play similar roles in taste transduction to that of transducin in phototransduction, i.e. to couple taste receptor activation to PDE regulation of cyclic nucleotide levels. Confirmation of this proposal comes from the quench flow experiments of Yan *et al.* (AChemS XXI). On the other hand, multiple lines of evidence suggest that IP_3 and Ca^{2+} are important second messengers in taste cell responses to bitter and sweet compounds. We have determined that phospholipase C β2 (PLCβ2) is selectively expressed in gustducin-positive taste cells. Andrew Spielman and colleagues have determined that gustducin's α-subunit does not activate PLCβ2; in collaboration with the Spielman laboratory we have determined that gustducin's Gγ subunit, Gγ13, mediates the denatonium-induced activation of PLCβ2 to increase IP_3 in taste tissue.

To test the *in vivo* roles of gustducin's subunits we generated homozygous α-gustducin null mice and determined that they have reduced behavioral and electrophysiological responses to bitter and sweet compounds—Gγ13 knockout mice being generated will be similarly tested. We conclude that heterotrimeric gustducin couples to taste receptors and acts as a principal mediator of both bitter and sweet signal transduction. Gustducin functions by a dual pathway in which its α-subunit regulates PDE and its βγ moiety activates PLC.

57. Bitter signal transduction in taste cells: antibodies specific for PLCβ$_2$ and for Gβ$_3$ inhibit the rise in IP$_3$ induced by denatonium

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Signal transduction of several bitter tastants has been correlated with phospholipase C (PLC) activation and a raise of inositol trisphosphate (IP_3) levels in gustatory cells (Akabas *et al.*, 1988, Science, 242: 1047–1050; Hwang *et al.*, 1990; Proc. Natl Acad. Sci. USA, 87: 7395–7399; Spielman *et al.*, 1996, Am. J. Physiol., 270: 926–931). Other possible mechanisms for bitter taste involve activation of phosphodiesterase (PDE) and changes in cAMP and cGMP concentration (Ruiz-Avila *et al.*, 1995, Nature, 376: 80–85; Rosenzweig *et al.*, 1999, J. Neurophysiol., 81: 1661–1665). The effector enzyme likely involved in the IP_3 transduction pathway has remained unknown. We found the novel PLC subtype β_2 to be specifically expressed in a subset of circumvallate taste receptor cells. The protein was located in the microvillar region of taste

buds. Furthermore, antibodies specific for PLCβ_2 blocked the denatonium-induced increase of IP_3 in rat taste tissue (Rössler *et al.*, 1998, Eur. J. Cell. Biol., 77: 253–261). Considering a role of the G protein $\beta\gamma$ complexes in regulation of PLCβ_2 activity, we identified the G protein subunit β_3 (Ray and Robishaw, 1994, Gene, 149: 337–340) in distinct taste bud cells of circumvallate papillae. Furthermore, Gβ_3-specific antibodies selectively inhibited the bitter tastant-induced formation of IP_3; a less pronounced inhibition was observed using Gβ_1-specific antibodies. A subsequent analysis of isolated taste bud cells by RT-PCR allowed us to identify individual cells which expressed PLCβ_2 together with the Gβ-subunit β_3, the Gγ-subunit γ_3 (Gallagher and Gautam, 1994, Methods Enzymol., 237: 471–482) and the Gα-subunit gustducin (McLaughlin *et al.*, 1992, Nature, 357: 563–569). These data support the concept that bitter-induced primary responses of taste cells are due to Gβ_3/γ_3-mediated activation of PLCβ_2. In a recent study, gustducin was found to be colocalized with the novel Gγ-subtype (Gγ_{13}) and Gβ_3 in taste receptor cells; the majority of cells expressed Gβ_1 in addition (Huang *et al.*, 1999, Nature Neurosci., 2: 1055–1062). Taken together these data suggest that distinct combinations of Gβ- and Gγ-subtypes may participate in the transduction of bitter stimuli.

58. Molecular model of the human sweet-taste receptor

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A molecular model of the human sweetness receptor (HSR) has been inferred from superpositions of 3D maps of sweetener interaction sites (themselves deduced from extensive SAR studies on highly-potent artificial sweeteners) onto known GPCRs assumed to be linked by common evolutionary origins. As a 7TMR model, the modelled HSR is formed of seven α-helical transmembrane domains (TMs), five of which (TMs 3–7) are binding. According to the model, TMs 3 and 7 have high homologies with rhodopsin; TM4 has the β_2-adrenergic receptor; and TMs 5 and 6 have the α_{2A}-adrenergic receptor. The seven TMs are in a crescent-shaped arrangement. The axes of the helices, within the binding domain limits, are parallel and arranged clockwise when viewed from outside. The interaxial distances of the adjacent antiparallel binding helices are ~1.05 nm; of TM3 and TM6, ~1.8 nm; of TM3 and TM7, ~1.7 nm. The five binding TMs form a roughly cylindrical pit, of ~1 nm in diameter, which constitutes the central binding cavity. The binding TMs bear 16 binding sites, among which 11 are considered as the 'key' binding sites (they recognize the main natural sweeteners, such as sucrose) and five are considered as 'accessory' (they contribute, together with the key sites, to the detection of certain highly-potent artificial sweeteners). The 11 predicted key sites, when identified by the Ballesteros–Weinstein numbering system, are: T3.33, E3.37, T4.53, T4.56, T4.57, T5.42, C5.43, T5.46, D6.52, T6.56 and K7.43; the five predicted accessory sites are: V4.49, V4.52, S5.41, G5.45 and Y6.55. These sites interact with the sweeteners through electrostatic interactions (via ionic interactions or H-bonds), steric interactions (via dispersion forces, often reinforced by steric fits) or π–π interactions. From this model, it is inferred that, for example, D-glucose (as D-glucopyranose) interacts with the HSR through the key sites of TMs 3, 6 and 7; D-fructose (mostly as β-D-fructopyranose) and xylitol through a bimolecular interaction, one molecule with the key sites of TMs 3, 6 and 7, the other with those of TMs 4 and 5; and sucrose through the key sites of TMs 3, 6 and 7 for the glucopyranosyl part, and of TMs 4 and 5 for the fructofuranosyl part.

Accessory Olfactory Mechanisms

59. Heterogeneity in the vomeronasal system of mammals

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The vomeronasal system of mammals is chemoarchitecturally dichotomous. Two populations of receptor cells have been identified in the vomeronasal sensory epithelium based on the family of receptor proteins they express on their membranes. These two receptor cell populations express different G proteins: the more basal population expresses Goa and the more apical population expresses Gia2. The Goa-expressing receptor cells project their axons to the posterior accessory olfactory bulb (AOB) whereas the Gia2-expressing cells project their axons to the anterior AOB. In all mammals studied to date the anterior AOB is Gia2-positive and the posterior AOB is Goa-positive. These two parts of the AOB are also chemoarchitecturally heterogenous with respect to their carbohydrate content as revealed both with lectin binding and immunoreactivity to monoclonal antibodies raised against carbohydrate moieties. However, species differences have been observed with respect to lectin binding, as with NADPH-diaphorase reactions and OMP immunoreactivity. The heterogeneity in the system extends through the accessory bulb to the terminations of mitral/tufted cells in the amygdala. Recent studies indicate that there are physiological and behavioral correlates to the dichotomy within the vomeronasal system.

60. Properties of the sensory neurons of the vomeronasal organ

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The membrane properties of the sensory neurons in the vomeronasal organ are particular (Trotier and Døving, 1996, J. Physiol., 490: 611). The frog vomeronasal receptor neurons have a membrane resistance around the resting membrane potential in the order of 200 kW/cm^2 which is 200 times higher than found for example in a squid giant axon (Curtis and Cole, 1938, J. Gen. Physiol., 21: 757). The reason for this feature is probably that these cells do not express channels for ions that normally let potassium leak through the cell membrane. The high membrane resistance explains why currents in the order of a few pA are sufficient to depolarize the cell. The high input impedance also leads to an unexpected role of the electrogenic Na^+/K^+ ATP-ase (sodium pump) in the creation of the membrane potential. Dependent upon the conditions the current created by the sodium pump is sufficient to hyperpolarize the membrane to –140 mV. A depolarizing current caused by an inward rectifying current I_h, balances the hyperpolarizing effect caused by the sodium pump. The I_h starts to be activated at –80 mV. Sampling of body fluids, e.g. urine, by an animal will expose the sensory neurons to an increased potassium

concentration. The reaction of the sensory neurons to increased potassium concentrations is described and the results discussed in relation to the particular properties of these receptor cells.

61. Activation of accessory olfactory bulbs after pheromonal stimulation in mice

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Pheromonal cues in mice stimulate the main olfactory (MO) epithelium and/or the vomeronasal (VN) organ. The stimulation of the MO system is thought to be relevant for behavioural modifications and is achieved by activation of receptors in the MO neurons, which send axons to the MO bulb and ultimately reach orbitofrontal neocortex via thalamic connections. At variance with MO projections, the VN system does not project to the neocortex: VN neurons reach the accessory olfactory bulb, which is connected with the hypothalamic medial preoptic area via the amygdala. The VN system is therefore suited to modulate neuroendocrine effects. In adult male mice urine, different androgen dependent substances can act as pheromones. Among them are the major urinary proteins (MUPs), a family of proteins that bind several small hydrophobic molecules, noticeably 2-*s*-butyl-4,5-dihydrothiazole and 2,3-dehydro-exo-brevicomin. MUPs with their ligands bound are excreted in urine and can act in two ways: (i) by slowing the release of the ligands in the air, as to lenghthen the duration of the olfactory trace; and (ii) by acting themselves as pheromonal stimuli. This notion is supported by data on the induction of neuroendocrine modifications, elicited by MUPs administration. We have previously shown that MUPs ligands are sufficient to act as male chemosignals that trigger behavioral modifications in conspecifics (Mucignat-Caretta *et al.*, 1998, Chem. Senses, 23: 67–70; Mucignat-Caretta and Caretta, 1999, Anim. Behav. 57: 765–769), while the protein without ligands can accelerate the onset of female puberty (Mucignat-Caretta *et al.*, 1995, J. Physiol., 486: 517–522). Since this effect is mediated by the VN system, we investigated the accessory olfactory bulb activation by revealing transcription of the immediate-early gene c-fos by mRNA *in situ* hybridization. Exposure of prepubertal female mice to MUPs purified from adult male mice urine induced a significant increase in the number of labelled cells, whereas a mixture of synthetic thiazoline and brevicomin resulted in a nonsignificant increase of labelled neurons.

62. Elephantine pheromonal transit to the vomeronasal organ

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(*Z*)-7-Dodecenyl acetate, the preovulatory urinary pheromone of the Asian elephant, Elephas maximus takes a circuitous route from its source in the female to male neuroreceptive cells. This transit involves a sequential series of discrete carriers with different requirements. Using solid phase micro extraction (SPME) followed by gas chromatography/mass spectrometry (GC/MS), trace and micromolar amounts of (*Z*)-7-dodecenyl acetate (Z7–12:Ac) were measured respectively in the headspace of native and protease-treated preovulatory serum. Z7–12:Ac was not detected in urine-free cervical mucus. Utilizing both SPME and evacuated canister headspace capture followed by cryogenic trapping, and subsequent GC/MS analysis, urinary Z7–12:Ac was quantified throughout the estrous cycle. Using a radiolabeled photoactivatable analog, [$^3H_2$]-(*Z*)-7-dodecenyl diazoacetate, the main urinary pheromone carrier was identified as a 66 kDa protein, with N-terminal sequences strongly homologous to albumins.

RT-PCR allowed the elucidation of the full cDNA sequence of elephant albumin. Albumin and pheromone binding experiments demonstrated a maximal binding at alkaline pH (8–10). Reduction in pH and/or treatment with protease increased detectable urinary Z7–12:Ac concentration, as pheromone was released from the albumin carrier. Male response to the urinary pheromone involves its transport toward the vomeronasal organ (VNO). The urinary-based, Z7–12:Ac–albumin complex mixes with trunk mucus: during the mechanical action of the flehmen, the trunk mucus/pheromone mixture is placed onto the mucus-laden incisive ducts leading to the VNO. Photoaffinity labeling allowed the identification of two closely related trunk mucus proteins homologous to known odorant binding proteins (OBPs) that bind the pheromone. Using antibodies against the elephant OBPs, tissues producing the proteins and their respective cDNA library clones were identified. The OBPs bind the pheromone tighter than the urinary albumin, with low discrimination between various lipophilic ligands and with a lower pH optimum. The acidic nature of the trunk mucus may effect the release of the pheromone from the urinary carrier protein, making it available for binding by the mucosal proteins. This phenomenon would effectively cause the pheromone concentration in the sensory organs to increase rapidly, rather than gradually. The overall effect is a significant increase of detection sensitivity, as observed in behavioral studies.

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63. The vomeronasal cavity in adult humans

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We observed the surface of the anterior part of the nasal septum of living subjects using an endoscope. In ~13% of 1842 patients without pathology of the septum, the vomeronasal pit was clearly observed on each side of the septum and in 26% of subjects it was observed only on one side. The remaining observations indicated either the presence of putative pits or, even, no visible evidence of a pit. However, repetitive observations on 764 subjects depicted changes over time, from nothing visible to well-defined pits and vice versa. Based on 130 subjects observed at least four times, we estimate that ~73% of the population exhibits at least one clearly defined pit on some days. The vomeronasal cavities were, in computer tomographies, located at the base of the most anterior part of the nasal septum. Histological studies indicated that the vomeronasal cavities consisted into a pit and, generally, a duct extending in a posterior direction under the nasal mucosa. Many

glands were present around the duct, which contained mucus. There was no sign of the pumping elements found in other mammalian species.

Most cells in the vomeronasal epithelium expressed keratine, a protein not expressed by olfactory neurons. Vomeronasal epithelial cells were not stained by an antibody against the olfactory marker protein, a protein expressed in vomeronasal receptor neurons of other mammals. Moreover, an antibody against protein S100, expressed in Schwann cells, failed to reveal the existence of vomeronasal nerve bundles that would indicate a neural connection with the brain. Positive staining was obtained with the same antibodies on specimens of human olfactory epithelium.

The lack of neurons and vomeronasal nerve bundles, together with the results of other studies, suggests that the vomeronasal epithelium, unlike in other mammals, is not a sensory organ in adult humans.

Ageing

64. Olfactory receptor cell function in aging epithelium

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Deficits in sensitivity, discrimination, identification and enhanced adaptation with slower resensitization have all been reported as age-associated changes in olfaction. Age-associated changes in calcium regulatory mechanisms have been observed in the CNS, and altered calcium homeostasis in olfactory receptor neurons (ORNs) could contribute to these age-associated olfactory deficits. To determine whether age-associated functional changes occur at the level of the ORN, we used calcium imaging techniques to evaluate the functional characteristics of >300 ORNs from human subjects ranging in age from 12–84 years, who are also given psychophysical tests. Odorants used include those known to stimulate adenylate cyclase or phospholipase C and to elicit an increase in intracellular calcium (ICa) in rat ORNs. Human ORNs respond to these odorants with either increases or decreases in ICa, and these changes can be linked to distinct transduction pathways, allowing us to test specific hypotheses about the effects of age or disease on neuronal mechanisms involved in calcium homeostasis and signal transduction.

Our data demonstrate that viable ORNs are as plentiful in biopsies from subjects 65 years and older as they are in subjects <40 years old. Further, we have found that, rather than being less sensitive, they are more likely to respond to odorant stimuli, but are less selective than ORNs from younger subjects, often responding to more than one odorant set. Chronic or more frequent elevation of ICa could shorten ORN lifespan and lead to a higher rate of ORN turnover, which has been observed in aged rats. In addition, loss of receptor cell selectivity could result in impaired discrimination, identification and inappropriate cross-adaptation. Subjects whose ORNs have also been characterized are now being tested using a cross-adaptation task with two unrelated odors included in the ORN assay. Preliminary data suggest that these odors show greater cross-adaptation in subjects whose ORNs were less selective, than in subjects whose ORNs were more selective. However, this phenomenon may not be purely age-related. These data may explain in part the finding that older subjects often experience greater difficulty in odor discrimination and identification tasks, and might also contribute to reduced perceptual sensitivity in real-world situations.

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65. Transitional areas between allo- and neocortex bear the brunt of the cortical pathology in Parkinson's disease

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Parkinson's disease is a disorder of the neuronal cytoskeleton involving multiple neuronal systems. Alterations develop in only a few susceptible neuronal types of the central, peripheral and enteric nervous systems. The pathology results from the gradual intraneuronal aggregation of the presynaptic protein alpha-synuclein. An abnormal and insoluble fibrillary material is formed which eventually appears as somatic Lewy bodies or as Lewy neurites in cellular processes of the diseased parent cells. Parkinson's disease always is accompanied by major extranigral pathology which, in the telencephalon, usually affects olfactory structures, the mesocortical insular and subgenual areas (autonomic loop components), the entorhinal region, hippocampal formation, amygdala and the temporal mesocortex (limbic loop components). In addition, autonomic regulatory centres and all of the non-thalamic nuclei sending projections to the cerebral cortex exhibit severe lesions. The most influential sources of input to the high-order centres of the limbic system are exteroceptive olfactory and neocortical information as well as interoceptive data from the internal organs. On the output side, the limbic system centres process and send out important direct and indirect projections to the prefrontal neocortex. Therefore, these centres can well be viewed as a neuronal bridge linking outside and inside worlds. They are ideally positioned to select information from the streams of exteroceptive and interoceptive data, to evaluate the significance of environmental and cognitive events, and to direct emotional responses and behaviour appropriately. The centres of the limbic loop thus play an integral role not only in regulating motivation and the initiation of affect-related movement, but also in the maintenance of emotional equilibrium, social behaviour, learning abilities and memory functions. Selective impairment of all of these structures which occurs in the course of Parkinson's disease eventually leads to olfactory dysfunction, deficits in autonomic responses to emotionally significant stimuli, malfunctions of the endocrine and autonomic systems, changes in personality and an insidious decrement in intellectual aptitude and pursuits.

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66. Odor memory in normal and pathological aging

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Although there is a growing volume of literature on odor memory in aging, few studies have aimed at assessing a broad range of odor-memory functions, including odor recall, in elderly. To meet

this request, we applied an odor test analogous to the California Verbal Learning Test (CVLT) in groups of elderly.

In a first study, healthy elderly were compared with healthy young adults on both the CVLT and the analogous odor test. The results showed that both immediate and delayed recall of odors, both free and semantically cued, as well as the ability to learn across trials is impaired in normal aging, perhaps more so for olfaction than for audition, which can be referred to poor use of semantic-clustering strategies and poor identification. For an understanding of odor memory in neuropathological aging, we compared patients with primarily cortical pathology, probable Alzheimer's disease (AD), with patients with subcortical pathology, Huntington's disease (HD), as well as with control groups. The results from this second study suggest that both AD and HD demonstrate impairment in (i) recall and learning of this task across trials (essentially no learning), irrespective of whether the task was immediate or delayed or whether it was free or cued; (ii) recognition memory; and (iii) identification. However, important differences between the two demented groups included that HD patients, compared with AD patients, retained more information over time, recalled odors more consistently across trials and were more susceptible to proactive interference in the recognition task.

The findings from these two studies suggest a decline in several aspects of memory in normal aging, perhaps more so for olfaction than for audition, and that odor memory is even more affected in certain types of pathological aging. Whereas both demented groups suffer olfactory memory loss, the pattern of loss differs depending on neuropathology.

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67. Olfactory function in aging and alzheimer's disease: insights from psychophysics and event-related potentials

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Neuropathology in olfactory areas of the brain in both normal aging and in Alzheimer's disease provided a strong rationale for investigating olfactory function. A series of psychophysical studies indicated significant loss of olfactory function in normal aging and more dramatic loss in Alzheimer's disease (AD). Unknown was the effect of aging and neurodegenerative disease on the temporal processing of olfactory information in the brain. Thus, we utilized olfactory event-related potentials (OERP) in investigations probing olfactory function in aging and Alzheimer's disease.

We first examined a healthy life-span population to determine effects of age, and then investigated patients diagnosed with probable AD at the UCSD Alzheimer's Disease Research Center, using the NINCDS–ADRDA criteria. An average DRS for the AD patients of 119 indicated mild to moderate dementia. Age and sex-matched controls were compared with patients. In the OERP experiments, odor stimuli were presented in an air-dilution olfactometer, incorporating features of Kobal's earlier designs, which delivered the test odorant with a rapid rise time (<20 ms), heated (to 35°C) and humidified (to 80% relative humidity). OERPs and auditory ERPs were elicited with a single stimulus paradigm. EEG activity was recorded at Fz, Cz and Pz. ANOVA was used to analyze differences in amplitude and latency of N1, P2, N2 and P3. Olfactory function was also assessed with psychophysical measures.

The results showed significant effects of normal aging on both psychophysical measures of olfactory function and the olfactory event-related potential. In the OERP experiments, amplitude and latency were significant indicators of olfactory impairment in normal aging and correlated with psychophysical measures of olfactory loss. In patients with AD impairment was reflected in longer latencies for later components (P2 and P3) of the olfactory event-related potential and impairment in odor identification. The latency differences between the AD and the age-matched controls were strikingly larger (200 ms) than the latency differences between the two groups for the auditory P3 (50 ms), suggesting the potential clinical utility of the OERP in the assessment of dementia. The results have both theoretical importance for understanding the impairment in olfactory function in aging and clinical populations and practical implications for assessment in these populations.

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68. Effect of aging on the olfactory network: what fmri combined with psychophysics can tell us

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The sense of smell decreases with age. Several brain areas are involved in different aspects of olfaction. We used fMRI to determine which olfactory brain areas are most affected by aging. We hypothesized a relationship would exist between psychophysical measures of olfaction and the volume of activated brain areas across age groups.

Whole brain Gradient Echo Planar Images were obtained with a 1.5T MR scanner while subjects trained to use velopharyngeal closure were exposed to a constant stream of air that was odorized with amyl acetate 12s ON, 40s OFF. Subjects were instructed to respond by a button press at the onset of each stimulus perception and to respond with another button press for the offset of each perceptual epoch. These responses were used to create reference vectors to which the variation in MR signal was correlated. Psychophysical tests including odor detection, odor identification and odor memory were administered to each subject in a separate session. Odor detection was assessed with the alcohol sniff test and with butanol detection thresholds. The San Diego Odor Identification Test was used to assess odor identification. Odor memory was measured using a recognition memory test.

For young subjects, brain areas showing statistically significant activations in more than half of the cohort were located bilaterally in the orbito-frontal cortex, insula and cerebellum, in the right cingulate gyrus, frontal operculum, hippocampus, and in the left

thalamus. The pattern of activation in the group of elderly subjects was much reduced: activation in more than half the cohort was seen in the insula and cerebellum bilaterally, in the left hippocampus and in the right thalamus. A repeated measures ANOVA showed a significant effect of age on the normalized number of activated voxels in the piriform cortex, the frontal operculum and the insula bilaterally and the right orbito-frontal cortex, areas that have been implicated in olfaction by several brain imaging studies. Psychophysical measures correlated to a different extent with olfactory relevant areas. The age effect on activation in areas of the brain subserving both taste and olfaction (insula, frontal operculum, orbito-frontal cortex) is of particular interest.

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Free Session I

69. Human brain magnetic fields evoked by food-related visual stimuli

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It is well known that visual information has a big role in the daily dietary life. To understand the brain sites responsible for vision related food images, we tried to analyse brain magnetic fields evoked by food pictures in comparison to non-food pictures.

We used ~100 pictures of food, e.g. fruits, vegetables and cooked foods, and ~30 pictures of non-food as visual stimuli, e.g. scenery, stationery and daily necessaries. These visual stimuli were presented randomly in front of each subject. Brain magnetic fields were measured with a 122ch whole-head neuromagnetometer, and averaged >80 times for food pictures and for non-food pictures.

Positions of signal sources on subject's MRI evoked by food visual stimuli were essentially the same as those observed for direct taste stimulation (Nagai *et al.*, 1998, Jpn. J. Taste Smell Res., 5: 371–374). The detected area was located in the insula cortex and operculum which were supposed to be the primary taste area (Small *et al.*, 1999, NeuroReport, 10: 7–14). The latencies of these responses were longer than ordinal visual evoked responses (>300 ms). Some subjects showed responses with latencies of longer than 500 ms in the orbitofrontal area which is supposed to be secondary taste area (Zald, 1998, Brain, 121: 1143–1154). The present study suggests that food related images induced by visual stimuli occur in the cortical taste areas which are activated by stimulation of the tongue with taste substances.

70. Comparison of preference-related brain electrical activity in response to odour

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Traditionally, research investigating preference responses to odours is qualitative, based on subjective reports and dependent on the subject's conscious detection of the odour stimulus. Previous research revealed changes in brain electrical activity associated with physiological responses to odours which were subjectively not detected (Owen, 1998, PhD Thesis). Preliminary studies with different odours suggested changes in responses between odours and associated with reported preference for the odours (Owen and Patterson, 2000, Int. J. Psychophysiol., 35: 30), supporting previous reports of changes in regional activation related to hedonic responses (Klemm *et al.*, 1992, Chem. Senses, 17: 347–361; Brauchli *et al.*, 1995, Chem. Senses, 20: 505–515). The current research was designed to further investigate these differences in responses. Subjective responses were correlated with objective physiological responses to investigate differences in perceptual responses to low concentration odours delivered during natural respiration.

Sixteen subjects (balanced for gender, age, smoking status, handedness and olfactory ability) participated in repeated recordings of brain activity responses to D-limonene (citrus smell). Brain electrical activity was recorded with a 64-channel EGI system (saline electrodes) during delivery of air or odour. Stimulus delivery was synchronized with inspiration using a continuous respiration olfactometer (Owen *et al.*, 1999, Biomedical Research in the 3rd Millennium, IEEE,Melbourne, pp. 65–68), and presented at a ratio of three air to each odour in a pseudo-random order for five minutes recording periods. Subjective responses to the odour stimulus were assessed pre-recording. Subsequently, subjects indicated if they perceived an odour during the recording and completed preference response ratings of the odour.

Subjects were placed in like and dislike groups based on their pre-recording preference responses to the odour stimulus. Neurophysiological responses to the test odour were examined and correlated with these preference groups. The brain activity responses of the odour differed in comparison to air. These responses were analysed using traditional EEG techniques to determine the relationship of the brain activity to the reported preferences. The power spectrum analysis for the like and dislike groups reflected differences in electrophysiological activation associated with preference responses, suggesting that odour preference may be reflected, in part, by these differences in the power spectrum in response to low concentrations of the odour. This analysis demonstrated a method of utilizing different techniques to better quantify the neurophysiological effects of odour inhaled during natural respiration.

71. Can olfactory bulbectomized rats detect odors?

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When the neonatal rat is bulbectomized the frontal cortex and olfactory peduncle grow forward to fill in the space vacated by the bulbectomy and axons of the reconstituted epithelium extend through the cribriform plate, penetrate the forebrain and make synaptic connections with forebrain neurons. We assessed whether these aberrant connections to the forebrain could mediate the detection of vapor stimuli.

Experimental rats were unilaterally bulbectomized at P2 and, at P77, were trained in an olfactometer to detect different concentrations of ethyl acetate and to detect cineole and two complex odors (gardenia perfume and chocolate chip cookie). These rats were retested after removing the remaining bulb on P95

and then studied using anterograde transport of horseradish peroxidase for connections between the olfactory epithelium and forebrain. Intact and adult bilaterally bulbectomized rats served as controls.

Anatomically, the frontal cortex and/or olfactory peduncle of each experimental rat contained numerous olfactory derived axons and glomerular-like clusters of axon terminals. Four of these 11 rats had olfactory connections to olfactory peduncle or olfactory cortex and each of these rats performed as well or nearly as well as controls on each of the odor detection tasks. Olfactory connections of the remaining seven rats appeared to be restricted to frontal pole cortex and, like adult-stage bulbectomized rats, each proved anosmic on the detection tasks.

These results indicate that, in the absence of the olfactory bulb, projections from the olfactory epithelium to olfactory peduncle or olfactory cortex can support detection of vapor stimuli.

72. Analyses of gustatory related human neural responses by taste-modifying process

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Recent studies on non-invasive recordings from human brain have shown the existence of taste-elicited activation areas in the cerebral cortex (Zald *et al.*, 1998, Brain, 121: 1143–1154; Small *et al.*, 1999, NeuroReport, 10: 7–14). To understand the mechanism of gustatory evoked neural responses, we tried to detected the stimulus latency of brain magnetic field evoked by different tastants which has different peripheral mechanism including taste-modifying process.

A new gustatory stimulation system was used for applying taste stimuli directly into subjects' mouth via tubing liquid-supply system. Brain magnetic fields were measured by 122ch whole-head neuromagnetometer. 500 mM sucrose and 50 mM citric acid were used for taste stimuli. We also examined the effect of miracle fruits (*Synsepalum dulcificum*) as a taste-modifying substance.

Positions of signal sources on subject's MRI were almost same area between sucrose and citric acid as taste stimuli. The detected area was the insula cortex or the operculum, which were supposed to be the primary taste area (Rolls, 1997, Crit. Rev. Neurobiol., 11: 263–287). Latencies of signals were different between sucrose and citric acid. Citric acid activated the primary taste area more quickly than sucrose. This would reflect a different transduction mechanism existing in the peripheral taste receptors (Kinnamon and Beidler, 1992, Ann. Rev. Physiol., 54: 715–731). Citric acid after chewing a piece of miracle fruits should the similar latency as sucrose did. It is suggested that taste sensation of sour taste would be changed to sweet on human brain system, which may explain the property of modifying a sour taste into a sweet taste after miracle fruit treatment (Kurihara and Beidler, 1969, Nature, 222: 1176–1179).

73. Implicit memory for odors: a new approach

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The incidental acquisition or perceptual priming of olfactory information has received little attention in olfactory research and the few studies that addressed implicit learning did not find clear evidence for its existence. In the present study chemosensory event-related potentials (CSERPs) were used to investigate whether the incidental presentation of two odors within an oddball paradigm would facilitate the automatic categorization of these odors at a later time. Since CSERPs allow to assess stimulus salience without the necessity of an overt verbal or motor response they are ideally suited to measure implicit processes.

Two groups of ten subjects (five women, aged 20–29 years) participated in two sessions. The priming group received the same two odors in both sessions [standard (P = 0.8), eugenol; deviant (P = 0.2), linalool]. The control group smelt the same odors as the priming group in the second session, but was presented with a different set of odors in the introductory session (standard, allylcaproate; deviant, phenylethyl alcohol). The odors were presented in a constantly flowing airstream to both nostrils of the subject non-synchronously to breathing. To assure incidental learning and retrieval conditions, subjects were instructed to ignore the odors and to concentrate on an auditory distractor task in both sessions. All subjects attended four blocks per session. The odors were presented within six sets of ten trials per block. The interstimulus interval was 8 s, the interset interval varied between 30 and 60 s. The EEG was recorded from Fz, Cz, Pz, F3, P3, F4, P4 in reference to linked mastoids. Subjective ratings of valence, quality and familiarity of the odors were obtained at the end of the second session.

A separate analysis of the four blocks revealed that both groups did not respond differently to the deviant odor until the second block: in the priming group the P3 amplitude increased in comparison to the first block, whereas in the control group the P3 amplitude actually decreased. The results suggest that the subjects generated a context-dependent implicit representation of the odors that, once activated, appeared to facilitate or respectively interfere with the categorization of the odors in the second session. The subjective ratings support this interpretation.

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74. Odor as a conditioned stimulus for the retrieval of emotional responses

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Despite the fact that many odours give the impression of being very familiar, our ability to identify them is very poor [the tip of the nose effect (Lawless and Engen, 1977, J. Exp. Psychol.: Hum. Learn. Mem., 3: 52–59; Cain, 1979, Science, 203: 467–470; Cain, 1984, Perfum. Flavor., 9: 17–21)]. Connections between odours and verbal/conscious labels are very weak, and yet associations between odours and experience and emotional memories may be established with only one exposure (Engen and Ross, 1973, J. Exp. Psychol., 100: 221–227). This has led to the suggestion that the function of odours may be to act as a retrieval mechanism for mood, memory

and emotion (Cain, 1984), rather than stored and retrieved as isolated memories. However, to date, to the best of our knowledge, no one has succeeded in providing experimental evidence to support these claims using physiological data free of demand characteristics.

The current study aimed to create a learned association between a unique and unfamiliar odor and an emotional response (mild anxiety) as reflected by mood and state ratings and measures of physiological (autonomic) arousal. Fifty-six participants were randomly distributed into three groups and undertook three experimental sessions, baseline, learning (paired with video segment to induce anxiety) and test, on three consecutive days. The experimental group (G1) received the same odor during learning and test while the two control groups received either a different odor (G2) or no odor (G3). Three different odors were used to counterbalance G1 and G2.The results indicated that subsequent presentation of the conditioned odour elicited significant increases in anxiety levels as indicated by EMG and heart rate parameters, and, less significantly, ratings of emotional state. However, no significant change was found in pencil and paper mood ratings. These changes were not found in the absence of odor (G3). Nor did they generalize to odors not present during conditioning (G2) which, if anything acted as conditioned inhibitors, or discriminative stimuli signalling the absence of aversive consequences. The results were taken to support the Encoding Specificity Hypothesis (Tulving and Thomson, 1973, Psychol. Rev., 80: 353–370), which states that anything present at the time of encoding can act as a contextual cue to aid the retrieval of learned material. A second experiment seeking to condition an relaxation response to odors will be reported on.

75. Electrogustometric taste detection thresholds imply a right cerebral hemisphere advantage in gustatory processing

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In this paper we review published evidence, and present new evidence, that aspects of gustatory function may be preferentially processed in the right hemisphere of the human brain. When automated electrogustometry was used to measure taste detection thresholds from the left- and right-sides of the tongues of 97 normal volunteers, a small but statistically significant advantage was obtained for the right side of the tongue ($P < 0.01$) (Stillman *et al.*, 2000, Clin.Otolaryngol., 25: 120–125). A similar advantage was obtained in two volunteers who undertook to monitor their taste detection thresholds daily for ~80 days. Furthermore the thresholds of both volunteers were more variable on the left than on the right. Neural pathways for taste project ipsilaterally to the cortex (Pritchard *et al.*, 1999, Behav. Neurosci., 113: 663–671), thus these behavioural outcomes are in keeping with recent evidence from imaging studies suggesting a functional asymmetry favouring the right hemisphere of the brain in the processing of taste information (Zatorre *et al.*, 1992, Nature, 360: 339–340; Small *et al.*, 1997, J. Neurosci., 17: 5136–5142). A growing body of evidence also supports the notion of a functional asymmetry favouring the right hemisphere of the brain in the processing of olfactory information (Zatorre and Jones-Gotman, 1990, Percept. Psychophys., 47: 526–531). However, there is uncertainty over which aspects of gustatory and olfactory processing are lateralized, and whether any are related to handedness (Zatorre and Jones-Gotman, 1990; Cer *et al.*, 1998, Ann. N.Y. Acad. Sci., 855: 575–578; Hummel *et al.*, 1998, Chem. Senses, 23: 541–544). Taste and olfaction each combine with other sensations from the oral cavity to produce the experience of flavour, and both are emotionally evocative senses. Thus it might be expected that patterns of hemispheric asymmetry should apply to both, and are likely to favour the right hemisphere.

76. Neurotrophin- and receptor-like immunoreactivity in mature hamster taste bud fields

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Neurotrophins and their molecular receptors are important for peripheral cell target innervation, cell maintenance and survival, and have been demonstrated in all sensory systems. In situ hybridization studies of the taste periphery in normal developing and adult rat and human tissues have shown that the neurotrophins, brain-derived neurotrophic factor (BNDF) and neurotrophin-3 (NT-3) may be differentially expressed in anterior tongue fungiform (F) and posteromedian circumvallate (CV) gemmal fields (Nosrat, *et al.*, 2000, J.Comp. Neurol., 417: 133–152). The degree to which their expression may be disrupted and/or sustained after gustatory nerve lesion is not known. In this regard, the hamster is an intriguing model for studying neurotrophin- and receptor-related molecular expression since the F taste buds, unlike posterior taste buds, do not wholly degenerate following denervation (Whitehead *et al.*, 1987, Brain Res., 405: 192–195; Oliver and Whitehead, 1992, Chem. Senses, 17: 529–542). This initial report in intact animals is an immunocytochemical fluorescent analysis comparing the immunoreactivity (IR) of F, CV and posterolateral foliate (Fo) taste buds to BNDF and NT-3, and their respective receptors, tyrosine kinase (trk)B and trkC.

Neurotrophin-IR is greater for NT-3 than BDNF in all bud fields, with an average of 100% of the F and Fo bud fields (100 buds) NT-3-immunoreactive versus 61% (121 buds) for BDNF. Also, the average number of immunopositive cells is greater in all bud fields for NT-3 ($F = 3.0 \pm 0.5$ SEM cells/bud, $Fo = 2.9 \pm 0.2$ and $CV = 2.8 \pm 0.4$) compared with BDNF ($F = 0.8 \pm 0.2$ cells/bud, $Fo = 1.3 \pm 0.1$, and $CV = 1.2 \pm 0.2$). Gemmal cell-IR is absent for TrkB or TrkC.

Receptor-IR of nerve fibers is more evident for TrkC than TrkB, consistent with greater NT-3-IR in buds. TrkC-immunopositive fibers are most prominent in the F field, where they are concentrated immediately subgemmally, in the central core region of the papilla.

The prominence of NT-3-IR across all three bud fields, and distinct expression of TrkC in the F field may bear on the issue of bud survival following neurectomy in mature hamsters, and is presently being studied.

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77. Vomeronasal information processing in medial amygdala

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We have previously shown using Fos-expression mapping, that pheromone stimulation of the vomeronasal organ in male hamsters activates neurons in the central vomeronasal/accessory olfactory pathway leading to the preoptic area. Key regions on this pathway are the anterior and posterior medial amygdala (MeA/P). MeA receives the majority of input from the accessory olfactory bulb and also convergent input from main olfactory projection areas. MeP receives massive input from MeA, contains receptors for the steroid hormones that are necessary for normal behavior and sends strong output to the medial divisions of the medial preoptic area. We now find that transmission of activity from MeA to MeP may depends on the type of vomeronasal-system stimulation, indicating that MeA may be an important filter for information passing though this pathway. Natural stimulation of the vomeronasal organ by female hamster vaginal fluid (HVF) or by flank-gland secretion, activated MeA and also strongly activated MeP. However, artificial stimulation of the vomeronasal organ using implanted electrodes, or of the accessory olfactory bulb by an injected metabotropic glutamate agonist, did not activate these areas in the same way. Activation of MeA at levels similar to those produced by natural stimulation was not accompanied by strong activation of MeP. This dichotomy was true for two types of natural stimulation and two types of artificial stimulation suggesting that it is not an artifact of a particular method of stimulation. We suggest that transmission of information from MeA to MeP may be dependent on the pattern of input. Artificial stimulation, which tends to activate all parts of the system synchronously, will clearly not duplicate the pattern of activation by natural stimulation, which activates vomeronasal sensory neurons selectively. Recent molecular studies have shown complex patterns of axon termination for vomeronasal sensory axons of different types. Recent Fos mapping studies have emphasized differences in activation patterns in the accessory olfactory bulb by different pheromonal stimuli. The patterns of accessory bulb output generated by these mechanisms may be interpreted in part in the anterior medial amygdala. We are now studying activation patterns in MeA for differences that may underlie the selective transmission.

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78. Effect of nasal dilators on olfactory function

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The aim of this study was to describe the effect that nasal dilators have on olfactory ability, nasal structures and sniffing strategies. Experimental psychophysical results demonstrate that nasal dilators increase odorant identification, lower odorant threshold, and increase perceptual odorant intensity. In additional experiments, magnetic resonance imaging (MRI) data demonstrates that the size of the nasal cavity in the region of the nasal valve is increased by ~20% when nasal dilators are worn. Also, smaller changes occur in the cross-sectional area in the bony part of the nose, perhaps as the level of nasal mucosal engorgement changes. During a sniff, pneumotachograph data demonstrates that the peak flow, maximum flow rate, volume and duration are all increased when nasal dilators are worn. The current results can most easily be explained by changes in the delivery of odorant molecules to the olfactory receptors. That is, the increase in the size and shape of the nasal vestibule and valve could in turn influence the distribution of nasal airflow in the nasal cavity. Thus, nasal dilation may increase the proportion of inspired odorant molecules that are directed to the olfactory mucosa and are, therefore, available for odorant perception. In addition, with the observed increase in the parameters of the sniff (e.g. sniff volume, sniff flow rate, sniff duration), there would be an increase in the number of odorant molecules available for detection, identification and perceptual intensity. These two mechanisms are not mutually exclusive. That is, they could be acting in concert to increase the delivery of odorant molecules to the olfactory receptors. From these results, nasal dilators appear to be a noninvasive technique that can be used to temporarily change human nasal anatomy and so they may provide a technique with which to better describe the relationship between nasal anatomy and olfactory ability.

Sweetness

79. Are temporal properties of amphipathic tastants related to their possible receptor-independent activation of signal-transduction pathways?

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Signal messengers such as cAMP, IP_3 and cGMP in intact taste cells following sweet-taste stimulation can be monitored in real time, in the sub-second range. However, many amphipathic (non-sugar) sweeteners are slow in taste onset and linger, and the molecular basis for these temporal properties is ill-defined.

We demonstrated that the amphipathic bitter tastants quinine and cyclo(Leu–Trp), and the non-sugar sweetener saccharin, translocate rapidly through multilamellar liposomes. Furthermore, when rat circumvallate (CV) taste buds were incubated with these tastants for 30 s, their intracellular concentrations increased 3.5- to 7-fold relative to their extracellular concentrations (Peri *et al.*, 2000, Am. J. Physiol. Cell Physiol., 278: C17–C25). The dynamics of this dramatic accumulation was also monitored *in situ* in rat single CV taste buds under a confocal laser-scanning microscope. Tastants were clearly localized to the taste-cell cytosol. Interestingly, saccharin and cyclo(Leu–Trp) stimulated a concentration-dependent reduction in cAMP level and pigment aggregation in melanophores of *Xenopus laevis*. Furthermore, such tastants were shown to be direct activators of G proteins *in vitro* (Naim *et al.*, 1994, Biochem. J., 297: 451–454).

We hypothesize that amphipathic sweet and bitter tastants, due to their permeation into taste cells, may stimulate downstream transduction pathways by means of receptor-independent mechanisms, in addition to their action on G protein-coupled receptors. Such phenomena may be related to the temporal

properties of these tastants and to their effect on cellular responses in cells of various tissues which are unlikely to contain taste receptors.

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80. Sensory advances in sweet taste chemoreception

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Sucrose is the sweetener of choice for conferring this important positive attribute of many foods, but perceived health concerns are expanding the use of non-sucrose sweeteners. Many alternative bulk sweeteners are available, but often do not confer the required sweetness level. Many intense sweeteners are available, but these do not have the required bulking and physical properties. In addition, many sucrose substitutes do not possess other required quality attributes, and suffer from non-sweet tastes and unwanted temporal profiles.

This part of the EU-supported project 'The Mechanistic Understanding of the Sweetness Response' carried out research on a number of levels. The work at Leatherhead Food Research association generated a comprehensive database of sweetness characteristics as a basis for physicochemical studies on the role of water in sweet taste chemoreception (Portmann and Kilcast, 1996, Food Chem., 56: 291–302). In addition, the properties of combinations of bulk/intense sweetener combinations were evaluated, and evidence for synergy found. This work was extended at the University of Wageningen, where psychophysical evaluation of interactive effects between intense sweeteners and aroma compounds was investigated (Nahon, 1999, PhD Thesis, University of Wageningen). Research at the University of Zurich was carried out to investigate the response of primates to different sweetener types, and to improve the understanding of mechanism of human response to intense sweeteners (Glaser, 1999, World Rev. Nutr. Diet., 85: 18–38). Examples of the results of the research will be given.

81. Hydration properties of sweet and bitter molecules and the role of water in their taste modalities

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Solution properties of sapid molecules are informative of their type of hydration (hydrophobic or hydrophilic) and on the extent of the hydration layer. Physicochemical properties (intrinsic viscosity and apparent specific volume) and NMR relaxation rates R_1 and R_2 for pure sweeteners, bitter molecules and their mixtures were found to be relevant in the interpretation of the effects of these solutes on water mobility.

Likewise, surface tension, contact angles with a hydrophobic surface and the adhesion forces to this type of surface of the aqueous solutions of sapid molecules were found to discriminate between their effects on water cohesion and between their taste qualities.

The interpretation of the two sets of independent experimental results, namely physicochemical and spectroscopic data, aids the elucidation of the role of water in sweet and bitter taste chemoreception.

82. Computational studies of sweet tasting molecules

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Results obtained so far from the computational studies in the TOSTQ programme will be described. Several different computational methods have been applied to the study of sweet tasting molecules. QSARs have been developed for specific families of sweet-tasting molecules, including the isovanillates and the sulfamates. Over 100 different molecular descriptors were calculated for each molecule and multiple regression equations were then obtained relating the relative sweetness of the molecules to a function of several parameters that were selected by using a genetic algorithm technique. QSARs were also developed from molecular field analysis using four probes with different properties. Several thousand interaction energies were calculated between the probes and each molecule, and the genetic algorithm was used to select the energies to form the most appropriate equation. The ability of the QSARs to predict the sweet taste values of molecules as yet unsynthesized or tested will be evaluated.

Molecular dynamics techniques have been used to study the behaviour of sweet-tasting molecules in water and evaluate the water-structuring ability of well-known sweeteners. The results have been compared with experimental values obtained for the solution properties of these molecules.

The sweet receptor is as yet unknown and methods have been developed for building a pseudo-receptor. This is done by overlapping the molecules and adding appropriate residues around the molecules to form a pseudo-receptor. The exact shape of the receptor and overlapping of the molecules can both be varied by fitting to experimental sweetness data. The designed pseudo-receptor can then be used to predict the sweet taste values of new molecules.

83. Interaction of taste and smell stimuli in determining overall flavour of foods

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Anecdotal evidence for the interaction of taste and smell is commonplace as generations of cooks and product developers have recognized that adding taste compounds like sugar enhances the volatile flavour of the dish. Scientific explanations for these interactions are less common. Physicochemical, as well as cognitive effects have been proposed. Using analytical techniques that

sample taste and aroma compounds *in vivo* during eating, it has been shown that cognitive effects seem to be responsible as the physicochemical effects are not significant in the systems studied. Using a simple apparatus, the sensory perception of flavour in the presence and absence of a volatile flavour has been monitored.

Development of the Chemical Senses

84. A taste for development: functional considerations

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The morphological and functional development of the rat gustatory system occurs primarily postnatally. As such, stimulus-induced processes likely play a major role in organizing the sense of taste. However, some very important events that direct development occur long before the gustatory system becomes functional. In order to examine some of the very early organizational processes, it has been necessary to institute experimental manipulations prenatally and then examine the effects as the system develops. Thus, by relating the neurobiological effects with prenatal manipulations, it is the hope that we can get a picture of events that serve to shape the long-term organization of the system during normal development. Moreover, as with all other sensory systems, it is important to identify processes that may have long-term (or permanent) detrimental effects on sensory and brain development. It is my goal in this presentation to provide background information about normal gustatory development that provides the necessary standard by which experimentally induced alterations can be compared, and to then describe findings resulting from early environmental manipulations. My focus will be on neurophysiological and anatomical development of the periperhal taste system and neuroanatomical development of brainstem taste nuclei.

85. Fungiform papillae—differences between children and adults

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Eight-year-old males are less sensitive than adults to sucrose and other tastants when a whole-mouth testing procedure is used (James *et al.*, 1997, Physiol. Behav., 62: 193–197). In contrast, when small localized regions of the tongue tip are stimulated, children of this age are more sensitive than adults to sucrose (Stein *et al.*, 1994, Devl Brain Res., 82: 286–294). In the latter study, the more sensitive areas in children had similar or greater densities of papillae than equivalent areas in adults. These papilla densities were determined at a low (×10) magnification and it was not possible to discriminate between taste- and non-taste-papillae. It is possible that the taste-bud densities of adults and young children differ and that this is the basis of the differences in sensitivity. The aim of this study is to compare the density of taste-pores on the anterior region of the tongue in adults and young males using a technique similar to that described by Miller and Reedy (1990, Physiol. Behav., 47: 1213–1219) which uses videomicroscopy to reliably measure taste-pore density.

Twenty young adult males and twenty 8- to 9-year-old males were studied. At the first session for each subject, two small (9 mm^2) regions near the tip of the tongue were stained with methylene blue which has been shown to stain the taste-pores (Brouwer and Wiersma, 1978, Histochemistry, 58: 145–151). Videomicroscopy at high magnification was then used to examine and record all the fungiform papillae and taste-pores in each small region. The same small regions were studied a second time on a different day. Video images were captured and analysed using NIH Image software. The parameters measured in each small area included the density of fungiform papillae, the diameters of the papillae and the density of taste-pores.

Children had a greater density of taste-pores on the tip of the tongue, however the number of taste-pores per papilla were similar in adults and children. The density of fungiform papillae in the two regions studied was greater in children than in adults and the papillae in children were smaller in diameter and more regularly shaped than those of adults. In view of previous findings (Hutchinson *et al.*, 1997, Proc. Aust. Neurosci. Soc., 8: 79) that the taste-sensitive area of the anterior tongue in 8-year-olds and adults is similar in size, it is likely that the greater sensitivity of small regions of children's tongues is due to a greater density of taste-buds. Children's higher thresholds to whole mouth stimulation may be due to a reduced capacity to assimilate the taste input from the whole mouth.

86. Development of olfaction, taste and flavor in humans

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A relatively neglected area of research of the chemical senses is the development of the human olfactory and gustatory senses. Although it has been known for several decades that humans can sense and respond with adult-like hedonic characteristics to common smells and tastes, our knowledge of their absolute sensitivity to chemosensory stimuli and their ability to discriminate between different tastes, odors or different concentrations of these stimuli is very limited. The primary reason for the lack of knowledge appears to be the difficulty in working with children of different ages, in particular, developing training and test methods. Furthermore, few comparative studies have been reported where adults and children are included in the same experiments and similar methods are used to obtain data.

Encouragingly, during the past decade an increasing number of studies are being reported and a clearer picture is developing of how the senses mature. In addition, the role of cognitive factors in influencing the decisions of children in chemosensory studies is becoming better understood and ways of overcoming these are being explored. This review will describe the current state of knowledge of the development of the human senses of smell and

taste, how children cope with the analysis of mixtures of smells and tastes ie flavor, and their ability to perceive fats.

87. Salt preference: effect of mother's morning sickness

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The determinants of the wide variations in salt preference exhibited by adult humans remain poorly understood. We have evidence that one factor which may contribute to salt preference in adulthood is prenatal exposure to the hydrational consequences of moderate to severe vomiting associated with maternal morning sickness. The present studies were prompted by experiments in rats (Nicolaïdis *et al.*, 1990, Am. J. Physiol., 258: R281–283) which indicated that dehydration during pregnancy, induced by multiple administrations of a diuretic, led to enhanced salt preference in adult offspring. The treatment and its effects were interpreted as a possible animal model of severe morning sickness. In a series of studies we assessed the salt preference of young adult college students and human infants and found that offspring of women who experienced moderate to severe vomiting as a result of early pregnancy sickness displayed significantly higher salt preference than those whose mothers had not experienced those symptoms.

These effects were first observed in studies of college students who completed surveys about their dietary salt intake or rated and consumed ten snack foods. Those subjects whose mothers reported moderate to severe vomiting reported a significantly higher level of salt use and ate more salty snacks than those whose mothers reported little or no symptoms (Crystal and Bernstein, 1995, Appetite, 25: 231–240).

Studies were then conducted with 16-week-old infants whose mothers reported either little or no vomiting (n = 15) or frequent moderate to severe vomiting (n = 14) during the first 14 weeks of their pregnancy (Crystal and Bernstein, 1998, Appetite, 30: 297–307). The infants' oral-motor facial reactions to each solution and their relative intakes of distilled water, 0.1 M and 0.2 M NaCl were used as measures of preference. Infants of mothers who reported no or mild symptoms had significantly lower relative intake of salt solutions than infants whose mothers reported moderate to sever symptoms. This pattern of results provides a potential explanation for some of the variability encountered when human infants are tested for their salt preference. Taken together, the findings support the hypothesis that maternal dehydration, induced by vomiting during pregnancy, can lead to enhanced salt preference in offspring. They further suggest that these effects are not only expressed in infancy, but may be remarkably durable if not permanent.

88. Earlier-than-early odour learning: human newborns remember odours from their pregnant mother's diet

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The ability to learn olfactory cues has been demonstrated in the foetuses of rats, rabbits and sheep (Hepper, 1988, Anim. Behav., 36: 935–936; Bilko *et al.*, 1994, Physiol. Behav., 56: 907–912; Schaal *et al.*, 1995, Behaviour, 132: 352–365). In these species, the introduction of a pure odorant into the amnion affects later infantile responses to that odour. These studies in nonprimate species highlight the fact that chemosensory information is part of the normal experience of the foetus. It is the aim of the present study to examine whether similar prenatal facilitative or inductive processes might operate in the formation of the earliest selective responses to odours in human infants. Taking advantage of the widespread use of anise flavour in the local Alsatian cuisine, and of the fact that it is readily perceived by newborns immediately after birth, we studied odour-elicited responses in infants born to mothers who had regularly consumed anise-flavoured sweets or drinks during late pregnancy and infants born to mothers who had not ingested such products during pregnancy.

Olfactory responsiveness was assessed in 24 neonates born to mothers who had or had not consumed anise flavour during the last two weeks of pregnancy. Both groups of infants were followed-up for behavioural markers of attraction (head orientation, mouthing activity) and aversion (negative facial responses) when exposed to the odour of pure anise and a control odor at the ages of 3 h and 4 days. The pattern of neonatal hedonic responses to the odour of anise depended on whether the pregnant mother ingested it: infants born to anise-consuming mothers evinced a stable preference for anise odour, whereas those born to anise nonconsuming mothers displayed aversion or neutral responses.

This study provides the first clear evidence that through their diet human mothers influence the hedonic polarity of their neonates' initial olfactory responses. Within the first 4 days after birth, infants not only recognize the individual signature of the amniotic fluid they inhaled during the last days before term (Marlier *et al.*, 1998, Child Dev., 69: 611–623; Schaal *et al.*, 1998, Behav. Neurosci., 112: 1438–1449), but they can also perceptually extract an isolated facet from this whole amniotic mixture. Thus, selective perception and learning of odour information is functional at least in the last gestational month.

89. Sensitive periods in early flavor learning

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Several animal model studies and some work in humans provide convincing evidence for sensitive periods during early life that influence a variety of metabolic, developmental and pathological

processes in later life. However, the extent to which early exposure to the sensory properties of foods influences later flavor preferences and food choice in humans remains a mystery, in spite of much speculation. Recent research in our laboratory has demonstrated that flavor experiences in milk (resulting from flavors transmitted from the nursing mother's diet to her milk) modify and serve to establish preferences during weaning. Complementing this research on the breast-fed infant, we are also currently investigating this important issue by using as a model system a class of infant formulas which are hydrolyzed protein based and thus have very pronounced and distinctive flavors which are unpalatable to older-aged infants and adults. We have shown that although infants younger than four months of age willingly accept substantial amounts of, and satiate while feeding, a novel, protein hydrolysate formula, older-aged infants reject this type of formula and the rejection occurs within the first minute of a feed. That this shift in acceptability can be ameliorated by prior exposure is supported by the finding that if infants receive exposure during this early period of acceptability, they will continue to accept these formulas for a considerable period of time thereafter. These observations implicate a sensitive period during development, occurring somewhere between the ages of three and seven months, during which the hedonic value of this formula, and perhaps other foods, is established. Data will also be presented that demonstrate that the type of formula that children are fed during infancy influences their flavor preferences when tested several years later. To our knowledge, the developmental change observed in the acceptance of protein hydrolysate formulas provides the best example of a sensitive period in flavor acceptability in humans thus far identified.

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90. Olfactory learning and the neurobiology of mammalian attachment

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The young of many altricial animals must form an attachment to their parent in order to orient to the parent and receive the care necessary for survival. Using a mammalian model of imprinting in which infant rats must learn an attachment to their mother, we have characterized this attachment and identified a potential neural circuit that may underlie attachment formation.

Neonatal rat pups have a unique propensity for learning an olfactory attachment that seems to be restricted temporally to a sensitive period (Wilson and Sullivan, 1994, Behav. Neur. Biol., 61: 1–18; Spear and Rudy, 1991). Forming an attachment to the odor during the sensitive period also causes dramatic, long-lasting changes in the brain. Both the behavioral and neural changes that occur during the sensitive period appear to be dependent upon the activation of the pontine noradrenergic nucleus locus coeruleus which releases norepinephrine (NE). Indeed, in sharp contrast to adult learning, NE is both necessary and sufficient for learning during the sensitive period. Research from our laboratory suggests that the termination of the sensitive period is controlled by the development of the locus coeruleus and the balance of specific autoreceptors activated by locus coeruleus recurrent collaterals (Wilson and Sullivan, 1994; Sullivan *et al.*, 2000, Behav Neurosci.; Nakamura and Sakaguchi, 1990, Prog. Neurobiol, 34: 505–526).

Another characteristic of olfactory learning during the sensitive period is that pups have difficulty in learning odor aversions, presumably to prevent pups from developing an aversion to maternal odor following rough treatment from the dam. Specifically, odors paired with moderately aversive stimuli such as shock (0.5 mA, 1 s) results in a subsequent preference for that odor. Following the termination of the sensitive period (10 days old), when pups begin to walk, odor-shock conditioning easily result in an odor aversion. Threshold for shock detection does not appear change ontogenetically (Stehouwer and Campbell, 1978, Exp. Psychol.: Anim. Behav Proc., 4: 104–119). Recent results from our lab suggest that the lack of amygdala participation in sensitive period learning may underlie pups' difficulty in learning odor aversions.

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91. Neuroethology of early olfactory learning in the rabbit

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We increasingly believe that to understand olfactory function it is necessary to understand olfactory learning, that is, the inflluence of experience on odor-guided behavior and its neural substrate (Hudson, 1999, J.Comp. Physiol. A., 185: 297–304). The European rabbit (*Oryctolagus cuniculus*) provides an unusually good opportunity to investigate this (Hudson *et al.*, 1999, in Box and Gibson, eds, Mammalian Social Learning, Cambridge University Press, pp. 142–157). Already *in utero* the young learn odors originating from their mother's diet, and without further postnatal experience, demonstrate a preference for these in food-selection tests at weaning and as adults. Furthermore, this experience is associated with specific enhancement in the sensitivity of the olfactory epithelium to the learned odorant. Newborn rabbits also show rapid and robust postnatal learning. If the mother's ventrum is scented just before the once-daily, 3 min nursing, the pups will learn to associate the novel odor with the maternal pheromone essential for the release of nipple-search behavior and sucking in a single exposure. When subsequently placed on the ventrum of a non-pheromone producing female or on a rabbit fur scented with the experimental odor, conditioned but not naive pups respond with the distinctive nipple-search behavior. This one-trial learning is robust, enduring for several days at least without further experience, it is mediated by the main rather than the accessory olfactory system and occurs most readily during a sensitive period of 2–3 days after birth. It thus provides an unusual opportunity to investigate processes supporting olfactory learning in a newborn mammal.

Present research is directed to identifying characteristics of the mother and suckling situation that act as reinforcers of this learning, and the role of neurotransmitter systems and neural structures in supporting the memory formation. A central question is whether the mechanisms involved in olfactory learning and memory at such an early age are fundamentally different from those known to operate in adult mammals.

Glutamate Receptors

92. A cloned taste receptor for umami and its functional properties

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The mechanisms by which taste cells detect glutamate and other umami stimuli has been studied through biochemical, electrophysiological and behavioral analyses. In mammalian taste buds, evidence has accumulated for the presence of ion channels gated by glutamate as well as metabotropic (G protein-coupled) receptors for glutamate. Ionotropic glutamate receptors were demonstrated in taste cells by several laboratories using patch-clamp and imaging methodologies, which did not distinguish between apical (chemosensory) and basolateral (modulatory or synaptic) functions for the receptors. On the other hand, behavioral experiments in rats indicated that umami taste may be generated through a receptor that can be activated by L-AP4, an agonist at a subset of metabotropic glutamate receptors. We previously showed by *in situ* hybridization, that the metabotropic glutamate receptor type 4 (mGluR4) is expressed in a subset of circumvallate and foliate taste buds in the rat. We have recently found the presence, in taste tissue, of an unusual variant mRNA for mGluR4. The variant includes a dramatically truncated 5′-end, such that a large portion of the coding sequence for the extracellular putative glutamate-binding domain is lacking. To assess whether this receptor mRNA is functional, we have expressed it in cultured CHO cells and examined the response of transfected cells following glutamate stimulation. The truncated receptor (which we term taste-mGluR4) couples negatively to a cAMP cascade in CHO cells. Importantly, it displays an EC_{50} for glutamate of ~300 μM, which is over 100-fold higher than the EC_{50} for the full-length 'brain-mGluR4'. Taste-mGluR4 is also activated by L-AP4, a compound that mimics the taste of MSG in rats. The concentrations of both glutamate and L-AP4 needed to activate taste-mGluR4 are quite similar to the thresholds concentrations at which nerve and/or behavioral responses can be detected, especially in very young rodents. Thus, functional properties of the cloned taste-mGluR4 receptor are similar to key features of umami taste, and allow us to propose that it serves as a taste receptor for umami. Further pharmacological properties of this receptor will be discussed, including its response to additional agonists and antagonists, and to the potential amplification of the response when glutamate and nucleotide monophosphates are co-applied to transfected cells.

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93. Glutamate transduction mechanism in mouse taste cells

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It has been proposed that taste cells may possess multiple receptors, including metabotropic (mGluR4) and ionotropic (NMDA) glutamate receptors, for transduction of umami taste presented by monosodium glutamate (MSG). In order to clarify the role of mGluR4 in transduction, we investigated the effects of MSG and 2-amino-4-phosphonobutyrate (L-AP4), a mGluR4 agonist, on taste cells by biochemical and electrophysiological methods in C57BL mice. The responses of the chorda tympani nerve (CT) to MSG were suppressed by gurmarin, a sweet response inhibitor, indicating that MSG response may be mediated by sweet receptors as well as umami receptors, while the CT responses to L-AP4 and the glossopharyngeal nerve responses to MSG were little suppressed by gurmarin, suggesting that these responses may be mediated by only umami receptors. In the biochemical study, concentrations of adenosine 3′,5′-cyclic monophosphate (cAMP) and inositol 1,4,5-triphosphate (IP_3) in taste tissue were measured by radiobinding assay kits. Stimulation with MSG significantly elevated both cAMP and IP_3 levels in the fungiform papillae. The increase in cAMP in response to MSG might occur through sweet receptors, because MSG was found also to stimulate sweet receptors, as mentioned above, which is known to result in cAMP increase (Nakashima and Ninomiya, 1997, Chem. Senses, 22: 338). On the other hand, the increase in IP_3 levels might relate to intracellular events mediated by mGluR4, since it has been reported that L-AP4 caused increment of $[Ca^{2+}]_i$ in taste cells (Hayashi *et al.*, 1997, Chem. Senses, 22: 699). Whole-cell patch clamp recording from isolated taste cells from circumvallate and foliate papillae showed that L-AP4 induced not only outward currents with a decrease in conductance but also inward currents with an increase in conductance at about resting potential. These inward currents reversed at +10 to +30 mV, suggesting that cation conductance was activated by L-AP4, which could lead to depolarization of taste cells. These results strongly support the idea that mGluR4 and then phospholipase C activation via G protein are involved in the transduction mechanism for umami taste, and also suggest the possibility that activation of phospholipase C may result in activation of cation conductance as well as $[Ca^{2+}]_i$ elevation through IP_3 production.

94. Ionic mechanisms of glutamate responses

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Umami is a unique basic taste, which displays a synergistic effect between glutamate and nucleic acid. The transduction mechanisms for umami are believed to involve membrane bound receptors on taste cells. However, details of the transduction mechanisms for umami are poorly understood. In mammals, umami taste appears

to be transduced by several receptor types that may be related to glutamate receptors of the vertebrate central nervous system. Recently, a metabotropic glutamate receptor 4 (mGluR 4)-like receptor was cloned and shown to be expressed in rat taste buds (Chaudhari *et al.*, 2000, Nature Neurosci., 3: 113–119). To elucidate the mechanisms for umami taste, the responses of isolated mouse taste cells to MSG and glutamate receptor agonists were examined using whole-cell patch-clamp recordings and optical Ca^{2+} imaging. Glutamic acid, L-AP4 (mGluR group III agonist), NMDA (NMDA type agonist), AMPA (non-NMDA type agonist) and ibotenic acid (mGluR group I, II and NMDA type agonist) were used. Data were obtained from taste cells isolated from female mice (811 weeks old) of the C57BL/6J strain, which have been shown to be highly sensitive to MSG. Under voltage-clamp at –80 mV and with K^+ in the pipette, application of MSG elicited inward currents with an increase in membrane conductance in some cells and outward currents with a decrease in membrane conductance in other cells (Oh *et al.*, 1997, ISOT XII abstract). L-AP4 elicited only outward current with a decrease in membrane conductance, NMDA elicited only inward current with an increase in membrane conductance, and AMPA did not produce a response. Interestingly, ibotenic acid, which has strong umami taste, elicited large inward currents. The inward currents resulted primarily from the opening of Ca^{2+} channels, and the suppressive outward currents from the closing of non-selective cation channels. Using Ca^{2+} imaging, stimulation with MSG, L-AP4 and NMDA resulted in increases in intercellular Ca^{2+} concentrations, while AMPA had no effect. These data suggest that MSG, L-AP4, NMDA and ibotenic acid all could result in release of neurotransmitter from taste cells.

95. Characteristics of umami responses in rats

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Umami is a Japanese word, introduced by Ikeda (1918), referring to the taste of monosodium glutamate (MSG), an essential taste effect of sea tangle which has been traditionally used in Japanese cuisine. It is accepted that umami is a unique taste independent of the classical four basic taste qualities. Nucleic acid derivatives such as inosine monophosphate (IMP) are also known as umami substances. Synergism, an enhancement of umami, occurs when MSG is mixed with IMP. The uniqueness of the taste of umami substances and the degree of synergism differ greatly among species of animals. Our previous study (Yamamoto *et al.*, 1991, Physiol. Behav., 49: 919–925) showed that rats could not discriminate the taste between umami substances and sweet-tasting substances, suggesting no particular receptors for umami substances in rats. We have also found that the chorda tympani plays a major role in mediating the taste of umami substances, followed by the greater superficial petrosal nerve, and the glossopharyngeal nerve has only a minor role. Recently, however, Chaudhari *et al.* (1996, J. Neurosci., 16: 3817–3826; 2000, Nature Neurosci., 3: 113–119) found that the metabotropic glutamate receptor, mGluR4, was expressed in circumvallate and foliate taste papillae in this species. Their behavioral experiment showed that MSG and an agonist for mGluR4, L-AP4, elicited similar taste in rats. 0We recorded chorda tympani responses of rats and obtained the following results. L-AP4 (5 mM) showed synergistic effects like MSG when mixed with 0.01 M IMP. An antagonist for mGluR4, MAP4 (40 mM), did not suppress the responses to 5 mM L-AP4 and the mixture of 5 mM L-AP4 and 0.01 M IMP. An anti-sweet peptide, gurmarin (50 μM), suppressed the responses to the mixtures of both 0.1 M MSG and 0.01 M IMP, and 5 mM L-AP4 and 0.01 M IMP. Although no synergism occurred for the mixtures of MSG and sweet substances, the responses to the mixtures of L-AP4 and sweet substances were synergistically enhanced, but they were not suppressed by MAP4, gurmarin or pronase E. These results suggest that umami receptors may not be simply understood by the established glutamate receptors such as mGluR4, and that there are more than two types of mechanisms for eliciting the synergistic effect of umami taste.

96. Genetics of umami substance acceptance

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One very useful tool for studying the genetic basis for individual differences in flavor acceptance is inbred strains of mice. We have investigated the 48 h intake of umami substances [monosodium glutamate (MSG); inosine-5′-monophosphate (IMP)] relative to water in 28 inbred strains of mice and have found enormous between-strain variability. Two of the strains with a pronounced difference in relative MSG and IMP intake [C57BL/6ByJ (B6) and 129/J (129)] have been used to conduct behavioral and genetic analyses. The behavioral analysis suggested that both sensory/taste and postingestive factors may contribute to the strain difference. The genetic analysis has shown that the B6 phenotype of high MSG acceptance was inherited as a recessive trait in the F2 generation. To detect genetic loci underlying the strain difference in MSG acceptance, we conduct a genome-wide linkage analysis genotyping the F2 mice. Our data show that the difference in MSG and IMP acceptance between the B6 and 129 mice is not related to salty or sweet taste responsiveness and most likely represents specific preference for umami compounds. The ultimate goal of these studies is to identify genes responsible for modulating intake of umami substances and to characterize/identify their mode of action, for example the involvement of taste and/or post-ingestinal factors.

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97. Responses to glutamate in the macaque

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The issue that has divided opinion over the status of umami as a basic taste is its relationship with the taste of sodium salt. Some researchers conclude that the umami quality elicited by monosodium L-glutamate (MSG) is independent of that evoked by NaCl or any other primary taste stimulus, and so qualifies as a basic taste. Others are not convinced that the taste of MSG is sufficiently separate from that of NaCl to justify such a designation. That issue is addressed here, informed by electrophysiological data from the hindbrain of rats and from the forebrains of macaques.

In a multidimensional space, generated from patterns of neural activity evoked in the rat's hindbrain by an array of taste stimuli, MSG does indeed lie near NaCl. Yet when sodium transduction is

disrupted by the lingual application of amiloride, the impact on the taste response to MSG is minimal and the pattern of activity it elicits is unaltered. Thus, MSG presumably generates a taste quality that transcends saltiness, one that is independently maintained even as saltiness is severely compromised. This conclusion would be in accord with the metabotropic transduction mechanism described for MSG, in distinction to the ionotropic mechanism hypothesized for sodium salts.

MSG is an effective stimulus throughout the macaque's taste system, with a central dynamic intensity range of 0.01–0.30 M. Approximately one-third of the taste cells at each synaptic relay respond to MSG at 0.1 M. It has been suggested that the primary taste cortex is the site of gustatory discrimination in the macaque. Similarities among neural response profiles here most closely resemble human reports of similarities among the same stimuli. Responses at other levels do not account for the fine discriminative capacity of humans, but relate more closely to the hedonic quality of the stimulus.

Thus, it is noteworthy that MSG evoked a neural response profile in primary taste cortex that correlated quite well with that elicited by NaCl, in accord with the pronounced salty component humans report for its taste. At succeeding synaptic relays, however, that relationship deteriorates, becoming increasingly distant and labile. At higher-order gustatory relays, MSG evokes a profile that is no more similar to those of any of the basic taste stimuli than they are to each other. This implies that MSG warrants independent status as a basic taste stimulus, serving as the prototype for the umami quality.

98. The representation of umami taste in the primate primary and secondary taste cortex

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There is a population of neurons in the primary taste cortex in the frontal operculum and insula and in the secondary taste cortex in the orbitofrontal cortex of the macaque with responses to sodium glutamate, and this population has responses which are different to the responses to the prototypical tastants glucose, sodium chloride, hydrochloric acid, and quinine. Extending this analysis, it was shown first that single neurons that had their best responses to sodium glutamate also had good responses to glutamic acid (e.g. 0.05 M); second that the responses of these neurons to the nucleotide umami tastant inosine 5′5-monophosphate (e.g. 0.0001 M) were more correlated with their responses to monosodium glutamate than to any prototypical tastant; third that there is a partly sensory-specific decrease in the responses of these neurons produced by feeding to satiety; and fourth that some of these neurons in the orbitofrontal cortex respond to umami taste and also to olfactory stimuli associated with the taste of glutamate.

These findings show that there is a representation of umami taste, which can be activated by the taste of monosodium glutamate in the mouth, in the primate primary and secondary taste cortex. The findings also show that the pleasantness of umami taste is represented in the primate secondary taste cortex. The neurophysiological finding of convergence of glutamate and corresponding olfactory stimuli onto some neurons in the orbitofrontal cortex suggests that umami flavour could be produced in a strong form by a combination of glutamate taste and corresponding olfactory stimuli, and this was confirmed in a human psychophysical study in which the savory odor was methylfuryl disulphide.

Neuroimaging studies in humans are showing that an insular (primary) and orbitofrontal (secondary) taste area can also be identified. These areas may also be activated by umami taste.

Sour Taste

99. The role of the proton in human sour taste: a psychophysical perspective

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The purposes of these psychophysical studies were to attempt to determine the role of the proton in human sour taste, to measure the relative impacts of the free proton and the undissociated proton on sour taste, and to test whether various acids stimulate different qualities of sour taste.

First, matching studies were conducted in which subjects were asked to discriminate between two compounds. The psychophysical experiments consisted of a series of forced-choice (duo–trio) trials. Across sets of trials, the concentration of one acid (the standard) was held constant, while the concentration of the other acid (the test acid) was varied semi-randomly. Comparisons were made among strong acids, strong acids versus weak acids, and among weak acids. Second, the contribution to sourness by the undissociated proton in weak acid solutions was tested. Initially, several weak acids were intensity matched to 3 mM HCl (a fully dissociating acid). The matched concentrations were next titrated with NaOH and the curves examined for points of intersection. In this manner, we could determine whether the oral environment or lingual epithelium functioned with an equivalent buffering capacity. That is, if undissociated protons (in the beaker) were dissociated in the oral environment, then there must be 'something' removing protons from the anion and therefore operating with an equivalent buffering capacity.

The results of the first matching method showed that: (i) all acids (both weak and strong) at low intensities are not discriminable; (ii) strong acids are at equal pH when they are indiscriminable; and (iii) weak acids (and weak versus strong acids) are not at the same pH when they are indiscriminable. These data further demonstrate that human subjects are able to discriminate among very small changes in the concentration of strong acids. The oral buffering method showed that although every subject could intensity match every acid, each subject differed slightly in the concentrations of their matches. Nevertheless, all subjects showed that their matched acid concentrations were titrated to the approximately the same degree at pH 4.0.

From the matching experiments several conclusions are possible. First, there is only one quality of sour taste. Thus, every acid is ultimately coded in the same way. Secondly, the anion is not contributing to the quality of the sour taste, which sharply contrasts acids with their salt counterparts. Thirdly, for strong acids, only the free proton concentration is important and the mouth is very good at tracking this. Fourthly, for weak acids, the undissociated protons are somehow contributing to the sourness. And, therefore, something in the oral cavity or lingual epithelium is counting all or some of these not-yet dissociated protons. Although

each subject appears to differ slightly in the degree to which this buffering occurs, all subjects express a lingual buffering capacity of pH ~4.0. Thus, we predict that when equal for sourness, taste cells experience the same pH from the acids because of this buffering effect. Support: This research was supported by R29 02995 to P.A.S.B.

100. Current concepts of sour taste transduction

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Sourness, or the taste perception of acidity, influences food selection in most species, including humans. Initiation of the perception of sourness involves a cascade of events that begins at the level of the taste receptor cell (TRC). Recognition that protons are the chemical signal for sourness presents the investigative challenge to understand how TRCs detect H^+ on the lingual surface and transduce that chemical information to neural signals. When TRCs are exposed to acid, several ion channels are activated, including potassium channels (Bobkov and Koleshikov, 1999, Neurosci. Lett., 264: 25–28), an NPPB-inhibited anion conductance (Miyamoto *et al.*, 1998, J. Neurophysiol., 80: 1852–1859) and a proton-gated sodium channel known as MDEG1 (Ugawa *et al.*, 1998, Nature, 395: 555–556). An important question remains unanswered, however. Does TRC detection of H^+ occur at the level of the cell membrane of TRCs or does detection involve intracellular events that then trigger the signal transduction pathway(s)? Early data obtained in isolated TRCs with the whole cell patch clamp technique indicated that H^+ may transit the cell membrane of TRCs through an amiloride-inhibited channel, which suggested that this electrical event initiated sour taste transduction (Gilbertson *et al.*, 1992, J. Gen. Physiol., 100: 803–824). However, a series of observations in intact animals and in individual TRCs have suggested that the detection of H^+ does not involve an amiloride sensitive channel. Our studies of chorda tympani nerve activity demonstrated that amiloride did not affect the CT response to application of H^+ to the lingual surface and that application of voltage pulses across the lingual surface did not influence the H^+-induced CT nerve response. Instead our data indicated that the cascade begins in the intracellular domain of TRCs, because the intracellular milieu of TRCs changes rapidly when isolated TRCs are exposed to changes in extracellular pH (pH_o). Measurements of intracellular pH (pH_i) using microfluorometry and BCECF, an intracellular pH sensitive fluoroprobe, have demonstrated that pH_i closely tracks changes in pH_o and that amiloride does not affect the pH_i response to altered pH_o (Lyall *et al.*, 1997, Am. J. Physiol., 273: C1008–C1019; Stewart *et al.*, 1998, Am. J. Physiol., 275: C227–C238). Since detection of H^+ normally occurs in polarized TRCs, we have recently developed a method of independently perfusing the apical and the basolateral domains of polarized TRCs in individual fungiform taste buds while measuring TRC pH_i fluorometrically. With this technique we have observed that the TRC response to extracellular pH changes is dependent on the side of the cell that is exposed to the changes. pH_i tracking is attenuated by a factor of 8 when the pH_o changes are induced on the apical side ($\Delta pH_i/\Delta pH_o = 0.09$) as compared with when they are induced on the basolateral side ($\Delta pH_i/\Delta pH_o = 0.69$). These results show that the apical TRC membrane has much reduced acid permeability, so that full activation of the TRCs from the apical side requires a drop in pH into the range capable of stimulating a sour response *in vivo*, where the threshold is a pH value below 4. Taken together these data indicate that detection of sour taste involves acidification of the intracellular domain of TRCs which then triggers signal transduction.

101. Sulfamate tastants

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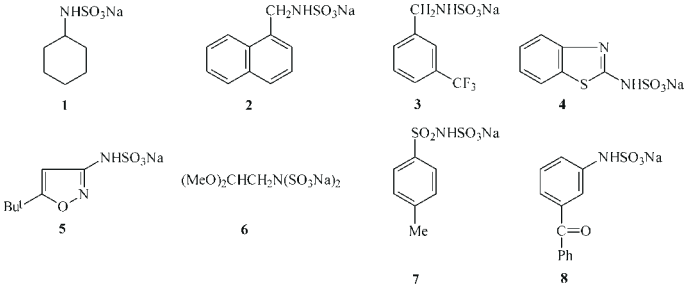
For some years we have had a programme in operation in Galway involving the synthesis of sulfamates ($RNHSO_3Na$), their taste evaluation and the development of structure–taste relationships (SARs). The work has focused principally on sweet-tasting sulfamates because of current interest in sodium cyclamate (**1**) analogues (EU–AIR scheme [AIR–CT94–2107]. See website at http:/www.fst.rdg.ac.uk/people/pparke/tostq.htm).



However, in our work it has been found that bitterness and sourness and various aftertastes also arise. Often two tastes are found in the same compound, e.g. sweetness/bitterness, sourness/sweet aftertaste. Surprisingly, saltiness has only been rarely found in the the sulfamates.

Some of the sodium salts that we have prepared are exclusively sour and some display a portfolio of tastes e.g. sour/bitter, sweet/bitter etc. Some of the compounds, e.g. **2**, **3**, **4** and **5**, display sourness only but their aqueous solutions give pHs which are in the alkaline region and others, e.g. **6**, **7** and **8**, show 'normal' (?) behaviour and have pHs < 7 in the acidic range.

A variety of tastant results including panel findings and accompanying pHs will be presented and discussed.

102. Cloning and expression of an ASIC1 from human taste tissue

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Acid sensing ion channels (ASICs) constitute a family of ion channels that mediate the perception of pain that accompanies tissue acidosis in sensory neurons. One member of this family, ASIC2a (MDEG1), was cloned from rat vallate and hypothesized to act as a sour taste receptor in rat taste buds (Ugawa *et al.*, 1998,

Nature, 395: 555–556). To investigate the possibility that an ASIC may function as a sour taste receptor in human taste buds, RTPCR was performed with taste tissue obtained from the fungiform papillae of human volunteers. Taste papillae from volunteers were removed after anesthetizing a small portion of the anterior tongue (Spielman and Brand, 1995, in Experimental Cell Biology of Taste and Olfaction, CRC Press, pp. 25–32). Using the published sequence of human brain ASIC1, primers were designed to amplify the entire coding sequence. The PCR product obtained was subcloned and sequenced, and found to differ from the human brain ASIC1 only in the substitution of glycine for aspartic acid at amino acid position 212. Searches for ASIC2a and ASIC3 in the human taste tissue were unsuccessful. Expression of human taste ASIC1 in *Xenopus* oocytes injected with complementary RNA produced rapidly-desensitizing inward currents in response to a shift in extracellular pH from 7.5 to 5.5, characteristic of the rat brain ASIC1 channels (Waldmann *et al.*, 1997, Nature, 386: 173–177). Amiloride applied in the bath solution during two-electrode voltage clamp analysis blocked the inward currents with an IC_{50} of 130 nM, nearly two orders of magnitude lower than the IC_{50} reported for rat brain ASIC1 in outside-out patches (Waldmann *et al.*, 1997). Recovery from block with 0.1 mM amiloride after three bath exchanges was 87% of the initial current. Immunohistochemical and *in situ* hybridization localization studies are in progress.

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103. The acid sensing ion channel BNC1 is not required for sour taste transduction

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Several acid sensing ion channel (ASIC) subunits have been molecularly identified in neurons, where they are believed to form H^+-gated cation channels that participate in the detection of painful stimuli associated with tissue acidosis. Reports indicate that at least one of these subunits, BNC1 (also known as ASIC2, MDEG1 and BNaC1), is present in rat vallate taste cells, suggesting a possible role in sour taste transduction (Ugawa *et al.*, 1998, Nature, 395: 555–556). Consistent with these findings, we have shown previously that ~80% of rat vallate taste cells respond to bath-applied citric acid (pH 5) with a rapidly activating inward current that desensitizes during sustained application of acid. The current reverses at ~+30 mV and is partially suppressed by amiloride (500 μM), suggesting that an H^+-gated cation channel may mediate the response (Lin and Kinnamon, 1999, Chem. Senses, 24: 570). To determine if BNC1 is involved in sour taste transduction, we have compared proton-gated currents in isolated vallate and foliate taste cells of BNC1 null mice (BNC1–/–) and their wild-type littermates (BNC1+/+). In the BNC1+/+ mice, rapid perfusion with citric acid (pH 4.5 or 5) at a holding potential of –80 mV elicited a rapidly activating inward current in 75% of the taste cells examined (n = 16). Similarly, 75% of taste cells of the BNC1–/– mice also responded to acidic stimulation (n = 20). There were no significant differences in the percentage of taste cells responding, peak current amplitudes, or rates of activation and desensitization between BNC1–/– and +/+ mice. To determine if the BNC1 null mice had similar sour taste preferences to their wildtype littermates, we conducted standard 48 h two-bottle preference tests using HCl solutions ranging from pH 1.5 to pH 3. No significant differences in preference ratios were detected between the BNC1–/– and +/+ mice in these experiments. In a second experiment we examined the effect of 50 μM amiloride on the taste preferences of BNC1–/– and +/+ mice to HCl at pH 2.4. No significant effects of amiloride were observed on sour preferences of any mice, and no differences were observed between wildtype and knockout mice. We conclude from these experiments that BNC1 is not essential for sour taste transduction.

104. Ionic mechanisms of sour responses in frog taste receptor cells

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It is commonly accepted that taste receptor cells (TRCs) do not utilize second messenger cascades for transduction of ionic stimuli causing salty and sour taste. Of diverse mechanisms reported so far, the direct modulation of ion channels (particularly, K^+ and amiloride-sensitive Na^+ channels) by external protons is believed to be the main contributor to sour responses (Lindemann, 1996, Physiol. Rev., 76: 719–766; Kinnamon and Margolskee, 1996, Curr. Opin. Neurobiol., 6: 506–513; Herness and Gilberson, 1999, Annu. Rev. Physiol., 61: 873–901). Although such a mechanism may effectively regulate TRC membrane potential, it is unclear how the non-specific modulation of ionic flux may encode sour modality. Theoretically, a proper change in K^+ and Na^+ concentrations or channel blockers known to taste bitter may polarize a cell to the same extent. The necessary specificity of apical membrane–acid stimulus interaction may require a membrane H^+ receptor (Ugawa *et al.*, 1998, Nature, 395: 555–556). A number of cellular systems, including nociceptive neurons, express proton-gated cation channels to monitor extracellular pH (Waldmann *et al.*, 1997, Nature, 386: 173–177). Our findings indicate that such a pathway may also be present in frog TRCs. Particularly, we found that isolated frog TRCs generated either hyperpolarizing or depolarizing receptor potentials in response to acid stimuli (HP TRCs and DP TRCs, respectively) (Bobkov and Kolesnikov, 1999, Neurosci. Lett., 264: 25–28). Bath solution acidification increased K^+ permeability in HP TRCs (most likely by activating H^+-gated K^+ channels), thereby leading to their hyperpolarization (Bobkov and Kolesnikov, 1999). Such channels were not found in DP TRCs that, in contrast, express K^+ and H^+ permeable cation channels activated by external millimolar K^+ as a ligand (Kolesnikov and Margolskee, 1998, J. Physiol., 507: 415–432). The responsiveness of DP TRCs to acid stimuli was strongly regulated by external K^+ due to the presence of these channels. Notably, the individual dissociation of taste disks yielded preparations each containing both HP and DP TRCs. This indicates that both cell subtypes coexist in a frog taste organ. Given that the intercellular space in a taste disk is very narrow (~0.01 μm), our estimate suggests that the pH activation of the K^+ current in HP TRCs may elevate extracellular K^+ by 1–10 mM for 1 s, leading to both

depolarization and enhanced pH sensitivity of DP TRCs. Thus, by exploiting paracellular pathways and working in concert, HP and DP TRCs may provide coincident detection, amplification and encoding of acid stimuli.

105. Rat vallate taste cells exhibit a hyperpolarization-activated cationic current which is enhanced by sour stimuli

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Hyperpolarization-activated currents have been implicated in repetitive firing in neurons and heart cells as well as in phasic activity such as beating of cilia. The successful cloning of these channels indicates the presence of four gene products (HCN 1–4, in humans) that form channels sharing cyclic nucleotide sensitivity, activation by hyperpolarization and permeability to Na^+ and K^+. We have recently observed an analogous current in rat CV taste cells. Here we describe its properties, which are similar to those of the HCN family of channels.

Whole cell recordings from taste cells were carried out in slices of rat lingual epithelium containing circumvallate papillae. The results are from 83 taste cells which exhibited a hyperpolarization-activated current (I_h). Hyperpolarization of the membrane potential results in a slowly developing inward current (see Figure 1). Kinetics and amplitude depend on membrane potential. The activation curve is sigmoid and fit by the Boltzmann equation (half activation near –100 mV). The activation time course could be fit by a single exponential. The time constant decreased with hyperpolarization. The reversal potential of I_h was near –30 mV, consistent with a mixed cationic current. I_h persisted during block of outward K^+ currents (by replacing intracellular K^+ with Cs^+), and was reduced in 15 mM extracellular Na^+. I_h was blocked by 1–2 mM extracellular Cs^+. In some but not all taste cells, citrate (pH 3–5, applied at the taste pore with a pico-spritzer) induced an inward current and an increase in conductance. Comparison of the steady state current-voltage relation of the cell in control and following citrate (pH 3–5) indicated the typical voltage-dependence of I_h. Citrate application enhanced I_h with an apparent depolarizing shift in activation. This I_h appeared to be present on the apical membrane of taste cells since inward current and enhancement of I_h could be observed in the absence of pH-effects on voltage-gated K^+ or Na^+ currents. Such effects were clearly noticeable when the stimulus jet reached the basolateral membrane.

Our results are consistent with a model in which mucosal H^+ shifts the voltage-dependence of activation in the depolarized direction, or directly gates the I_h-channel. In contradistinction, in other tissues I_h is not enhanced but suppressed by extracellular acidification. I_h enhancement is expected to result in an inward current. I_h may thus provide a novel mechanism of sour transduction in CV taste cells. The activation of I_h by sour stimuli will depend on the membrane potential and on intracellular cAMP, which also shifts the activation curve in the depolarizing direction. In chemosensory cells of the rat vomeronasal organ I_h appears to be involved in setting the resting potential. I_h may play a similar role in some CV taste cells. The slow kinetics of I_h are ideally suited for such a role.

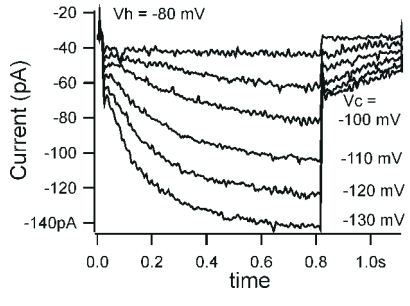

Figure 1

Clinical

106. Etiology, epidemiology and course of olfactory disorders

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Patients with olfactory disorders are often referred to the local ear, nose and throat clinic. In our county of 260 000 inhabitants, there are ~40 new patients per year, but it is supposed that many more people get impaired smell function, very often without knowing it. All patients have been investigated by physicians, specially trained in smell and taste deseases. We have found similar etiology of olfactory disorders as elsewhere—viral infections, nasal polyposis, head trauma and age—but sometimes no cause is found at all. After olfactory investigation with a threshold test, an identification test and a questionairrey, some patients were treated with corticosteroids. Although most patients with olfactory disorders did not improve, some patients benefited from the treatment. We have tried to relate different categories of chemosensory disorders to exposure to chemicals in work and to smoking habits.

107. Assessment of olfactory disorders using psychophysical, electrophysiological and imaging techniques

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Measurement of chemosensory event-related potentials (CSERP) is possible through the use of odorant delivery devices which produce accurately timed pulses of odorants within a flowing air stream at a given temperature without transient pressure artifacts. For clinical applications, H_2S or vanillin, stimuli believed to have little or no ability to stimulate intranasal trigeminal free nerve endings, are used to elicit olfactory event-related potentials (OERP). To produce stimulation of the intranasal chemosomatosensory system CO_2 is used at a concentration >40% v/v. Other than H_2S or vanillin, it has been demonstrated to specifically activate the trigeminal system without simultaneous olfactory activation. Late nearfield CSERP are measured by means of a standardized procedure that is sensitive to olfactory dysfunction seen in anosmic/hyposmic patients, Kallmann's syndrome, Parkinson's disease, MCS patients or patients with temporal lobe epilepsy. Cortical generators of these responses were identified in the area of the superior temporal sulcus. We also could

demonstrate that these areas are also activated using functional magnetic resonance imaging (fMRI). In addition, we found activity in the pyriform cortex and the fronto-orbital cortex which are thought to be the primary and the secondary olfactory corteces. These techniques are now finding their way into the clinical environment in combination with a newly developed psychophysical test system for odor threshold, discrimination and identification (TDI-test). This battery of tests provides a detailed assessment of patients with an impaired sense of smell.

108. Nasal airflow

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The airflow through the nasal passages is often asymmetrical with the dominant airflow alternating from one side of the nose to the other over a period of hours. This alternation in nasal airflow is commonly referred to as the 'nasal cycle' although there is little evidence to support any regular periodicity of changes in airflow. One question, which has often been debated, is whether the asymmetry in nasal airflow has any effect on olfaction. Recent work by Sobel *et al.* (1999, Nature, 402: 35) indicates that an asymmetry in nasal airflow may cause some disparity in olfactory perception and that this may improve olfactory acuity. This paper will discuss the factors influencing nasal airflow and describe the changes in nasal airflow associated with the nasal cycle in man. Our studies on nasal airflow in man indicate that there is much variation in the patterns of nasal airflow associated with the nasal cycle (Flanagan and Eccles, 1997, Acta Oto-Laryngol., 117: 590–595) and only a minority of the population exhibit reciprocal changes in nasal airflow. If asymmetry in nasal airflow does influence olfaction, then it will do so in a rather unpredictable way because of the great variability in patterns of nasal airflow between individuals.

109. Imaging in olfactory disorders

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This review summarizes the correlation between olfactory dysfunction and neuroimaging. Neuroimaging techniques offer a valuable means for evaluating and distinguishing disorders of olfaction. The new modalities and refined techniques allow us to pinpoint the anatomic and pathologic changes of many disorders of the sinonasal cavity and brain which may be related to olfactory deficits. From an anatomic point of view, olfactory deficits can generally be classified into peripheral causes or central (intracranial) causes. In the assessment of the peripheral causes of olfactory deficits, CT and MR imaging reveal anatomic information and structural changes, give a suggestion of differential diagnosis, and provide the road map which may be needed for surgical intervention. In the evaluation of the central causes of smell disturbances, MR imaging, functional MRI and volumetric assessments of olfactory-eloquent structures can provide the links between olfactory dysfunction and lesions in the brain.

110. Biopsies from the human olfactory epithelium

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Biopsy and analysis of the human olfactory epithelium is important in the understanding of olfactory dysfunction. In 1982, we developed an instrument and technique for the safe biopsy of olfactory epithelium in the intact, living human. This procedure allowed for morphologic study of the normal human epithelium, followed by a comparison of this normal ultrastructure to that in a variety of pathologic states. This technique has been in continuous use for the past 18 years and has been widely disseminated to other centers (e.g. NIH, Cincinnati, Boston, Baltimore, Philadelphia, San Diego in the USA; Germany; Korea; Japan; Australia). No major complications have been reported to date. The technique will be discussed and illustrative biopsies, normal and pathological, will be presented. Newer techniques for tissue processing will also be considered.

111. Distortion of olfactory ability: diagnosis and treatment

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Clinically, olfaction can fail in any of three ways: (i) decreased ability (hyposmia, anosmia) and two types of distortion, (ii) distorted quality of perceived odorants (troposmia) and (iii) perceived odor when no odorant is present (phantosmia). The last two conditions are usually much more upsetting to a person's quality of life than a simple loss. A loss of olfactory ability often accompanies a distortion. The pathophysiology of troposmia is probably a decreased number or function of primary neurons so that an incomplete characterization of the odorant is made. In phantosmia, there is either an abnormal signal or inhibition from the primary neurons that is perceived by the central olfactory system or peripheral olfactory neurons 'trigger' a central process. The clinician's goal is to carefully define the problem (e.g. taste versus smell, real versus perceived, one versus two nostrils), to perform the appropriate exam and testing, and to provide therapy if possible. A metabolic problem like trimethylaminuria needs to be considered in the diagnosis. Treatment includes assurance with no active therapy (because many of these will naturally resolve), topical medications, systemic medications, anesthesia to the parts of the nose or tongue, and surgical excision of olfactory epithelium. The endoscopic operation to excise olfactory epithelium is a difficult operation, often involves repair of a CSF fistula, and is only rarely indicated (16 procedures in 11 years). Our experience with 16 nostrils is that all except one are free of their phantom odor perception and five operations needed to be repeated after an initial failure. Although the goal of the operation is to destroy all the olfactory tissue, eight of the nostrils have had a return of olfactory ability after the surgery.

112. Olfaction and neurodegeneration

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Most neurodegenerative diseases are associated with some impairment of olfaction particularly idiopathic Parkinson's disease (IPD)

and Alzheimer's disease (AD). Despite current antipathy, the olfactory defect may be aetiologically more important than the associated disorder of movement or cognition Olfaction was examined in the following neurodegenerative conditions: IPD, drug-induced PD, progressive supranuclear palsy (PSP), multiple system atrophy (MSA), corticobasal degeneration (CBD), motor neuron disease (MND) and AD. We used the University of Pennsylvania Smell Identification Test (UPSIT) and obtained olfactory evoked potentials (OEPs) in response to H_2S gas. Pathological studies were performed on olfactory bulbs in IPD and MND. IPD: 126/155 (81%) patients had significantly abnormal UPSIT scores compared with controls: $P < 0.0001$. 12/37 (32%) had prolonged OEP latency with normal amplitude measurement on OEP but 27 had absent or unclear readings. In drug-induced PD, 5/10 had abnormal UPSIT scores. For MND 9/58 (16%) were abnormal on UPSIT. OEP were performed in 15 patients. In nine the responses were normal for latency and amplitude measurements. In AD UPSIT scores were abnormal in all eight patients examined. OEP was normal in the four subjects who could be tested. In the remaining conditions—PSP, MSA and CBD—only UPSIT was performed. Olfaction was normal in all except for the MSA group. All olfactory bulbs from IPD patients showed typical Lewy bodies. There was excess lipofuscin deposition in the MND olfactory bulb neurons. Most neurodegenerative disease is associated with olfactory disorder. Olfaction has not been studied in diffuse Lewy body disease but in Huntington's chorea there is often marked impairment. The significance of the association is unclear but it might provide a clue to the underlying mechanism of disease.

113. Olfactory disorders in internal medicine

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Abnormalities of smell have been reported in a variety of internal disease. Endocrine diseases, including adrenal cortical insufficiency, Cushing syndrome, congenital adrenal hyperplasia, chromatin-negative gonadal dysgenesis, hypogonadotropic hypogonadism, various menstrual abnormalities, pseudo-hypoparathyroidism and hypothyroidism, as well as detoxification disorders such as chronic liver failure and chronic renal insufficiency are among the most common causes of olfactory disease associated with internal disease. Recently we could demonstrate that 63.6% of a sample of patients with cirrhosis of the liver ($n = 50$) were hyposmic and 4.5% of them were even anosmic. Global psychometric measurements exhibited a positive correlation with olfactory sensitivity; the highest coefficient of correlation was found for the odor identification test. Zinc levels, serum bilirubin levels and other standard liver function parameters showed no correlation The most interesting finding was that the aetiology of the chronic liver failure had no influence on the degree of olfactory loss. A similar experience was made in patients with chronic renal disease. In concordance with others, we found an elevated threshold for odor perception which was dependent on the degree and the duration of renal impairment. Acute removal of uraemic toxins by dialysis did not correct olfactory disturbances, suggesting a long-lasting effect of uraemia on olfactory function. However, after renal transplantation, a normal odour perception returned in most patients, indicating the capacity of the olfactory system to recover once the concentration of uraemic toxins remains below a critical threshold. The knowledge of the olfactory deficit associated with internal disease, having a high incidence, contrasts the generally accepted distribution of the etiology of olfactory disorders, attributing them mainly to nasal disease (21.5%), prior upper respiratory disease (20.5%) or head trauma (22.5%), only 20% usually being classified as idiopathic or as miscellaneous causes. These discrepancies indicate the need for more research in this field.

114. Anti-inflammatory and surgical therapy of olfactory disorders

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Head trauma, upper respiratory tract viral infections and sino-nasal disease each account for ~20–25% of olfactory dysfunction. In the case of head trauma the olfactory disturbance is commonly thought to result from shearing of the olfactory nerve filaments with consecutive degeneration of the olfactory epithelium. After viral infections loss of olfactory cells has been demonstrated. In both situations the olfactory loss is sensorineural and therefore difficult to treat. Allergic rhinitis, polyposis and chronic sinusitis, on the other hand, may cause hyposmia by blocking the nasal airway and preventing odorants from reaching the olfactory receptors. Contrary to sensorineural disorders, conductive disorders are often amenable to treatment. The presentation will present both surgical and medical methods of treating sino-nasal disorders with special focus on the improvement of olfactory symptom

Plasticity in the Olfactory Pathway

115. Plasticity in the primary olfactory projection: paths sensory axons take to find their target glomeruli.

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It has been demonstrated that olfactory sensory neurons which express a specific odorant receptor project their axons to a stereotypical pair of glomeruli in the olfactory bulb (reviewed in Mombaerts, 1999, Annu. Rev. Neurosci., 22: 487–509). Furthermore, odorant receptor expression has been shown to be necessary for glomerular targeting by axons of olfactory sensory neurons but it is not sufficient for correct targeting (reviewed in Mombaerts, 1999). To further understand the stereotypic and apparently highly specific topographic projection of axons expressing the same odorant receptor, we have examined the primary olfactory projection within the olfactory nerve and glomerular layers of adult mice that concurrently express the M72 odorant receptor gene and a reporter gene consisting of the green fluorescent protein (GFP) fused to tau. A key objective was to determine whether fasciculation of axons is a important determinant in establishing the olfactory pathway. Serial reconstructions of the axonal projections of M72-IRES-tauGFP-expressing neurons were generated on the confocal microscope. Within the olfactory nerve layer, axons were generally found to be loosely aggregated in the outer regions of the layer. However, as axons approached the glomerulus they projected deeper into the olfactory

nerve layer and fasciculated prior to entering the neuropil. While the vast majority of axons entered the glomerulus from the directly apposed nerve fiber layer, a small subset of axons were observed (in all animals examined) to project atypically through the glomerular layer. These axons entered the glomerular layer in anomalous regions and then projected around and between glomeruli, before finally entering the correct glomerulus. Therefore axons that initially miss their target glomeruli are able to correct their mistake by projecting along atypical paths. Importantly, because single axons could follow atypical paths to the target glomerulus, it appears that fasciculation itself is not a prerequisite for correct targeting. It remains to be determined what guidance cues are utilized by axons that allows them to correct errors in targeting.

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116. Reinnervation of the olfactory bulb and functional capacity after recovery from lesions of the olfactory epithelium

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The olfactory system maintains perceptual stability throughout life despite its capacity for wholesale or piecemeal replacement of sensory neurons. The epithelium is precisely mapped onto the bulb according to two principles: zonality (or rhinotopy) and receptotopy. Thus, the mapping of the projection is a candidate substrate for odor encoding. Perceptual stability may depend on the maintenance of precise connectivity between the receptor sheet and the bulb despite turnover. Conversely, a deterioration of function, for example, due to aging, head injury or epithelial insult in humans, may occurs when that precision is lost. We have assessed both reinnervation of the bulb and odorant encoding following large-scale turnover of the neuronal population to determine the limits on accuracy and mechanisms that may subserve it.

Inhalation of MeBr, a selective olfactotoxin, destroys both neurons and non-neuronal cells in the olfactory epithelium, but leaves the olfactory nerve undamaged. The epithelium rapidly reconstitutes both neuronal and non-neuronal populations. The division of the epithelium into discrete, concentric zones, assayed by OCAM/mamFas II or odorant receptor expression, is also restored to normal or near-normal. Newly generated olfactory neurons rapidly reinnervate the bulb; new axons fill glomeruli by 3 weeks after lesion and have matured by 6–8 weeks. Zonality of the projection recovers to be indistinguishable from normal. However, receptotopy is not restored to pre-lesion precision. For example, after lesions that result in a permanent reduction in the population of neurons (due to respiratory metaplasia during reconstitution), typical glomeruli at the posterior margin of the bulb are relatively hypoinnervated or remain permanently denervated. Receptotopic convergence is distorted under these conditions; neurons that normally project onto the necklace glomeruli instead innervate numerous other glomeruli that are not in the necklace. Likewise, neurons that normally target a pair of typical glomeruli further anterior in the main bulb project to more than the usual two after recovery from the lesion. In contrast, if a substantial number of neurons projecting to the necklace are spared, the newly born neurons return to the appropriate glomeruli and do not innervate ones outside of the necklace. In keeping with the distortion of axon targeting after regeneration and reinnervation, behavioral performance on a five-odorant identification task that was learned prior to lesion is also markedly abnormal. The lesioned animals take longer to recover to criterion performance after a 2 month post-lesion recovery period than do non-lesioned animals rested for the same period. In addition, the processing of odorant stimuli by lesioned-recovered animals is different, as demonstrated by multi-dimensional scaling analysis.

There are limits on the anatomical accuracy of the reinnervation of the bulb after wholesale turnover of pre-existing neurons, and these limits have behavioral consequences. The preservation of fibers along which the newly growing axons can track by selective fasciculation may be critical to the restoration of the receptotopic precision that is characteristic of the pristine state and required for full function.

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117. Chemorepulsive semaphorins and the control of primary olfactory axon development and regeneration

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Mounting evidence indicates that in addition to short-range surface bound axon-guidance cues, long-range diffusible molecules contribute to the accurate patterning of axonal projections. As has been shown for short-range cues, secreted long-range cues can be either positive (attractive) or negative (repulsive) in nature. Most of the secreted chemorepulsive molecules identified so far belong to the family of phylogenetically conserved semaphorins. Recently two gene families, the neuropilins and plexins, were shown to serve as receptors for the secreted semaphorins. We have studied the role of sema3A, the prototype secreted semaphorin, in the developing and the lesioned olfactory system. During embryonic development neuropilin-1 positive primary olfactory axons appear to be consistently excluded from sema3A positive areas, while penetration of these axons into the developing olfactory bulb correlates to a local decline in sema3A expression levels. In the mature olfactory system sema3A and neuropilin-1 are expressed in a pattern that suggests the involvement of sema3A-neuropilin-1 signalling in maintenance of primary olfactory projections. Neuropilin-1 is expressed by immature and nearly mature primary olfactory neurons, while the neuropilin-1 ligand sema3A is present in second order target neurons of primary olfactory neurons, the mitral- and tufted cells. Bulbectomy results in the appearance of numerous sema3A positive fibroblast in the bulbar cavity. These fibroblasts encapsulate regenerating olfactory axons and apparently contribute to the failure of these axons to regenerate deep into the bulbar cavity. Current studies focus on axogenesis, synaptic stability and primary olfactory axon regeneration in sema3A knockout mice (obtained from Dr. Taniguchi) and in sema3A transgenic mice with postnatal neuronspecific overexpression of sema3A.

118. OMP finds a partner

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The highly restricted pattern of cellular expression, developmental regulation and phylogenetic conservation of sequence has led to the use of olfactory marker protein (OMP) expression as a hallmark of maturation of olfactory receptor neurons (ORNs). Evidence for function derives from studies of OMP-null mice generated by homologous recombination. These mice exhibit altered behavioral and electrophysiological activities (Buiakova *et al.*, 1996, Proc. Natl Acad. Sci. USA, 93: 9858–9863; Youngentob and Margolis, 1999, NeuroReport, 10: 15–19). The altered EOG observed in the OMP-null mice can be restored to near normal by delivery of OMP via an adenoviral vector (L. Ivic *et al.*, in preparation). However, determination of the mechanism by which OMP influences these activities has been elusive. Our preliminary biochemical data implied the existence of protein that could act as an OMP partner. To identify this putative OMP-partner, T7-phage libraries expressing cDNAs from olfactory neuroepithelium as fusions with phage coat protein were used in an iterative panning strategy to screen for phages expressing fusion proteins that could interact with OMP. Phage plaques were picked and their inserts sequenced. All of the phage plaques selected had in-frame inserts, 90% of which had identical nucleotide sequences (342 bases). The deduced amino acid sequence matched the first 84 amino acids of a previously described cDNA. This cDNA predicts a 15kDa protein, which is similar to the molecular weight that we previously observed as potential OMP partners in radiolabeled gel overlays.

Sequence features of this protein suggest potential sites for transition metal binding and for post-translational modification. These sites suggest mechanisms by which the OMP-partner could interact with cytoplasmic OMP to influence ORN electrophysiology. For this hypothesis to be valid, OMP and its partner must be co-localized in ORNs. This has been confirmed by *in situ* hybridization in both the olfactory and vomeronasal neuroepithelia.

To confirm the interaction between OMP and OMP-partner, we synthesized peptides that together span most of the predicted protein sequence of the OMP-partner. These were tested for their ability to interact with OMP. At least one of these peptides can be chemically cross-linked to OMP, indicating proximity in solution. Further support for this derives from NMR studies. The peptides were individually titrated into samples of [^{15}N]ratOMP and the binding monitored by the observation of changes in the ^{1}H and/or ^{15}N chemical shifts of resonances in the 2D ^{1}H–^{15}N HSQC NMR spectra. One peptide showed a specific interaction with OMP, further confirming the specificity of the interaction. Preliminary analyses using other techniques confirm this interaction. These data argue convincingly that we have identified an OMP-partner whose characterization will provide insight to the mechanism of OMP function.

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119. The cell biology of the glial cells of the olfactory system

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The olfactory ensheathing cell (OEC) is the major glial cell of the olfactory system, ensheathing the olfactory axons on their course towards the olfactory bulb. It has become a cell of great interest due to its ability to remyelinate axons in demyelinating lesions and support regrowth of neurites. To characterize the cellular and molecular properties of these cells in more detail, we purify OEC using the glial marker, O4, and fluorescence activated cell sorting (Franceschini and Barnett, 1993, Devl Biol., 155: 337–350). Using a panel of glial markers, we have shown that with time in culture the purified OECs developed into two antigenically and morphologically distinct cell types, termed Schwann-cell like and astrocyte-like. Conditioned medium from type-1 astrocytes contains a potent mitogen and survival factor for OECs which turns out to be an neuregulin isoform (Pollack *et al.*, 1999, Eur. J. Neurosci., 11: 769–780). We have generated a clonal OEC cell line by infection with the retrovirus containing the temperature sensitive mutant gene of the large T antigen. This cell line was able to remyelinate an experimentally-created demyelinated lesions of the rat spinal cord (collaboration with Dr R.J.M. Franklin, University of Cambridge) (Franklin *et al.*, 1996, Glia, 17: 217–224) with peripheral-type myelin supporting the concept of a myelinating Schwann cell-like OEC. A second cell type containing intermediate filaments was found in the lesion that did not ensheaths axons reflecting the plasticity of primary OECs and this clonal OEC cell line. Recently, we have identified the human OEC, which labels with similar markers to the rat OEC possesses Schwann cell like properties, and is able to remyelinate experimentally created demyelinated lesions. Further studies are ongoing on the property of the OEC with particular reference to the Schwann cell which shows these cells have similar but distinct properties. These studies have shown that *in vitro* the OEC, unlike the Schwann cell can integrate into an astrocyte-rich environment.

120. Glial cells in the receptor-axon sorting zone: anotherneuron-glia interaction plays a key role in olfactory development

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Olfactory receptor neurons (ORNs) tuned to respond to particular odor properties project their axons into the brain to terminate in particular glomeruli. The glomeruli are not organized in a pattern that closely reflects the spatial distribution of ORNs. Therefore, the topographical mapping mechanisms that have been so well studied in visual systems may not be very useful in developing olfactory systems. We are studying the issue of ORN axon guidance and targeting in the moth *Manduca sexta*, where the anatomy of the developing adult antennal system offers special advantages.

In *Manduca*, we have recognized a special 'sorting zone', located at the base of the olfactory (antennal) nerve as it enters the

antennal lobe of the brain. Here ORN axons shed the neighbor–neighbor relationships established in the antenna and make new associations with axons destined for the same glomeruli in the antennal lobe. The sorting zone contains a dense cluster of glial cells, which BrdU experiments reveal arise from the antennal lobe. The ingrowth of ORN axons triggers the production of this cluster.

In previous experiments, we discovered that a different set of glial cells, the neuropil-associated glia of the antennal lobe, must be present in order for ORN axons to induce the formation of glomeruli. We now have used the same paradigm to reduce the population of glial cells in the sorting zone, in order to test the importance of these glial cells. We have discovered that when glial numbers are severely reduced, ORN axons do not sort properly into the fascicles destined for particular glomeruli. Many axons, in fact, pass right through their normal target, the antennal lobe and project along second-order pathways to higher brain centers.

We conclude that glial cells in the developing antennal system not only aid in the construction of glomeruli, but also in some way interact with the ingrowing ORN axons to cause them to reorganize in a way essential to normal targeting. Current studies are exploring the nature of the axon–glia interaction.

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Environmental

121. Adverse effects of environmental odors: annoyance responses and symptom reporting

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Industrial, municipal and agricultural activities are often associated with the emission of odorous compounds or odor mixtures which affect the population. Adverse effects of such environmental odor exposure are typically discussed under the category of annoyance. Both the scientific understanding of odor annoyance as well as science-based odor regulation have long been hampered by the difficulties of exposure assessment on the one hand and the assessment of adverse population effects, including symptom reporting, on the other. Since about 1990, however, progress has been made and lawful exposure–response associations as well as their modification by person-related factors have been elaborated; these will be reviewed.

Assessment of odor exposure in such studies is typically done by means of either dispersion modelling or systematic field inspections using trained observers. The outcome of such efforts is either in terms of average odor concentrations or odor frequencies as the exposure index for defined areas. Effect assessment in the affected population is typically done by means of questionnaires covering odor annoyance, symptom reporting and relevant covariates known or suspected to influence the exposure–response association.

Systematic exposure–response contingencies have been established for odor annoyance responses and symptom reporting for a variety of industrial sources (e.g. Cavalini *et al.*, 1991, J. Environ. Psychol., 11: 123–142; Steinheider and Winneke, 1993, J. Environ. Psychol., 13: 353–363; Steinheider *et al.*, 1998, J. Psychophysiol. Suppl., 64–79). However, the precision of annoyance prediction from odor exposure measures rarely exceeds $r^2 = 0.17$ in such studies. This is partly due to the fact that person-related factors, such as age, perceived health or stress-coping styles, modify exposure–response associations. Furthermore, other influential factors either related to stimulus attributes, e.g. pleasantness/unpleasantness of odorant mixtures, or related to the effects of memory traces from past experiences modifying annoyance-related sensory information processing have not yet received adequate attention. Recent findings and approaches bearing on these uncertainties will also be discussed.

122. Hypersensitivity to environmental chemicals: possible mediation by inflamed airways

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Some individuals claim to have special sensitivity to environmental chemicals. These people may claim hypersensitivity to just one agent, such as environmental tobacco smoke, or to a broad and even shifting range. Experiments have largely failed to corroborate such sensitivity. For example, people with chemical sensitivity who claim to notice odors before others have no better measured olfactory sensitivity. A failure to find an explanation for the complaints of these individuals has often led them to search for explanations outside orthodox medicine, with attendant pitfalls. An intersection of clinical and research observations led us to the suspicion that the problem of hypersensitivity may lie in actual hyperchemesthesis in the upper airways, including the sinuses. For example, some patients with recurrent sinusitis find odors more vivid and annoying only when their sinusitis becomes active. Treatment with antibiotics brings the odors down to normal, non-annoying levels. In screening non-symptomatic subjects for inclusion in studies of environmental chemicals, we have found (i) much more low-level inflammation than expected and (ii) more reactivity among these subjects in physiological assays of the airways and eyes. Existing inflammation may hold the key to important individual differences in how people react to environmental odors.

123. Improving animal welfare: development of active and passive flux measurement of odours and gases in animal houses

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Poor ventilation in livestock buildings causes build up of both odour and noxious gases in the environment, and leads to stress and ultimately poor health and suffering in the animals. Passive flux measurements for ammonia, based on Ferm tubes, are currently implemented, but a need exists for automated flux measurements for a number of volatile species found in livestock buildings. In collaboration with Silsoe Research Institute, we have been developing automated flux measurement systems that will offer a means of measuring flux, indirectly for some species and directly for others, in the immediate vicinity of livestock buildings. In order to do this, we have adapted a number of passive and active sampling techniques, as well as sensor types, to create a distributed

sensor network capable of being deployed around and within a livestock building, to measure flux. In this presentation we describe the sensors developed for ammonia, oxides of nitrogen, methane and malodorous compounds, and the circuitry involved in creating a distributed sensor network.

There are at least three sensor systems that are commonly used for detecting and quantifying gas concentrations in environmental monitoring: solid state sensors, electrochemical sensors and light-based sensors. Combined with these, diffusive or passive samplers are known as the cheapest method of monitoring air quality and can give a good overall picture of average pollutant levels in an area. The low cost per tube permits sampling at a number of points in the area of interest. Although results from single-point passive samplers are not as precise as those from automatic point monitors, the accuracy and reproducibility of the measurements has increased over recent years. We have adapted these techniques to flux measurements.

In our case we adapted both active gas-sensing devices and diffusive sampling devices to enable flux monitoring of a number of volatile chemicals in livestock areas, and we are currently in the process of developing a compact, portable distributed system of sensors and electronics suitable for agricultural use.

124. Measurement of odor and odorants from swine facilities

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Complaints of odor nuisance and health symptoms related to odor exposure have become more frequent in communities with confined animal facilities, wastewater treatment plants, and biosolids recycling operations. Health complaints frequently include eye, nose and throat irritation, headache, nausea, diarrhea, hoarseness, sore throat, cough, chest tightness, nasal congestion, palpitations, shortness of breath, stress, drowsiness and alterations in mood (Schiffman *et al.*, 1995, Brain Res. Bull., 37: 369–375; Schiffman, 1998, J. Animal Sci., 76: 1343–1355; Schiffman, 2000, J. Agromed., 7, in press). Proceedings of a workshop held at Duke University on April 16–17, 1998, co-sponsored by Duke University, the Environmental Protection Agency (EPA) and National Institute on Deafness and Other Communication Disorders (NIDCD), suggested three possible paradigms to account for health complaints from exposure to odor (Schiffman, 2000). In the first paradigm, the symptoms are induced by exposure to odorants (compounds that induce odor) at concentrations that also cause irritation. The cause of symptoms is actually the irritation rather than the odor; the odor which occurs simultaneously with the irritation simply serves as an exposure marker. Thresholds for irritation are higher than those for odor perception. In the second paradigm, health symptoms are induced by odors in the absence of irritation. This typically occurs with exposure to odorant classes such as organic amines and sulfur-containing compounds. In the third paradigm, a co-pollutant (such as bioaerosols consisting of endotoxin, dust from food, airborne manure particulates, allergens, microorganisms or toxins) that occurs in odorous emissions is responsible for the reported health symptoms.

This presentation will describe psychophysical and analytical experiments designed to quantify the levels of odors and odorants associated with swine facilities, and to determine if these levels are sufficient to induce health complaints via paradigm 1 above. Human psychophysical experiments were performed near swine facilities and in the laboratory to measure nasal lateralization thresholds for emissions from swine facilites. Irritation, unlike odor, can be localized to one nostril or the other. The lowest concentration at which an odorous sample can just be lateralized (the nostril receiving the stimulus can be determined) constitutes the irritant threshold (Wysocki *et al.*, 1997, Am. Ind. Hyg. Assoc. J., 58: 704–712; Cometto-Muñiz, 1998, Ann. N.Y. Acad. Sci., 855: 648–651). Lateralization thresholds were found for many air samples from swine facilities, which indicates that these odorous emissions constitute an irritant. Gas chromatography and mass spectrometry were also performed to obtain estimates of the levels of volatile organic compounds that are present in irritant air samples.

125. Agriculturally derived odors: irritants, cognitoxins or just plain nasty smells

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Odors emanating from agricultural activities are creating conflicts between farmers and their neighbors. To some, the experience downwind is that of a toxic chemical. To others, the malodorants are pure irritants. Others may note the presence of a malodor and correctly attribute its source to a farming operation and may not be offended. Still others may be unaware of the odor's presence, having become adapted to its presence long ago. We have been investigating the perception of environmental odors and their amelioration in the laboratory, in model situations and in real-life settings.

Collaborative studies in our laboratories have employed both human sensory and chemical analytical techniques to investigate the complex nature of odors emanating from swine slurry (SS) and spent mushroom compost (SMC). To those not intimately familiar with swine operations, SS is an intense and extremely unpleasant odor, which has been confirmed in sensory analyses. Odors from SMC are far less unpleasant and highly dependent on the sample. Using psychophysical methods, we can dissociate pure odor from chemosensory irritation in the nose. This allows us to address issues related to subjective irritation versus true sensory irritation. Other approaches rely upon slight deception, namely, providing false information about chemicals to determine whether otherwise harmless odorants can become toxins simply through cognitive bias (cognitoxin). Using gas chromatography with olfactometry allows us to identify the offensive odorants in complex mixtures derived from agricultural operations.

Many malodorants at high concentrations are subjective irritants, but they may not be true sensory irritants, at least at the exposed concentrations. Negative cognitive bias can exacerbate the reports of odor intensity and irritation, whereas positive bias has the opposite effect. Furthermore, long-term exposure to odorants/irritants can result in long-term adaptation. Addition of

compounds approved for use by humans to ameliorate fecal odors (e.g. in colostomy bags) to SS or SMC reduces the malodor; however, powdered active charcoal appears, at present, to be the single most effective malodor counteractant.

Studies of environmental odors and their control require a multidisciplinary approach to understand the myriad facets of complexity. Sensory and analytical methodologies can provide meaningful data; however, without additional education of the general population, such information will remain within academic journals or, at best, text books.

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126. Health effects of environmental odours and bioaerosols due to composting

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Some studies have shown that health effects may result from an exposure to environmental odours (Shustermann *et al.*, 1991, Environ. Health Perspect., 94: 25–30; Steinheider *et al.*, 1998, J. Psychophysiol. Suppl., 64–79). In these studies the exposure to environmental parameters was not measured regularly. Composting sites are an example of plants releasing odours and bioaerosols containing microorganisms (e.g. *Aspergillus fumigatus* and *Actinomycetes*) into the surroundings.

Table 1 Reported annoying odours and measured microorganisms in the residential environment

	A_{near}	$A_{distanf}$	B_{near}	$B_{distant}$	C_{near}	$C_{distant}$
Annoyed by odours (%)	80.19	25.76	87.98	17.39	41.05	11.76
Type of annoying odours (%)						
Disgusting	9.26	0	10.13	0	2.74	0
Faecal	75.93	43.24	50	54.17	36.99	33.33
Compost-like	14.2	35.14	37.34	4.17	42.47	0
Other	0.62	21.62	2.35	41.67	17.81	66.67
Total microorganisms and *Actinomycetes* (CFU/m^3)	10^6	–[a]	[b]	–	[b]	

[a]Not measured.

[b]Like uphill of the plant.

In a study concerning the environmental and public health relevance of composting sites, residents living near three sites (A_{near}, B_{near}, C_{near}) were studied and compared with residents living in the same district but distant from the site ($A_{distant}$, $B_{distant}$, $C_{distant}$). The perceived general health status (complaints and previous doctors' diagnoses) was accessed with a special environmental health questionnaire; tendency to show somatic symptoms by the SOMS2 (Rief *et al.*, 1992, Diagnostica, 38: 228–241) and health-related quality of life by SF 36 (Ware *et al.*, 1994, The Health Institute, New England Medical Center, Boston, MA). At the same time, residents were also asked whether annoying smells occurred in their neighbourhood, how they could be characterized and the exposure to microorganisms in the air of the residential area was measured (Table 1).

The results of the three questionnaires were analysed by logistic regression. Hints for an influence of residential bioaerosols on general health, tendency to somatize and health-related quality of life were found. Odour annoyance alone, even if stated as disgusting, did not show these influences. Epidemiological studies concerning health effects of industrial odours should also consider measuring data characterizing the exposure of study population to substances in the environment.

Imaging

127. Odor coding and associative plasticity in the olfactory system of the honey bee

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Olfactory reception in insects is to a large degree accomplished by the antennae. From there, receptor neurons project to and terminate in the antennal lobe (AL), a neuropil that is composed of spherical compartments (glomeruli) and considered a structural as well as functional analogue to the vertebrate olfactory bulb. The glomeruli are regarded to be functional units in the sense that they are part of a chemotypic odor representation in the AL. Optical imaging experiments using calcium-sensitive dyes applied to the whole neuropil have revealed that different odors elicit characteristic patterns of glomerular activity. This combinatorial spatial code is bilaterally symmetrical and species specific. However, the spatial representation of odors in the AL is not rigid but rather depends on the associative content of the respective odor. In a differential conditioning paradigm bees were trained to associate a certain odor with a sucrose reward. A second odor was presented without reward, leading to a 'negative' associative content of this latter odor. Before and after training the spatial activity distribution within the AL was optically recorded during presentation of both test odors. Whereas the response to the unrewarded odor reamined unchanged, an increase of the responses to the rewarded odor was observed as a consequence of associative learning. Moreover, the spatial activity pattern did not only change quantitatively but also qualitatively leading to a greater dissimilarity between the activation patterns elicited by both odors after training and thus possibly to a greater discriminability.

How do single antennal lobe neurons, especially those leaving the AL and projecting into higher brain centers, contribute to these spatial odor representations?

Single cells were recorded intracellularly during presentation of different odor stimuli. After staining the cells with the calcium-indicator Fura-2, the spatial activity distribution elicited by different odors within the cells' dendritic regions were optically recorded. Subsequent 3D reconstruction of the stained cells

allowed an assignment of the respective dendritic regions to single identified glomeruli. The recordings so far indicate that the spatial activity distributions across the glomeruli derived from recording single projection neurons correspond to the activity patterns recorded in the whole neuropil. The latter can thus be regarded as the spatial odor representation leaving the AL. However, the response time course of single neurons is more complex than the compound response of the whole neuropil. The responses of projection neurons can include periods of both excitation and inhibition. These dynamical properties are most likely due to AL-intrinsic network interactions.

128. Study of flavor representation in the human brain with fMRI

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In everyday life, the word 'taste' commonly refers to a global perception, the flavor, resulting from the interaction of three major components: (i) chemical stimulation of the taste buds of the tongue; (ii) chemical stimulation of the olfactory epithelium, mainly through the retronasal pathway; and (iii) chemical, thermal and tactile stimulation of the somatosensory system, both on the tongue and on the nasal epithelium. Different studies were undertaken with fMRI, focusing on either one or two of these modalities, in order to understand the relative influence of each component on the global cortical representation of flavor.

A first study was undertaken in France in order to study cortical activation in response to the interaction between pure taste and chemical lingual somatic stimulation. Twelve right-handed subjects participated in this study and underwent an fMRI experiment (3 T MR scanner) with six stimuli. Four stimuli were chosen to be pure gustatory compounds—NaCl (salty), aspartame (sweet), HCl pH 2.4 (acid) and quinine (bitter)—and two were chosen to present both a taste and a lingual somatic component: aluminum potassium sulfate (acid and astringent) and HCl pH 1.6 (acid and pungent). Results showed that no additional activated area was associated with the presence of the chemical somatic stimulation, suggesting a high level of convergence between the two kinds of information. However, multidimensional analysis performed on the numbers of activated voxels across regions of interest allowed discriminating between both modalities. Two different sub-regions could be identified in the insular lobe for taste experiments: the superior part of insula was found coactivated with adjacent opercula whereas the inferior insula was found associated to angular gyrus activation in the left hemisphere. On the other hand, the addition of a somatic component to the taste stimulation resulted in the discrimination from all other areas of the activation of the Rolandic operculum, known to receive direct projections from the lingual nerve.

A second study was conducted in San Diego to study the olfactory perception. Six subjects participated in a preliminary fMRI study (1.5 T MR scanner) and tested two olfactory stimuli among amyl acetate (banana), ethyl butyrate (fruit gum) and citral (citrus). The stimuli were dissolved in water and swallowed by the subjects so that the olfactory stimulation occurred through the retronasal pathway. Results showed activated areas corresponding to previous results acquired on humans with imaging techniques and to elctrophysiological data on non-human primates, including piriform cortex, parahippocampal gyrus, orbito frontal cortex and insula.

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129. Olfactory processing in healthy subjects measured with positron emission tomography

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How the brain processes different olfactory functions is currently unknown. To map the underlying networks we employed PET measurements during monorhinal and birhinal passive smelling of odorless air (AS), of single odors (OS), during discrimination of odor intensity (OD-i) and odor quality (OD-q), and during tests of odor recognition memory (OM).

Twenty-nine healthy, right-handed females (22–29 years) participated in three series of PET studies in which rCBF was measured with an ECAT Exact HR scanner in 3D mode. Several different types of odorous stimuli were used, allowing us to evaluate whether processing of different odours involved different cerebral regions.

During the OD-i the same odor was presented consecutively in different concentrations, whereas in OD-q different odors were employed in suprathreshold concentrations. OD-i and OD-q were tested by asking the subjects whether the previous odor was stronger or weaker (OD-i), same or different (OD-q) than the previous. In the OM task 10 target and 10 distractor odors were presented 60 min after the encoding. The task was to determine for each odor whether it was presented during the encoding or not. The control condition for OS was AS, and for OD-i, OD-q, and OM the collapsed data from AS and OS. The significance level (general linear model) was calculated according to Ledberg *et al.* (1998, NeuroImage) and SPM96 ($P < 0.05$, corrected).

The two statistical approaches yielded similar results. (i) OS activated right and left orbitofrontal, right and left piriform cortex and amygdala, right thalamus, left insular cortex and anterior cingulate. (ii) The pattern of activation varied with the category of odorous stimuli. (iii) A *post hoc* region of interest analysis showed that each hemisphere was engaged in the processing of right-sided as well as left-sided odour stimuli. (iv) OD-i engaged left insular cortex and right cerebellum. These two areas were activated also by OD-q, which, in addition, activated right subiculum, caudate, the orbitofrontal cortex–frontal operculum and the midbrain. (v) OM involved right pyriform cortex, the orbitofrontal cortex, right thalamus, right middle temporal gyrus, right prefrontal cortex, right cuneus, the cerebellum and midbrain.

Odorous stimuli are mediated bilaterally in the brain, but probably with somewhat different patterns, depending on the type of stimulus. The olfactory functions are mediated by a pool of task

independent olfactory core areas, from which one or several regions are recruited during each odor related task. A stepwise recruitment of task-dependent regions outside this pool, which are all directly or indirectly connected to the core regions, subserves in a hierarchical order discrimination of odor intensity, odor quality and odor recognition memory.

130. Differential involvement of left and right orbitofrontal cortex in familiarity and emotional judgements of odours

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Few studies have examined the functional neuroanatomy underlying the cognitive operations involved in olfactory judgements. In a previous work, we asked normal subjects to perform different olfactory tasks (detection, familiarity, edibility), while regional cerebral blood flow (rCBF) was measured with PET (Royet *et al.*, 1999, J. Cogn. Neurosci., 11: 94–109). We demonstrated that the familiarity and edibility judgements activated different cerebral areas. We have further examined the neural correlates of these tasks and additionally examined two other olfactory tasks: intensity and hedonicity. All five olfactory tasks induced rCBF increases in the right orbitofrontal cortex (OFC). However, right OFC activity was highest during familiarity judgements and lowest during the detection task. By contrast, left OFC activity increased significantly during hedonic judgements, but not during either odor detection or edibility judgements. This converges with previous PET studies demonstrating left OFC activation during exposure to emotionally valenced odorants (Zald and Pardo, 1997, Proc. Natl Acad. Sci. USA, 94: 4119–4124; Zald and Pardo, 2000, Int. J. Psychophys., 36: 165–181). These data support a model of parallel processing in the right and left OFC in which the relative level of activation depends on whether the judgement involves odor recognition or emotion.

To test further this hypothesis, we specifically examined neural correlates of responses to emotionally valenced olfactory stimuli. We compared olfactory response patterns to those induced by emotionally valenced visual and auditory stimuli. For each sensory modality, rCBF measured during presentation of both pleasant and unpleasant stimuli was compared with that measured during presentation of neutral stimuli. For all three sensory modalities, emotionally valenced stimuli led to increased rCBF in the OFC, the superior temporal gyrus and the superior frontal gyrus, in the left hemisphere. Emotionally valenced olfactory and visual, but not auditory stimuli produced additional rCBF increases in the hypothalamus and the subcallosal gyrus. Only emotionally valenced olfactory stimuli induced bilateral rCBF increases in the amygdala. These findings suggest that pleasant and unpleasant emotional judgements recruit the same core network in the left hemisphere, regardless of the sensory modality. However, this core network is activated in addition to a number of circuits that are specific to individual sensory modalities.

131. Magnetoencephalographic imaging of gustatory brain areas

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Several areas in the human cerebral cortex have been assumed to be the primary gustatory area (area G) (Ogawa, 1994, Neurosci. Res., 20: 1–13). By means of magnetoencephalography, we have successfully located area G at the transition between the inner face of the operculum and the insula in the human cerebral cortex (Kobayakawa *et al.*, 1996, Neurosci. Lett., 212: 155–158), but posteriorly to the central sulcus (Kobayakawa *et al.*, 1999, Chem. Senses, 24: 201–209), in contrast to that in macaque monkeys (Ogawa, 1994). We also found activation in the central sulcus by NaCl stimulation with almost the same latency as that in area G, but less frequently (Kobayakawa *et al.*, 1999). In the present study, we investigated the gustatory evoked magnetic fields (GEMs) originating from area G in response to various concentrations of NaCl to examine the role of area G in recognition of stimulus intensity.

Taste stimuli were 100 mM, 300 mM and 1 M of NaCl desolved in deionized water. Stimuli were applied to the tongue of the subject by using a taste stimulator with rapid-rise time (Kobayakawa *et al.*, 1999). The temperature of the taste stimuli and rinsing water was maintained at the same level as that of the tongue to avoid thermal stimulation. A 400 ms duration of each stimulus and a 30 s inter-stimuls interval were used. The tongue was continuously rinsed with deionized water during ISI Five neurologically normal adults, aged 22–35, participated in the experiment. The 64-channel whole-head SQUID system was used to measure GEMs. In a single session consisting of 40 trials, each subject received a given concentration of NaCl only. Subjects were asked to show the perceived intensity by using their fingers after each trial. Equivalent current dipoles were calculated and located on anatomical MRI of subjects.

In most sessions (18 out of 19), the averaged first peak of the GEMs (peak latency = 177 ms) were yielded in area G. The magnitude of the dipole in area G increased with increased concentrations ($F = 17.67$, $n = 4$, $P < 0.01$). Averaged GEMs in all 64 channels were superimposed on the same sheet to measure the GEM onset latency. The averaged onset latency was 118 ms in response to 1 M NaCl, and it remained unchanged with changes in concentration. Though the reaction time to NaCl decreases with increases in concentration (Saito *et al.*, 1998, Ann. N.Y. Acad. Sci., 855: 493–497), the present results indicate that the onset latency of the GEMs to NaCl was independent of the concentrations used in the present experiment. This might partly be due to the fact that the concentration range used was too high to affect the onset latency. GEMs to more dilute concentrations remain to be studied.

132. Comparison of brain activity induced by stimulation and imagination

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Previous studies using PET and functional MRI (fMRI) have demonstrated that brain activation occurs not only following actual visual, auditory or motor stimuli, but also following the imagination of these stimuli or activities. The aim of this study was to compare brain activation in response to odor stimuli with the response to the imagination of the same odor by fMRI.

Imaging was performed in 13 healthy volunteers using a 1.5 T MRI scanner capable of echo planar imaging (Siemens, Vision). Twenty-two axial images were obtained with a matrix size of 128 × 128 pixels and a slice thickness of 6 mm. Activation maps of those brain areas involved in olfactory processing and the processing of odor imagination were derived using correlation analysis technique (SPM'96b). Stimulus delivery was provided by a specialized olfactometer which allowed rapid delivery of odorants (onset <20 ms) with a defined delivery rate, temperature and humidity. The odorant Eugenol was presented to the left nostrils in four 800 ms bursts within an 'ON' period of 48 s. This was followed by a 42 s 'OFF' period when non-odorous air was delivered. During a second 'ON' period the subjects were asked to imagine the previously smelled odorant. This second 'ON' period lasted for 48 s, like the first one. This procedure was then repeated three times.

Group analysis of 13 subjects revealed surprising similarity in cortical activation during stimulation and imagination with/of Eugenol. Due to the type of analysis, the areas activated in this experiment were mainly secondary/tertiary cortical areas related to association. Intensity of activation during the two conditions was similar except in the medial frontal gyrus and the cingulate gyrus, which were more highly activated during the imagination task.

133. Measuring brain activation due to pleasant odours using fMRI

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fMRI is a non-invasive brain imaging technique in which blood oxygenation level-dependent (BOLD) contrast is used as an indirect marker of neuronal activation. In the study described here, we used fMRI to investigate the areas of the brain responsible for olfactory processing during presentation of vanillin (2.2 p.p.m.) and super-threshold tea aroma.

Eight subjects participated in the study. T_2^*-weighted, coronal images were obtained with 8 mm slice thickness, 128 × 64 matrix size, in plane resolution of 3 mm and 27 ms echo time using a 3 T scanner. Ten multi-slice images were generated every 1.67 s. Odourants were delivered bi-rhinally in five, 0.5 s duration bursts within an ON period of 5 s, followed by a 15 s OFF period in which a constant flow of air, without odorant, was delivered. This procedure was then repeated for 40 cycles, alternating between the tea and vanillin odour. Use of a specialized olfactometer (Burghart GmbH, OM4, Wedel, Germany) allowed delivery of odorants at a constant rate, temperature and humidity, with rapid stimulus switching. After each scanning session, the subject was required to rate both the pleasantness and the intensity of the odours on a scale from +2 to –2. Activated areas were identified via correlation analysis, using a reference function formed from the convolution of the stimulus waveform and a gamma-variate function (4.2 s time to peak). Isotropic, 3 mm resolution, multi-slice inversion recovery echo-planar data sets with grey matter nulled were used for anatomical localization. The mean signal intensity change and number of active pixels were then measured for each of the activated areas.

Significant areas of activation were found bilaterally in the inferior frontal gyrus, in the left superior temporal gyrus, left orbitofrontal cortex, cingulate gyrus, right secondary (SII) cortex and bilateral pre-motor areas, in agreement with previous studies using PET and fMRI (Zatorre *et al.*, 1992, Nature, 360: 339–340; Levy *et al.*, 1997, JCAT, 21: 849–856; Yousem *et al.*, 1997, Radiology, 204: 833–838). Similar brain regions were activated by both odours in all subjects, however, tea induced a larger activation consistent with the more intense subjective response which was measured for the tea aroma (1.3/0.6). Activation was found in the orbitofrontal cortex, an area where activation has been hypothesized to be associated with the pleasantness of the odorant (Rolls *et al.*, 1999, NeuroReport, 10: 453–459), in three subjects in response to the tea odorant and two for the vanillin; in these subjects the pleasantness ratings were between 0.5 and 1. Three subjects indicated that the odorants caused a painful sensation, which seemed to be related to trigeminal effects. Little orbitofrontal cortex activation was found in these subjects. In the majority of brain areas, the measured activation showed a significant attenuation over the 40 cycles of the experiment, consistent with effect of habituation. Activation in SII was, however, found to be relatively constant over the duration of the experiment.

134. Cortical activity evoked by focal electric-taste stimuli

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The central processing of electric-taste in humans has not been previously investigated with modern imaging techniques, and little data exists on the cortical processing of gustatory information from discrete peripheral fields. We hypothesized that electric-taste would have a similar central representation as chemical taste, and that taste processing would occur primarily ipsilateral to the side of the tongue stimulated. Functional magnetic resonance imaging (fMRI) was utilized to observe cortical hemodynamic responses following focal electric-taste stimuli delivered to the right versus left portion of the anterior tongue.

Functional responses to stimulation of the anterior tongue with 1 s electric-taste (25–50 μA) stimuli were observed in 11

right-handed normal adults. Activated areas were identified based on the correlation of each voxel's time course with template functions derived from activation of auditory cortex or visual cortex. For group-averaged analysis, the anatomical and associated activation maps were first warped to Talairach coordinates.

Unexpectedly, the location and hemisphere activated were usually identical regardless of the side of the tongue stimulated. Electric-taste evoked activity in the probable primary gustatory area in the superior insula, in the anterior insula/frontal operculum, and in Brodmann areas 6 and 44 was predominately in the right hemisphere. More ventral insular activation occurred more equally in both hemispheres. Significant activation also occurred in the superior temporal lobe and inferior parts of the post-central gyrus, and scattered in the inferior frontal lobe. The pattern of central activation was more consistent with the results of previous studies that have used chemical taste stimuli rather than somatosensory lingual stimuli, which suggests that electric-taste activated the gustatory system. The right-hemisphere dominance of evoked activity suggests that cortical gustatory processing is related to non-verbal aspects of the response to the stimulus, such as memory or recognition.

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Free Session II

135. Floral odorants: influence of nucleophilic moiety on odour properties

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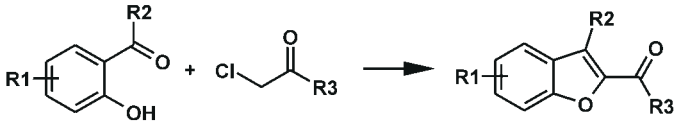
Most hydrocarbons do not exhibit interesting or well defined odours. However, examples are known of alkenes endowed with pleasant fruity, green and floral notes. Taking the megastigmatrienes as references, we have prepared structurally similar molecules by Wittig reactions between cyclohexanone or other ketones and triphenylphosphonium derivatives of alkyl or alkenyl bromides. Fruity and green notes are present in most of the compounds synthesized.

In a second series of compounds, the effect of the functional group on the floral odour has been studied by replacing aldehydic and alcoholic functions with vinyl or alkyl groups. Reference compounds were hydroxycitronellal, lilial, cyclamen aldehyde and other common floral-smelling molecules. The aldehyde function was easily converted by the Wittig reaction with methyltriphenylphosphonium bromide into the vinyl derivative, which in turn afforded, by catalytic hydrogenation, the corresponding saturated compound. Most of the compounds synthesized exhibited pleasant odours, including floral, green and fruity notes. In some cases the main characteristic of the reference odorant were retained in the corresponding hydrocarbons.



This research provides further information on the odour of hydrocarbons and contributes to the understanding of structure–odour relationships in floral and fruity odorants. Hydrocarbons endowed with fresh and pleasant odours could replace aldehydes as additives in detergents for use in the environment.

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136. Response recovery in mouse olfactory receptor cells

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Exposure to odour activates a G protein-coupled cascade in olfactory receptor cells (ORCs), culminating in an influx of Ca^{2+} through cyclic nucleotide-gated (CNG) channels. The resulting rise in intraciliary Ca^{2+} concentration evokes an additional excitatory Ca^{2+}-activated Cl^- current. We investigated mechanisms of Ca^{2+} removal from the cilia and recovery from odour-induced adaptation in isolated ORCs using the suction pipette technique and rapid solution changes at 37C. ORCs were stimulated twice in succession for 1 s with the odour cineole and were exposed either to normal Ringer or a low Na^+/choline solution during the 5 s recovery interval between the two odour exposures.

The 1 s odour stimulus led to an inward receptor current which terminated quickly at the end of stimulation in Ringer. After a 5 s recovery period in Ringer the response to the second exposure had recovered to 81 ± 3% (mean ± SEM, five cells) of its original value. The processes governing response recovery were investigated by exposing the cell during the recovery period between the two odour stimuli to a low Na^+/choline solution designed to incapacitate Na^+/Ca^{2+} exchange. Under these conditions the response to the first odour stimulus was prolonged, decaying more slowly than in Ringer; such response prolongation during exposure to low Na^+/choline solution being seen in a total of six cells. Since no abrupt reduction in junction-corrected current was seen at the moment of solution change, little current can have been carried by Na^+ at that time since the CNG channel conducts choline poorly. The prolonged current therefore seems likely to have been carried by Ca^{2+}-activated Cl^- channels, indicating that $Ca^{2+}{}_i$ remained elevated for an extended period when the external Na^+ concentration was reduced. This result suggests that, as in the frog (Reisert and Matthews, 1998, J. Gen. Physiol., 112: 529–535), Na^+/Ca^{2+} exchange is present in the cilia of mouse ORCs, and plays an important role in extruding the Ca^{2+} which enters during stimulation. Nevertheless, the gradual current decay seen even in low Na^+/choline solution suggests that other mechanisms may also contribute to Ca^{2+} extrusion. When cells were stimulated again following a 5 s recovery period in low Na^+/choline solution, the response to the second stimulus was reduced to 56 ± 5% of its original magnitude, a value significantly different from that obtained when recovery took place in Ringer (paired *t*-test, $P = 5\%$). This depression of the response when recovery took place in low Na^+/choline solution indicates that in the mouse, as in the frog, extrusion of Ca^{2+} by Na^+/Ca^{2+} exchange is important not only for response termination but also for the recovery from adaptation.

137. Differential neural activity in the human amygdala evoked by odorant and non-odorant stimulations

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In epileptic patients entering into a preoperative assessment program for possible surgical treatment, the use of stereotactic electro-encephalography (SEEG) with depth electrodes usefully contribute to localize a suspected site of seizure onset. Taking advantage of this procedure, we have recorded the amygdala SEEG activity in response to olfactory stimulations of 14 patients with partial epilepsy while they were engaged in two tests. Firstly, patients were submitted to a passive stimulation test for which they were asked to smell 12 different odorants and instructed to focus their attention on the stimulus. No explicit cognitive task was required during this test. Secondly, patients were submitted to a suprathreshold detection test including eight odorant stimulations of butanol and eight non-odorant stimulations presented in a counterbalanced order. They had to decide whether the stimulation was odorant or not. For both tests, odorants were presented with an inter-stimulus interval of 1 min.

On the one hand, the SEEG recordings obtained for both tests revealed that each odorant stimulation induced reproducible olfactory evoked potentials (OEPs) in the amygdala. The latter ones were large and could be observed in response to a single odorant. The OEP waveform, consisting in a negative potential followed by a positive potential, was similar to that commonly described for scalp potentials. The OEPs were followed by frequency increases of SEEG rhythms that lasted from 1 to 5 s, and that had previously been described as spindles. On the other hand, the recordings revealed that the non-odorant stimulations of the suprathreshold detection test were ineffective in eliciting OEPs, but induced all the same spindles in the amygdala. From these findings, we can conclude that the OEPs are specific responses to olfactory stimulation and that spindles are rather responses related to breathing. Additional findings showed an effect of stimulus repetition on the peak latencies, which may be attributed to stimulus predictability, that is, to early processes involved in selective attention. Not only does the human amygdala produce differential responses to odorant and non-odorant stimulations, but also its speed of processing appears to be sensitive to recent experience with an odor.

138. Olfactory coding in the moth antennal lobe

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The functional significance of olfactory glomeruli in olfactory coding has been studied in a number of organisms. In both insects and mammals, receptor neurons expressing identical chemical sensitivity have been shown to target the same glomerulus, as in moths (Hansson *et al.*, 1992, Science, 256: 1313–1315; Hansson, 1997, in Cardé and Minks, eds, Insect Pheromone Research. Chapman & Hall, New York, pp. 164–183), or the same pair of glomeruli, as in mammals (Mombaerts *et al.*, 1996, Cell, 87: 675–686). To continue our studies of the moth primary olfactory centres, the antennal lobes, we have expanded the investigations of glomerular targetting in receptor neurons to studies of projection (antennal lobe output) neuron dendritic innervation patterns and imaging of calcium release in the glomerular array. In an earlier study of the sphinx moth *Manduca sexta*, results implied an overlap between receptor neurons and projection neurons processing information regarding the same pheromone component (Hansson *et al.*, 1991, J. Comp. Neurol., 312: 264–278). In the honey bee, optical imaging studies have shown how different odours are often coded by combinations of several glomeruli, even though a few odours were coded by single glomerulus activations (Sachse *et al.*, 1999, Eur. J. Neurosci., 11: 3970–3982).

In the cabbage looper *Trichoplusia ni*, seven physiological types of pheromone-specific receptor neurons target separate glomeruli in the sept-partite male-specific macroglomerular complex (MGC) (Todd *et al.*, 1995, Physiol. Entomol., 20: 349–361). Projection neurons processing pheromone information were investigated and stained intracellularly (Anton and Hansson, 1999, Proc. R. Soc. Lond., 266: 1813–1820). Only a fraction of these innervated the same glomerulus as the corresponding receptor neuron type. A clear difference between neurons processing information regarding major and minor pheromone components was observed: dendritic branches of major pheromone neurons showed a considerably larger overlap with axonal branches of receptor neurons expressing the same specificity than did neurons involved in processing of information regarding minor components. Some projection neurons responding to pheromone stimulation were also found to exclusively innervate glomeruli situated outside the MGC.

In recent studies of calcium release we have observed that this activity correlates well with earlier results from activity-dependent receptor neuron staining, i.e. a glomerulus targeted by a specific receptor neuron type is also the site of calcium release when these neurons are activated. Additionally, we observed that a large number of odours activate calcium release in a single glomerulus. Combinatorial coding thus seems to be less common than what has been shown for the honey bee.

139. Locust aggregation pheromones: plasticity in central processing

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Male desert locusts, *Schistocerca gregaria*, in the gregarious phase produce an aggregation pheromone which attracts other adult males and females, but not nymphs of any developmental stage. The major component of this aggregation pheromone is phenylacetonitril (PAN) (Torto *et al.*, 1994, J. Chem. Ecol., 20: 1749–1762). In the present study, we observed age-dependent changes in aggregation behaviour also in adult locusts. Our goal was to investigate if and how central processing of the aggregation pheromone changes in parallel with behavioural and endocrine changes observed during development and with age in adult locusts. Intracellular recordings from projection neurons were performed to characterize single neurons in the antennal lobe (AL) while antennae were stimulated with aggregation pheromone.

The response profiles of AL neurons changed clearly during development in gregarious locusts. The proportion of component specific neurons (responding to one aggregation pheromone component only) decreased from first instar to adult locusts, while the proportion of generalist neurons, responding to a large variety of components, increased. This result is consistent with the finding that the number of olfactory sensilla increases during development, while the number of AL neurons stays constant as far as is known, leading to a larger convergence of olfactory receptor neurons on each AL neuron in late developmental stages (Ignell *et al.*, 1998, J. Comp. Physiol., 183: 453–465). Also, the proportions of neurons processing information regarding specific components changed. In adult locusts, a larger proportion of AL neurons responded to the major adult pheromone component (PAN) than in nymphs. On the other hand, the proportion of AL neurons responding to a general green leaf volatile, E2-hexenal, did not change during development.

In adult locusts we observed a very low number of AL neurons responding to PAN in 4-week-old individuals compared with 1- to 2-week-old locusts, in parallel with a decreasing attraction to PAN with age. Antennal receptor neurons, however, did not change in sensitivity. Both changes in behaviour and in the responsiveness of AL neurons were correlated with the juvenile hormone level in adult locusts. We conclude from our data that age-dependent changes in central olfactory processing are modulated by juvenile hormone. If the observed changes in olfactory processing during development are also influenced by hormones remains to be shown.

140. Asian food preferences revealed by data mining multiple studies in Japan, Singapore, Indonesia and Australia

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Multiple studies of olfactory, gustatory and trigeminal perception and preference have been carried out over three years in four Asian countries, using different food products but asking a number of common questions in an identical acquisition protocol. The data from these studies were 'mined' for trends and insights into food preferences that might be common or specific to the cultures concerned. Such information is sought by food product developers in approaching the emerging and potentially huge food markets of Asia.

Sensory evaluations were conducted on adult consumers in: Japan, two studies on 44 products and 285 people; Singapore, four studies on 40 products and 200 people; Indonesia, two studies on 56 products and 105 people; and Australia, three studies on 32 products and 125 people. Data were obtained from unstructured scales of perceived sensory intensity for taste (sweet, sour, salty, bitter), overall olfactory strength and trigeminal sense (oral burn), as well as overall liking of the sensory attributes and the whole product.

A correlation matrix for each study showed the extent to which these common attributes co-vary with overall acceptance of the product. By overlaying these for all the studies it could be seen that certain attributes are drivers in certain countries but not in others.

In Japan, perceived taste of sweetness, saltiness and umami were common drivers of food preference. As predicted, Japanese consumers considered attenuated trigeminal components of flavour (low level of acidity and low chilli burn) as very important. Australian consumers revealed by contrast an optimal, medium level of spiciness that determines preference. Singaporean consumers revealed that perceived sourness and chilli strength positively determined preference. Drivers of overall liking of products for Indonesian consumers included chilli, pepper and odour.

A person's food preferences are largely determined by experience, which in turn is generated by their culture. The highly variable importance of chilli in the diets of four cultures studied here (low in Japan; low to medium in Australia; medium in Singapore and Hong Kong and high in Indonesia) suggests that chemosensory experience may create a bias toward one or more of the chemosensory modalities in driving food preferences.

While previous research has generally shown very little difference between members of cultures in chemosensory perceptual judgements, this overview of eleven studies in the four countries in which a common psychometric procedure was used in all four, suggests that culturally driven experience builds up in different chemosensory modalities such that preference becomes driven in different ways in each culture.

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141. Disposition of 141 odorants and 16 major semantic descriptors in a 3D olfactory space

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The classification of odors according to their quality discrimination is an old challenge, often solved by using rules that are more empirical than experimental. In the Siena ECRO congress (1998), we showed that a 3D olfactory space for 141 odorants was able to explain 88% of the experimental psychophysical variance (Callegari *et al.*, 1999, Chem. Senses, 24: 56–57). In the present state, this olfactory space also displays 16 major semantic descriptors, represented by 16 correspondent colors. As shown in the 1998 ECRO congress, the 3D space reveals a hemisphere-like shape, reminiscent of the topographical coding hypothesis of olfactory information at the olfactory bulb level. In the current improved version, each color appears as reasonably well localized into the space, suggesting a continuum rather than a mosaic of clusters. Two versions of this olfactory space are available: one physical, in plastic material, and the other computerized, easier to handle. The space shown in the Brighton ECRO/ISOT congress is the second one.

Initiated by Harper *et al.* (1968, Perf. Essent. Oil Rec., 59: 22–37), the systematic characterization of odorants by a limited number of semantic descriptors was developed by Dravnieks (1985, Atlas of Odor Character Profiles, Data Series 61. ASTM, Philadelphia, PA), which fixed 146 standard descriptors. However, factor analysis of the data obtained with this method gives rise to a rather cumbersome 17-dimensional space explaining 89% of the variance, which is difficult to handle (Jeltema and Southwick, 1986, J. Sens. Stud., 123–136). On the other hand, multidimensional scaling (MDS) applied to direct measurements of similarities were initiated by Woskow, who also yielded a 3D space explaining 86%

of the variance, but for a limited number of odorants (Woskow, 1968, in N. Tanyolaç, ed., Theories of Odor and Odor Measurements. Istanbul, pp. 147–188). Practically, the geometrical growth of experiments for this type of procedure, prevents applying it to >25 odorants. Callegari (1998, Thesis, University of Bourgogne, Dijon) combined the advantages of both procedures to characterize the olfactory quality, by using an algorithm, based on the ratio model of categorization of Tversky (1977, Psychol. Rev., 84: 327–352), which from semantic profiles, leads to similarities comparable to that obtained directly by human subjects. The final space results from the MDS applied to the similarity data derived from the semantic profiles of Dravnieks for 141 odorants. In addition, the 16 kept descriptors out of 146, were selected on criteria concerning at least two odorants with a percentage of applicability (PA) greater or equal to 32 (range 0–100). In these conditions, 56 odorants (40% of the whole) are represented by these 16 descriptors.

Poster Communications

142. GPI anchored proteins in the chemoresponse of paramecium to folate

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Peripheral membrane proteins can be attached to the cell surface through glyco-phosphatidylinositol (GPI) lipid anchors. These proteins can be involved in signal transduction, and often are found in aggregates of surface signaling molecules (Brown and London, 1998, Ann. Rev. Cell Devl Biol., 14: 111–136). The folate binding protein for folate uptake is often used as a marker for the special lipid domains where GPI-anchored proteins are concentrated.

The *Paramecium* chemoresponse to folate appears to be mediated by a GPI-anchored protein, acting as the receptor for folate (Paquette *et al.*, 2000, J. Exp. Biol., in press). Cells that are transformed for antisense expression of a gene, PIG-A, which codes for an enzyme in the first step of GPI anchor synthesis show few GPI anchored proteins, reduced PIG-A transcripts and reduced lipid intermediates. The transformed cells also display decreased responses to folate and glutamate, but not to other stimuli. A polyclonal antiserum against a mammalian folate binding protein is a very specific blocking antiserum for the folate behavioral response; other responses are unaffected by the antiserum. Antigen pre-absorption renders the antiserum ineffective in blocking folate response. The same antiserum recognizes primarily one protein among the *Paramecium* GPI-anchored proteins, and also binds in a pattern on the surface of unpermeabilized cells. Immunoprecipitation using a chicken anti-folate binding protein antiserum that we developed results in isolation of one protein at 30 kDa, and this protein is GPI anchored. It is our future goal to understand how this receptor couples and participates in signal transduction.

Two genes for another enzyme for GPI synthesis, PIG-K, have also been cloned and present additional targets for down-regulation of GPI anchoring. Both PIG-K genes are expressed. The sequence of PIG-K is being used to design more antisense vectors for down-regulation of GPI anchoring. Other reagents that we are using in the study of GPI-anchored proteins include fumonisin, which inhibits ceramide synthesis, and *Clostridium* alpha toxin, which requires GPI-anchored protein receptors to kill cells.

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143. Expression of *c-fos* in the piriform cortex in response to an olfactory learning: an immunohistochemical study in the rat

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The piriform cortex (PCx) is the main area of the primary olfactory cortex and subsequently receives dense inputs from the olfactory bulb. Several lines of evidence suggest that the PCx is a non-homogeneous structure where differential information processing could occur. According to its anatomical organization and its functional characteristics, the PCx is assumed to play an important role in olfactory memory. One way to identify brain areas exhibiting learning-related changes in neuronal activity is to examine the expression of the immediate early genes such as the proto-oncogene *c-fos*. We used immunohistochemical detection of the protein product Fos within the PCx following a two-odor discrimination learning test. Olfactory discrimination training was performed in a four-arm radial maze. Three groups of rats were used: trained (T), pseudo-trained (P) and naive (N). All animals were water-deprived. The T-rats had to learn to discriminate between two odors delivered in two differents arms. One odor of the pair only was water rewarded. Operant conditioning consisted of 20 trials per day. Discrimination was considered as acquired when the animals reached 80% of correct choices. Rats were sacrificed 1 h after the last training session when this criterion was reached. The P-rats were always randomly rewarded with water. The N-rats represented the home cage control (i.e. no behavioral training) group in order to determine the baseline level of Fos. Among this group, some rats were stimulated by either one odor or the other of the pair to determine the effect of odorous stimulation upon Fos expression. Quantitative analysis of the Fos-immunoreactive cells is in progress to compare the patterns of PCx activity in the different groups of rats.

Fos immunoreactivity will be also examined in other structures presumed to be involved in olfactory learning, such as entorhinal cortex or hippocampus.

144. OMP gene deletion alters mucosal inherent activity patterns

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In a companion behavioral study (see Youngentob *et al.*, this meeting) we provide evidence that odorant quality perception is altered in OMP-null animals. Considerable neurophysiological evidence indicates that different odorants produce different spatiotemporal patterns of neural activity at the level of the mucosa. The apparent mechanism for this spatiotemporal differentiation of odorants is the regional variations in the sensitivity of the receptor neurons to different odorants. More importantly, the relative

position of the neurophysiologically determined 'hot spots' (that portion of the activity pattern which makes each odorant unique relative to the others) on the mucosa predicts the relative position of these same odorants in a behaviorally determined perceptual odorant space, thereby suggesting that the mucosal activity patterns serve as the substrate for the perception of odorant quality. Previous evidence has demonstrated a marked decrease in EOG responsivity of the mucosa to odorants, as well as an alteration in response and recovery kinetics in mice lacking the gene for OMP. These observations suggest, therefore, that the absence of OMP would result in an alteration in the odorant-induced spatiotemporal activity patterns that are characteristic of different odorants. This, in turn, would alter the spatiotemporal patterning of information that results from the mucosal projection onto the bulb, thereby changing odorant quality perception.

To test the hypothesis that odorant-induced mucosal activity patterns are altered in mice lacking the gene for OMP, we optically recorded the fluorescent changes in response to odorant stimulation from both the septum and medial surface of the turbinates of both OMP-null and control mice, using a voltage-sensitive dye (Di–4-Anepps) and a Dalsa 128 × 128, 12 bit CCD camera. To maintain continuity with the previous behavioral study, the odorants 2-propanol, citral, carvone, ethylacetoacetate, and propyl acetate were again used. Each odorant was randomly presented twice to each mucosal surface in a Latin Square design. The results of this study demonstrated that, for each of the two mucosal surfaces evaluated in both mouse strains, there do indeed exist different spatial activity patterns for different odorants. More importantly, however, these patterns differed between OMP-null and control mice. These data suggest, therefore, that the alterations in mucosal activity patterns serve as the substrate for the behaviorally observed changes in odorant quality perception in the null mutant.

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145. Deciphering the human olfactory subgenome

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The ability of the olfactory apparatus to discriminate a wide range of odorants is provided by a very large repertoire of G protein-coupled, seven transmembrane helix receptors—olfactory receptors (ORs). The multigene superfamily coding for these receptors, also termed the olfactory subgenome, constitutes up to 1% of the gene count in the human genome, as well as in the genomes of other vertebrates. We initiated a systematic effort to evaluate the composition of this subgenome in humans, both experimentally and by data mining performed on the large-scale sequencing data derived from the Human Genome Project.

The experimental pipeline starts with the design of OR-specific degenerate oligonucleotide primers of various redundancies, corresponding to the most conserved regions of the OR protein (transmembranal helices 2 and 7). The design of such primers is based on a special algorithm that we developed. Fragment of OR coding region is then amplified from human genomic DNA, and spans between TM2 and TM7. These are termed olfactory receptor sequence tags (OSTs). Libraries of OSTs are subsequently subjected to oligonucleotide fingerprinting—a procedure designed to identify and compare DNA clones by their patterns of oligonucleotide hybridization. A clustering algorithm is used to identify sets of OSTs corresponding to the same gene. This allows us an effective, non-redundant sequencing of genes from the OST libraries. OSTs provide the first insight into the olfactory subgenome size and sequence diversity, further serving as a basis for the retrieval of the OR full coding regions from the large-scale sequencing data. The cumulative results are stored in HORDE, the human olfactory receptor data exploratorium (http://bioinformatics.weizmann.ac.il/HORDE). OSTs and other sequences serve as query for BLAST searches against large-scale sequencing data in GenBank, using an iterative procedure for expanding our OR collection. Sequences corresponding to the ORs coding regions are automatically retrieved and compared with the database. We currently use a cutoff value of 99% identity at the amino acid level for accepting new entries into HORDE, except cases in which distinct genomic loci with higher identity are known.

The presently available >300 OR genes belong to 11 distinct families and 73 subfamilies. Family 7 is the largest, potentially as a result of expansion that happened late in mammalian evolution. About 53% have frame interruptions and therefore are considered as pseudogenes. The established experimental and computational devices can be applied to analyze the composition of an olfactory subgenome in any vertebrate species, an ultimate need for the study the evolutionary history of the olfactory genes superfamily, and for full understanding olfactory discrimination.

146. Odorant receptor expression in mature [OMP(+)] and immature [GAP-43(+)] olfactory sensory neurons

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Odorant receptors (OR) are expressed in a zonal configuration in the mammalian olfactory epithelium (OE). This patterning in the OE may contribute to specific innervation patterns within the olfactory bulb (OB). Furthermore, glomerular targeting appears to be contingent upon receptor choice (Mombaerts *et al.*, 1996, Cell, 87: 675–86). This raises a question as to when in the life cycle ORs are expressed. Evidence indicates that olfactory sensory neurons (OSN) express ORs in the absence of the OB (Sullivan *et al.*, 1995, Neuron, 15: 779–789) but does not preclude whether OR expression is an early or late event in OSN differentiation.

In the OE, OMP expression is considered a marker of neuronal maturation. In contrast, immature neurons (those that are still extending axons) express GAP-43. We are interested in determining whether ORs are expressed in mature and/or immature OSNs. To accomplish this we performed *in situ* hybridization with DIG-labeled riboprobes for eight ORs encompassing the presumptive zones in the OE in combination with immuno-histochemistry (IHC) using anti-OMP antiserum.

In addition, we examined tissue from transgenic mice containing

the P2-IRES-tau-lacZ construct (Mombaerts *et al.*, 1996). We performed IHC with a β-gal antibody and either an OMP or a GAP-43 antibody to further examine the issue of OSN maturity relative to OR expression. We found that at least 90% of P2(+) OSNs are also OMP(+) in control P2 mice. This corresponds well with our data indicating that no more than 10% of P2(+) OSNs are also GAP-43(+). One month after unilateral bulb ablation (OBX) the total number of P2(+) OSNs is dramatically decreased on the lesioned side. However, the percentage of P2(+)/GAP-43(+) OSNs is greatly increased in post-OBX OE. This was expected given the abbreviated life span and accelerated proliferation of OSNs on the OBX side.

Our data are consistent with and extends previous observations on OR expression after OBX (Konzelmann *et al.*, 1998, Cell Tissue Res., 294: 421–430). Our study demonstrates definitively that ORs are expressed prior to the maturational state defined by OMP expression. We also confirmed that the P2 OR is expressed in the absence of a target. However, we cannot yet rule out the possibility that the OB may have a more subtle effect on OSN maturation and OR expression.

147. Odorant binding proteins participate to the discrimination of agonist and antagonist compounds in the noctuid moth, mamestra brassicae

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General odorant binding protein 2 (GOBP2) are one subclass of the odorant binding proteins (OBPs) characterized in Lepidoptera (Vogt *et al.*, 1991, J. Neurobiol., 22: 74–84). The distribution of GOBP2s in different subsets of sensilla from those of pheromone binding proteins (PBPs) (Laue *et al.*, 1994, Naturwissenschaften, 81: 178–180), together with their sequence conservation between species, led to suggest a role in the detection of general odors.

In *Mamestra brassicae*, a recombinant GOBP2 (MbraGOBP2) was expressed in *Escherichia coli* and gave positive binding with three tritiated pheromone analogues (Maïbèche-Coisné *et al.*, 1998, Eur. J. Biochem., 258: 768–774). This poor specificity was inconsistent with data obtained on native MbraGOBP2, which showed positive binding only with *cis*-hexadecenol, Z11–16:OH (Bohbot *et al.*, 1998, Biochem. Biophys. Res. Commun., 253: 489–494). This compound acts as an antagonist to pheromone-mediated male attraction in this species. In order to better understand the role of GOBP2 in *M. brassicae*, we performed binding studies on purified MbraGOBP2 and compared its expression pattern with those of the MbraPBP1 in antennae by *in situ* hybridization.

MbraGOBP2 was purified by RP-HPLC, then incubated with tritiated analogues of pheromonal compounds and behavioural antagonist Z11–16:OH. The protein showed high affinity for the latter compound and no affinity for the pheromone components.

The expression patterns of MbraGOBP2 and MbraPBP1, the protein that specifically binds the major pheromonal compound, *cis*-11-hexadecenyl acetate (Z11–16:Ac), were studied by *in situ* hybridization. The digoxygenin labelling was restricted, in both cases, to the long sensilla trichodea, which house two neurons: the neuron with large spikes responds to Z11–16:Ac and the neuron with small spikes responds to the Z11–16:OH (Renou and Lucas, 1994, J. Insect Physiol., 40: 75–85).

The expression in a functionally defined population of sensilla, together with binding specificity and previous electrophysiological data, suggest an unsuspected role for the MbraGOBP2. This protein could be involved in the transduction process for the behavioural antagonist to which neurons are specifically tuned and always co-compartmentalized in long trichodeal hairs, with neurons responding to the major pheromonal compound, Z11–16:Ac. These data are consistent with the involvement of odorant-binding proteins in the fine discrimination between the occurrence of pheromone and antagonist, related to avoidance of interspecific mating mistaken.

148. Olfactory receptor neurons have enhanced olfactory marker protein immunoreactivity in the nasal cavity ipsilateral to naris occlusion

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Olfactory marker protein (OMP) is a 19 kDa cytosolic protein of unknown function that is expressed in mature olfactory receptor neurons (ORNs) (e.g. Margolis, 1972, Proc. Natl Acad. Sci. USA, 69: 1221–124; Farbman and Margolis, 1980, Devl Biol., 74: 205–215). Studies in OMP-null mice suggest that this protein may modulate olfactory signal detection or transduction (Buiakova *et al.*, 1996, Proc. Natl Acad. Sci. USA, 93: 9858–9863). Recently, olfactory bulbectomy has been shown to enhance OMP immunoreactivity in the remnant ORNs that survive this procedure (Carr *et al.*, 1998, J. Neurobiol., 34: 377–390). Given that bulbectomy also increases neurogenesis in the olfactory mucosa, it has been suggested that OMP may play a mitogenic role (Carr *et al.*, 1998). To test these competing hypotheses for the function of OMP, we studied OMP immunoreactivity in unilaterally naris-occluded mice. In this preparation, neurogenesis is decreased in the olfactory mucosa ipsilateral to the naris-occlusion while olfactory stimulation is presumably decreased or eliminated on the occluded side (Farbman *et al.*, 1988, J. Neurosci., 8: 3290–3295). On the first day after birth, anesthetized mice had either the left or right naris occluded by cauterization. After an 11 day survival period mice were perfused with 4% paraformaldehyde and their nasal cavities were prepared for OMP immunocytochemistry. Nasal cavities were imbedded in JB-4 plastic or paraffin, sectioned in the coronal plane at 4–8 μm, and processed with standard ICC procedures for OMP immunoreactivity. Fifty ORNs which expressed OMP immunoreactivity were analyzed on each side of the nasal cavity in three different sections, representing the rostral, medial and caudal portions of the cavity in each animal. Densitometric measurements were made on each selected cell using an image analysis system to quantify the differences in OMP immunoreactivity. On average, all sections in all animals showed significantly greater OMP immunoreactivity on the occluded than on the patent side of the nasal cavity. These results are consistent with the hypothesis that decreased olfactory stimulation that accompanies naris occlusion caused an up-regulation of OMP and provide further evidence that OMP is part of the olfactory transduction pathway. Since naris occlusion decreases neurogenesis in the ipsilateral nasal cavity, our results are inconsistent with the idea that OMP promotes neurogenesis.

149. Cellular distribution of *Drosophila* odorant receptors

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Although insects have proven to be valuable models for exploring the function, organization and development of the olfactory system, the receptor molecules which interact with odorants had not previously been identified in any insect. We developed a novel search algorithm, used it to search the *Drosophila* genomic sequence database, and identified a large multigene family encoding putative G protein-coupled odorant receptors (Clyne *et al.*, 1999, Neuron, 22: 327–338). The family is highly divergent from previously identified proteins, including odorant receptors from other organisms.

Nearly all the DOR (*Drosophila* odorant receptor) genes are expressed in one or both of the olfactory organs: the third antennal segment and the maxillary palp. In addition, individual genes are expressed in subsets of olfactory receptor neurons (ORNs), and different genes are expressed in different subsets of ORNs, as expected for odorant receptors. A question critical to olfactory coding is the question of how many receptors are expressed in a given ORN. We are addressing this question in the simpler of the two *Drosophila* olfactory organs, the maxillary palp. Single unit physiology has shown that the 120 ORNs on the palp can be classified into six functional classes based on odor response spectra (de Bruyne *et al.*, 1999, J. Neurosci., 19: 4250–4532). Investigation of the number of DOR genes expressed in the palp has identified nine genes expressed in the palp from 27 tested. This indicates that at least one class of ORN will express more than one DOR gene, and we are testing this hypothesis directly using double-label *in situ* hybridization.

We are also determining the subcellular localization of DOR proteins by raising antibodies to several proteins. An antibody raised against a particular DOR protein reacts with a single band on Western blots of antennal protein extracts. Immunofluorescence labelling shows that this DOR protein is localized within a subset of olfactory sensilla, exactly as would be expected for an odorant receptor protein expressed in the dendrites. To confirm this localization we are generating transgenic flies expressing DOR: guanine fusion proteins under the control of the particular DOR gene's promoter. As RNA expression analysis indicates that this DOR gene is expressed during development, we are also using the antibody to determine the intracellular location of the DOR protein during development.

150. Co-expression of calcium signaling component in vertebrate taste bud cells

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In all vertebrates, the sensation of taste is mediated by specialized cells in taste buds. Some taste stimuli received by G protein-coupled receptors are transduced through intracellular signaling pathways initiated by the activation of G proteins. Previous physiological studies have indicated that taste transduction utilizes two or more signaling pathways. The Ca^{2+} signaling cascade of phospholipase C (PLC) followed by IP_3-dependent Ca^{2+} release from intracellular stores has been reported to be activated by many bitter tastants such as denatonium benzoate and by some non-sugar sweeteners such as saccharin and SC-45647 in mammals. In catfish, it is known that several amino acids, including L-alanine, as well as denatonium, induce an increase in the IP_3 concentration in taste buds.

In order to investigate the molecular mechanism of Ca^{2+} signaling pathways common to the vertebrate gustatory systems, we analyzed the expression of their molecular components. We first identified a PLC subtype expressed in the taste buds of pond loach (*Misgurnus anguillicaudatus*), designated DPLC2, which is closely related to mammalian PLC2 shown recently to be expressed in rat taste buds (Rossler *et al.*, 1998, Eur. J. Cell Biol., 77: 253–261). The taste-bud-specific expression of PLC2 in a fish species as well as the rat strongly suggests that PLC2 mediates the tastant-induced second messenger response in taste buds, which is common to vertebrates. Next, we examined the correlation of gene expression of the candidate components leading to PLC2 activation in rat circumvallate papillae, including the G proteins Gi2 (Kusakabe *et al.*, 2000, Chem. Senses in press) and gustducin (McLaughlin *et al.*, 1992, Nature, 357: 563–569), and a G protein-coupled receptor, TR2 (Hoon *et al.*, 1999, Cell, 96: 541–551). As a result, it was shown that the mRNAs for PLC2 and Gi2 coexist in the same cells, and PLC2- and Gi2-positive cells include both gustducin-positive cells and TR2-positive cells. However, no correlation was found between the expressions of TR2 and gustducin, as reported previously. Our results thus indicate that a taste transduction pathway comprising TR2, Gi2 and PLC2 occurs in a subset of taste cells.

151. An original method of ligand binding study applied to a novel heterologous rat odorant-binding protein variant

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The study of odorant binding onto odorant-binding proteins (OBP) requires either a spectific property of the protein (e.g. binding of a fluorescent probe) or the use of radiolabeled molecules. With large amounts of recombinant OBP, we finalized a general test which can be applied to any volatile odorant or pheromone.

Recombinant rat OBP-1F was secreted by the yeast *Pichia pastoris* and purified by liquid chromatography in a highly pure form. Its binding properties were studied using 1-aminoanthracene (1-AMA) as a fluorescent probe according to Paolini *et al.* (1999, Biochim. Biophys. Acta, 23: 689–698). The protein was observed to bind 1-AMA with a dissociation constant of 0.6 ± 0.3 μM. Fluorescence experiments revealed that 1-AMA was efficiently displaced by airborne molecules including usual solvents such as EtOH and DMSO, while MeOH was a far less efficient competitor. The assay was employed to test a classical odorant, IBMP (2-isobutyl-3-methoxypyrazine). We observed that IBMP was able to chase half of the 1-AMA molecules from OBP-1F at 0.3 μM, a concentration far lower than EtOH (45 mM) and DMSO (50 mM).

We set up a biomimetic assay (volatile-odorant binding assay, or VOBA) to study the uptake of airborne odorants without radiolabeling and conceived to understand the odorant capture by OBPs in the nasal mucus under natural conditions (Briand *et al.*, 2000, Eur. J. Biochem., 267: 3079–3089). Recombinant OBP-1F was set to a physiological concentration (1.65 mM) and incubated overnight in a sealed glass chamber containing a pure undiluted odorant which evaporated freely. The odorant was then extracted from the precipitated protein with chloroform and analyzed by gas chromatography. VOBA permitted observations on the binding of airborne odorants of different chemical structures and odors (2-isobutyl-3-methoxypyrazine, linalool, isoamyl acetate, 1-octanal, 1-octanol, dimethyl disulfide and methyl thiobutyrate).

Uptake of airborne odorants in nearly physiological conditions strengthens the role of OBPs as volatile hydrophobic odorant carriers in the mucus of the olfactory epithelium through the aqueous barrier towards the chemo-sensory cells. The displacement of 1-AMA by various organic volatile solvents usually employed in odorant-binding experiments led us to suggest technical cautions in olfaction studies. Moreover, the ability of EtOH to efficiently bind OBP-1F raises the question of how it can interfere with the aroma perception in alcoholic beverages. In addition, to elute the use of radiolabeled odorants, VOBA has the great advantage of avoiding any solvent which can disturb the odorant uptake.

152. Suptype-specific binding characteristics of vertebrate OBPs

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Odorant-binding proteins (OBP) are one of the most abundant classes of protein found in the mucus of the olfactory epithelium. This proteins are thought to transfer hydrophobic odorous compounds through the aqueous barrier towards the chemo-sensory cells (Pelosi, 1994, Crit. Rev. Biochem. Mol. Biol., 29: 199–228). Several OBP subtypes have been identified in various vertebrate species. Amino acid sequence homology among these proteins is often very low. The unexpected diversity of OBPs in the nasal mucus suggests that OBP subtypes could reveal different binding properties for a wide spectrum of odorant molecules. Three different subtypes of OBPs have been cloned in the rat and their primary structure deciphered. To evaluate their binding properties, these OBP subtypes were expressed as His-tagged fusion proteins in *Escherichia coli*, thus allowing production of large amounts and efficient purification.

To approach the question of whether different OBP subtypes reveal different ligand binding properties, a spectroscopical binding assay using various fluorescence chromophores was employed (Löbel *et al.*, 1998, Eur. J. Biochem., 254: 318–324). The fluorescence dye 1-aminoanthracene (1-AMA) was identified as strong ligand for OBP1 and OBP3, in contrast the chromophore 1,8-anilinonaphthalene (1,8-ANS) specifically interact with OBP2. In addition surface plasmon resonance (SPR) technology was used to explore the biospecific interaction of these globular proteins with small odorous compounds. Displacement experiments using odorants of different structural classes (e.g. aliphatic and heterocyclic compounds) revealed distinct ligand specificity of the OBP subtypes. Amino acid residues creating the ligand binding specificity were analyzed by site-directed mutagenesis studies. This type of analysis is supposed to give some new insight into the structure/function relation of odorant binding proteins.

153. Investigation of the signal transduction pathway in rat vomeronasal receptor neurons: the putative role of polyunsaturated fatty acids

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The detection of pheromones, chemicals that allow social communi-cation between animals of the same species, is mediated by the vomeronasal organ (VNO). The signal transduction pathway in the VNO is still unknown. However, it may differ from the olfactory system in various aspects, because some of the main compounds of the olfactory sensory transduction apparatus are not expressed in vomeronasal tissue.

Recently, Liman *et al.* (1999, Proc. Natl Acad. Sci. USA, 96: 5791–5796) found that an ion channel of the transient receptor potential (TRP) family is exclusively expressed in microvilli of VNO receptor neurons and they hypothesized an involvement of these rTRP2 channel proteins in vomeronasal sensory transduction.

The activation of TRP channels is poorly understood. While it is widely accepted that vertebrate TRP channels play a key role in capacitative calcium entry, there are several indications that polyunsaturated fatty acids (PUFAs) are involved in the activation of certain TRP channels *in vivo*.

Here, we investigated the effects of PUFAs on freshly dissociated vomeronasal receptor neurons of the rat, by using whole cell voltage clamp recording and Ca^{2+}-imaging techniques. We found that application of both arachidonic and linoleic acid elicited an inward current of ~250 pA amplitude after a delay of several seconds with a relative slow onset kinetic. Both fatty acids showed differences in their specific potency. In Ca^{2+}-imaging experiments we observed that superfusion of isolated VNO neurons with 30 μM arachidonic acid induced a transient rise in $[Ca^{2+}]_i$ in the knob and soma. This Ca^{2+}-signal could not be blocked by U73122, a specific inhibitor of phospholipase C, but was abolished in Ca^{2+}-free medium, indicating that this Ca^{2+}-response is not store-operated.

Further studies should be performed to investigate whether this arachidonic acid-induced inward current is due to an activation of TRP channels and whether this putative signal transduction pathway is used for pheromone detection *in vivo*.

154. Large-scale expression and structural features of a novel rat odorant-binding protein variant secreted by *Pichia pastoris*

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Studies of odorant-binding protein (OBP) interactions with odorants and olfactory receptors need large amounts of proteins that can be engineered. The yeast *Pichia pastoris* is known to

provide secreted disulfide-bonded proteins that are properly processed.

After characterization of a novel OBP variant isolated from the rat nasal mucus, the corresponding cDNA was cloned by reverse transcription–PCR (Briand *et al.*, 2000, Eur. J. Biochem., 267: 3079–3089). Recombinant OBP-1F, the sequence of which is close to that of previously reported rat OBP-1 (Pevsner *et al.*, 1988, Science, 241: 336–339), has been secreted by *P. pastoris*, using either the yeast prepropeptide signal from the *Saccharomyces cerevisiae* α-mating factor peptide with Glu-Ala-Glu-Ala as a spacer or its native rat signal peptide. Yeast cells were transformed using a standard electroporation method. The comparison of the highest producing clones showed that the construct using the yeast α-mating factor prepropeptide signal was five times more efficient than the construct using the native rat signal peptide.

N-terminal sequencing and mass spectrometry were applied to recombinant OBP-1F expressed with the natural rat signal peptide. The measured mass (18 134.0 ± 1.0 Da) was in perfect agreement with the natural protein and with that deduced from the cDNA sequence, with two disulfide bridges formed, as ascertained by the sulfhydryl group titration. Circular dichroism showed that recombinant OBP-1F was folded in a secondary structure awaited for a lipocalin. Calibrated exclusion–diffusion chromatography of purified OBP-1F gave an apparent molecular mass of 31.2 kDa, demonstrating dimerization of the recombinant protein at neutral pH, as already described for OBP-1 (Löbel *et al.*, 1998, Eur. J. Biochem., 254: 318–324). The two SS bond pairings (C44–C48 and C63–C155) were demonstrated by mass spectrometry of the peptides resulting of the OBP-1F proteolytic digestion. The recombinant protein was purified by ion-exchange chromatography and gel filtration in a highly pure form, cleared from minor contaminants due to natural yeast secretion. After purification, we obtained 100 mg/l of culture of recombinant OBP-1F secreted with the α-mating factor peptide (Briand *et al.*, 2000).

The overproduction of recombinant OBP-1F, which is exactly identical to the native molecule, should allow structural and mutational analysis in order to understand the relationships between structure and biological function of this carrier protein.

155. Neuroanatomical support for a cross-talk between nutrition and olfaction: orexins and their receptors are present in the olfactory system

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The recently discovered orexins (ORX) (Sakurai, 1999, Regul. Pept., 85: 25–30) have been reported to stimulate food intake; these findings seem related to the highly specific localization of ORX-containing neurons in the lateral hypothalamus, the wide distribution of ORX fibres throughout the brain and finally the up-regulation of ORX peptides shown in fasting rodents. The ORX system comprises two peptides ORXA and ORXB derived from the same pre-proorexin (preproORX) as well as two receptors (ORXR1 and ORXR2) which belong to the G protein-coupled receptor family. A cross-talk between nutrition and olfaction seems quite obvious, since food odours are able to trigger a cascade of anticipatory modifications of the digestive tract. Conversely, it could be anticipated that the animal nutritional status could modulate transduction of food odours to the brain. A number of peptides implied in the control of food intake and/or their receptors have been found in the olfactory bulb (NPY fibres and cell bodies; NPY Y1 and Y5 receptors; orexin fibres; leptine receptors). However, there are no available data concerning the possible presence of such peptides and their receptors in the olfactory epithelium.

RT-PCR was used to detect mRNA coding for preproORX, ORXR1 and ORXR2 in rat olfactory bulb and epithelium, as well as in hypothalamus as a positive control and in kidney as a negative one. In all tissues except kidney, we found PCR products of the expected size for preproORX and the two receptors. Using immunocytochemistry, we have looked for the localization of ORXA, ORXR1 and ORXR2 in the rat olfactory system. As already described, we found some varicose ORX fibres in the olfactory bulb, mainly in the glomerular zone. We also found some thick ORXA fibres in the lamina propria underlying the olfactory epithelium and surrounding the olfactory axon fascicles. A specific punctated staining for ORXA appeared at the surface of the epithelium, near the microvilli of the supporting cells and the dendritic knobs of olfactory receptor cells. The staining observed for ORXR1 in the olfactory epithelium paralleled that observed for ORXA. We also found some fibres labelled for ORXR2 only in the lamina propria. Interestingly, the staining observed using an antibody against $G_{\alpha olf}$ resembled that observed for both ORXA and ORXR1.

Our results suggest that ORX and their receptors are synthesized in the olfactory epithelium; the probable cellular localization of both proteins near the dendritic knobs where the olfactory receptors are located (Menco *et al.*, 1997, J. Neurocytol., 26: 691–706) suggest a possible role for ORX in the modulation of odour transduction at the level of olfactory receptor cells. Our data provide for the first time a molecular basis for a nutrition/olfaction interaction.

156. Olfaction and neural no synthase: some elements from mice lacking the *nNOS* gene

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Considering the activity level and localization, NO synthase has been proposed to take part in the processing of olfactory signals in the main olfactory bulb, and of conspecific odors in the accessory bulb (Breer and Shepherd, 1993, Trends Neurosci., 16: 5–9). We have examined these possibilities in male mice, 13 ± 1 weeks old, having the *nNOS* gene deleted on a C57BL/6J background. Forty-one mice with ascertained genotypes were used: 12–/–, 16+/– and 13+/+ animals. During 9 min sessions we measured the time spent in one side of a double open field pervaded by two non-mixing airstreams (2.5 l/min), at the output of a dilution olfactometer. Six olfactory choices were offered successively: air/isoamyl acetate (IA), air/food odor (FO), air/familiar conspecific male urine (FU), FO/IA, air/unfamiliar male Ajax mouse urine (UU) and UU/FU. The mouse groups were tested randomly, matching for the right and left sides within each group.

The three groups significantly preferred air to IA or FU, and FO to IA or air. The –/– mice differed significantly from the others by spending even more time in the FO side versus air, and in that IA in one side did not increase the controlateral time score for FO, which might be connected to the digestive problems in that group (Huang *et al.*, 1993, Cell, 75: 1273–1286). In the +/+ mice, specifically, the

UU side score was significantly greater when measured against air than against FU, and significantly greater than the score for FU/air. The number of boli deposited per session did not depend on the genotype; it was maximal for UU/air and minimal for FO/air.

The *nNOS*-deleted mice seemed to detect, discriminate and process FO and IA accurately, and to present hedonic/emotional reactions to FU and UU as control mice do. The lack of any relative preference for UU in the +/– and –/– groups however gives a first indication that *nNOS* could well be involved in the processing of complex pheromonal signals.

157. High-throughput detection of mRNAs highly enriched in the lobster olfactory organ

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We have used representational difference analysis (RDA) of cDNA (Hubank and Schatz, 1994, Nucleic Acids Res., 22: 5640) to amplify cDNAs derived from mRNAs enriched in the olfactory organ of the clawed lobster, *Homarus americanus*. The cloned second difference product from the RDA was subjected to sequential cross hybridization until all species in the product were identified, yielding 27 unique clones. We used Northern blots, cDNA dot blots or RNA dot blots to confirm that all 27 clones were enriched at least twofold in the olfactory organ compared with the tissues used to drive the RDA subtraction: brain and second antenna. This high fidelity is characteristic of RDA of cDNA. Sequencing revealed that 11 of the clones had significant similarity to six known sequences. (RDA often amplifies multiple non-overlapping fragments of a cDNA.) These were: an ionotropic glutamate receptor (two clones), dopamine β-hydoxylase (three clones), a tubulin isoform, a calcium-binding protein (two clones), a serine protease and an α2-macroglobulin (two clones). The other 16 sequences had no similarity to known sequences. Experiments to identify which cell types of the olfactory organ express each clone are in progress.

Surprisingly, no products were obtained in the third difference product from the RDA. The third difference product typically yields only cDNAs from highly specific mRNAs. Although absence of a product in an RDA is, by itself, not evidence for a lack of differences in mRNA abundance, other evidence also argues that there may be few mRNAs uniquely expressed in the lobster olfactory organ. This evidence includes behavioral tests with odorants on spiny lobster lacking functional olfactory organs (Steullet *et al.*, 1999, Chem. Senses, 24: 613) and observations that mRNAs enriched in the olfactory organ often show weak expression in tissues, such as the dactyl, containing concentrations of contact chemoreceptors. We suspect that many of the genes involved in the function of olfactory receptor neurons are also used by neurons in other, primarily contact type, chemoreceptor organs.

158. A new GPCR from the human lingual cDNA library

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It is generally accepted that at least a part of sweet and bitter taste signals is transduced by G protein-coupled receptors (GPCRs). It has been proposed that such receptors could display high degree of homology with olfactory receptors. Abe *et al.* (1993, J. Biol. Chem., 268: 12033–12039) showed using RT-PCR strategy that a family of olfactory receptor-like mRNAs is expressed in rat tongue. One of these mRNAs, coding the protein named GUST27, was present in epithelial cells of the rat tongue, including taste buds. Additionally, GUST27 colocalized with α-gustducin a taste-specific G protein, suggesting that it could be involved in taste transduction. Similar approach allowed Matsuoka *et al.* (1993, Biochem. Biophys. Res. Commun., 194: 504–511) to obtain cDNA clones highly similar to olfactory receptors from bovine taste tissue.

In order to investigate the expression of GPCRs in human tongue degenerated primers corresponding to transmembrane domains 2 or 3 (for 5′ primer), 6 or 7 (3′ primer) were used to amplify olfactory receptor-like cDNAs in RT-PCR experiments. A single band of ~520 bp could be amplified from the human foetal tongue Gene Pool™ (Invitrogen) using primers RS2 and RAS4 hybridizing to TM3 and TM7, respectively. After cloning into the pGEMT-Easy vector (Promega), restriction analysis demonstrated the presence of several clones. Among the 29 sequenced clones containing inserted cDNAs of ~520 bp, six (20.7%) were artefacts due to mispriming, 10 (34.4%) were expressed pseudogenes and 13 (44.8%) coded putative olfactory receptors-like proteins.

Three of the latter had already been cloned and were identified as TPCR24, HGMP07I and OR10A1. The others were new and, consequently, were named JCG1 to JCG9. A homology search using BLAST software enabled us to locate the complete coding sequence of JCG2 and the partial coding sequence of JCG9 on chromosome 11 (locus 11q25). No homology was observed with recently reported putative bitter receptor sequences (Matsunami *et al.*, 2000, Nature, 404: 601–604; Adler *et al.*, 2000, Cell, 100: 693–702). The full-length cDNA for JCG2 encoding heptahelical transmembrane protein of 312 amino acids was cloned by RT-PCR. The tagged protein was expressed in COS7 and HEK-293 cells.

159. Expression of galectins 1 and 3 and olfactory marker protein (OMP) in human olfactory epithelium

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Due to their regulatory functions and modulating effects in various tissues, galectins, a family of endogeneous lectins, have been the subject of intensive research in the past years. Their appearance in a wide range of organisms has led to insights into mechanisms of cell regulation. Since the olfactory epithelium is a rare example of a regenerating nerval tissue, we examined the expression of galectins 1 and 3 in human olfactory epithelium.

The expression pattern of galectins 1 and 3 was investigated in relation to olfactory marker protein (OMP) using confocal laser immunofluorescence in human specimens and post-mortem biopsies. OMP expression was found in olfactory receptor neurons (ORN) in the olfactory mucosa and in fibers of the olfactory nerve crossing the submucous connective tissue. Galectin-1 was expressed

in both the connective tissue of the nasal cavity and in the basal layer of the olfactory epithelium. In contrast, galectin-3 expression was limited to cells of the upper third of the olfactory epithelium. Expression of both galectins 1 and 3 occurred in OMP-positive cells. However, between areas of galectin-1 and galectin-3 expression in the lower and upper portions of the epithelium, OMP-positive ORN did not stain for both galectins.

Considering the potential role of galectins 1 and 3 in cell differentiation and maturation, the differential localization of galectines in the olfactory epithelium appears to be consistent with a significant role of these molecules in the physiological turnover of ORNs.

160. Characterization of I_h channels expressed in olfactory receptor neurons of the perch

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Recently, a family of genes encoding subunits for hyperpolarization-activated and cyclic nucleotide-gated cation channels (HCN) has been cloned from the mouse and the human (Santoro *et al.*, 1998, Cell, 93: 717–729; Ludwig *et al.*, 1998, Nature, 393: 587–591). The channels encoded by this gene family exhibit the general features of a mixed Na^+/K^+ current called I_h or I_f. Functionally, I_h is important in setting the resting membrane potential, and in controlling cell excitability and rhythmic electrical activity patterns. The presence of I_h currents was determined for several sensory tissues, including olfactory receptor neurons of the rat (Vargas and Lucero, 1999, J. Neurophys., 81: 149–158) and the lobster (Corotto and Michel, 1994, J. Neurophys., 72: 360–365).

To analyze the expression patterns of I_h channels we performed the reverse transcription–polymerase chain reaction on different tissues of the perch *Perca fluviatilis*, including olfactory epithelium and retinal tissue, using degenerate primers directed against HCN sequences. The isolated cDNA fragments were characterized by sequence analysis and were subsequently grouped into different HCN subtypes. In addition, we report the indentification of I_h currents in dissociated olfactory receptor neurons of *P. fluviatilis*. By using the patch-clamp technique we measured the voltage dependence and sensitivity for cyclic nucleotides of the corresponding I_h channel. In conclusion, we provide the first evidence that different HCN subtypes with specific expression patterns do exist in lower vertebrates. Further studies will investigate the relationship between native I_h curents and the different HCN subunits expressed in sensory tissues.

161. Development of axonal projections in the mouse olfactory system

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Detection and discrimination of odorous molecules is based on specific odorant receptor proteins located in the ciliary membranes of olfactory sensory neurons. The repertoire of genes encoding such receptors is extremely large, numbering as many as 1000 genes for some mammals. To gain an insight into how the system is designed to encode information about a stimulus, the axonal projection pattern of olfactory neurons expressing distinct genes from a subfamily of highly related receptor genes (mor37) was analyzed. A gene targeting strategy in mice allowed the co-ordinated translation of an odorant receptor along with a marker protein, permitting the visualization of the cells, including their axonal projections. Using either tau lacz or GFP as axonal markers, two different receptors could be visualized in the same individual by double-labeling.

Using this approach, a synchronous onset of expression for three genomically linked receptors at embryonic stage E11.5 was observed. Each receptor was expressed in a different subset of sensory neurons. The following day (E12.5) labelled axon terminals first reached the mesenchyme which separates the nasal cavity from the differentiating telencephalon. 14.5 days after gestation single stained axon fibers entered the outer nerve layer of the presumptive olfactory bulb. A convergence of nerve fibers initially occured at embryonic day E17. Axons from neurons expressing distinct receptors started to form protoglomerular structures at a similar anterior-ventral position; interestingly at this developmental stage the different axon populations intermingle to a very high degree. On the subsequent days, a separation of axonal terminals into distinct glomeruli was observed, that was completed at postnatal day 9.

162. Functional expression of olfactory receptors and perception of odorants in heterologous systems

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Olfactory perception of odorant molecules occurs via transmembrane receptors mainly coupled to a G protein named G_{olf}. We had two major goals. One was the study of the functional relationship between olfactory receptor and odorants. An expression system was therefore developed in mammalian cells so as to characterize the functional response of receptors to odorants. The rat I7 olfactory receptor was used in order to validate the system. The presence of rat I7 messenger RNA was checked in stably transfected cells, and membrane expression of the receptor was revealed either by expressing a fusion receptor–GFP protein or by specific anti-peptide antibody receptor labelling. Stimulation of cells with rat I7 reportedly preferential ligand octanal induced no measurable cAMP elevation, but the intracellular calcium concentration increased in a dose-dependent manner following a bell-shaped curve over a concentration scale of 10^{-9} to 10^{-6} M. The response to odorant stimulation consists of a transitory increase of intracellular calcium followed by a prolonged desensitization. In contrast, the neighbouring ligand nonanal triggered a calcium response for higher odorant concentrations, but it did not result in receptor desensitization. Moreover, heptanal did not evoked a calcium response in the system. This cellular system thus displays an important discrimination between close ligands, which makes it a precious tool for screening the characteristics of receptor–ligand interactions. Furthermore, it allows the examination of the differential effect of the transduction pathway drugs on the calcium response and its desensitization, depending on the odorant used.

Our second goal was to screen a whole range of odorants on

olfactory receptors so as to detect new related pairs. For that purpose, we took advantage of the homologies between transduction cascades in yeast and mammalian cells. The pathway normally used for reproduction, with the α factor activating Ste2 receptor resulting in cellular cycle arrest, was hijacked: the modified system allows for growth on a selective medium upon stimulation of a heterologously expressed receptor by its ligand, thus offering a positive screening of the related pairs. Both olfactory receptor and chimeric Gpa1-Gαi2 or Gpa1-Golf protein are introduced into the modified yeast cells by plasmids. Expression occurs under galactose induction of pGal1/10 promoter. Validation of the system was performed by screening rat I7 receptor response to a range of odorants: the growth response was detected for heptanal only, in the range between 5×10^{-8} and 5×10^{-5} M, in the presence of the Gpa1-Gαi2 protein only. Induction conditions appear to be critical for expression, and the solvents of odorants have a toxic effect for the highest odorant concentrations. The change from octanal to heptanal as the preferential ligand for rat I7 receptor may be due to the expression system itself, in that yeasts are in continuous contact with the odorants, and thus integrate a succession of stimulation and desensitization periods.

163. OMP gene deletion results in an alteration in odorant quality perception

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Previous work suggests that odorant quality perception might be altered in mice lacking the gene for OMP. The loss of OMP results in a marked reduction of the EOG response to odorants that is coupled with a slowing of response and recovery kinetics. Thus, it could be suggested that these defects would degrade the odorant-induced spatial activity patterns that are characteristic of different odorants, as well as alter the differential temporal activation of the mucosa. These altered spatiotemporal patterns would, in turn, alter the organized and stereotyped patterning of information that occurs at the level of the mucosal projection onto the bulb. Such alterations would be important because one model of odorant quality encoding has proposed that a stimulus is encoded along multiple dimensions as a vector of *n*-dimensions. It has been suggested that the glomeruli of the olfactory bulb are an obvious candidate for the *n*-dimensions that encode odorant quality at this level. Thus, degrading or disrupting the neural activity of the bulb would be expected to alter odorant quality perception.

To test the hypothesis that odorant quality perception is altered in OMP-null animals we have trained and tested seven mice (three OMP-null and four background controls), using our five odorant identification confusion matrix task (AOCM). Using standard operant techniques, mice were trained to differentially report (i.e. identify) the odorants ethyl acetoacetate, carvone, propanol, propyl acetate and citral. Following criterion training, animals received 40 testing sessions, using a standard 5 × 5 confusion matrix design, in order to acquire data for the comparison of odorant quality perception between these two groups. On average, control and OMP-null animals performed at equivalent levels (mean ± SD: 91.47 ± 1.82 versus 91.93 ± 0.53, respectively). These results demonstrate that, despite the altered neurophysiological activity known to occur in OMP-null mice, these animals can perform the identification task at levels comparable to controls. The composite matrix for each animal (both OMP-null and control) was compared with every other animal, yielding a dissimilarity matrix of animal AOCM responses. A multidimensional scaling (MDS) analysis of the dissimilarity data yielded a three-dimensional solution, with each animal (both OMP-null and control) occupying a point in MDS space. Preliminary statistical analysis (MANOVA) provides strong evidence for an effect of genotype in determining the location of an animal in MDS space. These data suggest, therefore, that, compared with controls, odorant quality perception is altered in OMP-null animals. Moreover, they support previous neurophysiological and behavioral sensitivity measurements which indicated an alteration in neural function in the null mutant.

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164. Oxytocin suppresses GABAergic synaptic transmission between cultured olfactory bulb neurons through a presynaptic mechanism

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One of the well-known functions of neuropeptide oxytocin (OT) is to cause the secretion of milk and contraction of the uterus in female mammalian animals during parturition and lactation. During the last two decades it has become apparent that OT may in addition play a role in brain function. We have provided behavioral and electrophysiological evidence suggesting that OT originating in the hypothalamic paraventricular nucleus acts on the olfactory bulb following its release partly into the cerebrospinal fluid, and that it induces maternal behavior (Yu *et al.*, 1996, Neuroscience, 72: 1073–1082; Yu *et al.*, 1996, Neuroscience, 72: 1083–1088). However, the precise mechanism by which OT acts on bulbar neurons remains to be elucidated. To examine the effects of OT on the synaptic transmission between mitral/tufted (M/T) and granule (GR) cells, we applied the whole-cell recording technique to primary culture.

The olfactory bulbs were dissected from embryonic day 20–21 rat embryos. After mechanical dissociation with a fire-polished Pasteur pipette, the cell suspension was plated at a high density (5×10^5/cm^2) onto coverslips coated with polyethylimine. The neuronal growth medium contained 90% Dulbecco's modified eagle medium with 5% horse serum and 5% newborn calf serum. Cultures were maintained in a water-saturated atmosphere (95% air and 5% CO_2) at 37°C and electrophysiological recordings were made after 21–28 days in culture. Presumptive M/T and GR cells were identified based on morphological and immunohistochemical criteria (Trombley and Westbrook, 1990, J. Neurophysiol., 64: 598–606). Whole-cell recordings were performed at room temperature with an EPC-9 patch-clamp amplifier.

OT (100 nM) suppressed spontaneously occurring $GABA_A$ receptor-mediated IPSCs recorded in M/T cells. OT had no significant effect, however, on the membrane current evoked by exogenous application of GABA in M/T cells and on macroscopic currents in M/T or GR cells. OT also decreased the frequency of

miniature IPSCs recorded in the presence of 2 μM tetrodotoxin but did not affect the overall amplitude distribution. The effect of OT was mimicked by the selective OT receptor agonist Thr^4, Gly^7-OT (100 nM). These results suggest that OT suppresses GABAergic synaptic transmission from granule cells to mitral/tufted cells through a presynaptic mechanism.

165. Multiple adrenergic receptors are expressed in posterior taste receptor cells and act to inhibit outward potassium currents

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Recent studies have provided evidence that multiple transmitters are operative in mammalian taste buds including serotonin, GABA, acetylcholine, glutamate and norepinephrine (NE). Moreover, in addition to neuronal elements, taste receptor cells also respond to neurotransmitters. For example, we have previously reported that chloride currents in rat posterior taste receptor cells are enhanced by β-adrenergic stimulation (Herness and Sun, 1999, J. Neurophysiol., 82: 260–271). Here we present evidence that outward potassium currents in taste receptor cells are inhibited by adrenergic stimulation and that taste receptor cells express multiple subtypes of adrenergic receptors.

Taste receptor cells from circumvallate and foliate papillae of rat were dissociated and examined using whole-cell patch clamp recording technique. NE was effective in reducing outward potassium currents at tested concentrations in a dose response manner (0.01–50 μM). These inhibitions were similar to protein kinase A-dependent cAMP induced inhibitions previously reported (Herness *et al.*, 1997, Am. J. Physiol., 272: C2005–C2018). Therefore, the involvement of β-receptors was tested. Isoproterenol, a β-agonist, was effective in reducing outward current and was blocked by the β-antagonist propranolol. Two alpha agonists were also tested, phenylephrine, an α1 agonist, and clonidine, an α2 agonist. Phenylephrine was mostly non-responsive, producing an inhibition (76 ± 5% current remaining) in only 9/55 cells. Clonidine reduced potassium currents to 79 ± 1% (n = 39/58 cells) and also inhibited sodium currents (79 ± 1%). Yohimbine, an α2 antagonist and known bitter stimulus, was strongly effective in reducing potassium current stimulus and hence could not be tested to counter the action of clonidine. Neither NE, cAMP or forskolin was effective in inhibiting inwardly rectifying potassium current in these cells.

Reverse transcriptase–PCR was performed on RNA extracted from lingual tissue containing circumvallate or foliate taste buds using primers specific for α and β adrenergic receptor subtypes. PCR products were observed for primer sets with β1 and β2 but not for β3 receptors. PCR experiments for all alpha subtypes are in progress.

To address the question of where endogenous NE may arise, immunocytochemistry (ICC) on posterior taste buds was performed using a commercially available antibody for NE. Several immunopositive cells were observed per taste bud.

Collectively, patch clamp, PCR and ICC data suggest that a subset of taste receptor cells may contain the neurotransmitter NE which can be released onto neighbouring cells expressing adrenergic receptors. Further experiments will be needed to assess the physiological role for the presence of multiple types of adrenergic receptors in taste receptor cells.

166. Developmental changes in and putative functions of serotonin receptor expression in the rat olfactory epithelium

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The adult mammalian olfactory epithelium (OE) is characterized by neurogenesis throughout life and retains the capacity to regenerate olfactory sensory neurons. Proliferative stem cells (globose basal cells) within the OE are capable of self-replacement and give rise to new neurons. Proliferation density, however, decreases postnatally. Yet the mechanisms underlying the regulation of stem cell proliferation are poorly understood.

To gain insights into these mechanisms, we investigated the expression of the serotonin receptor type 3,5-HT_3. Serotonin (5-HT) has been implicated in the morphogenesis of the central nervous system and craniofacial structures, as well as in proliferation of T cells and in neurogenesis. Its actions are mediated by multiple receptor subtypes. The pattern of the 5-HT_3 receptor mRNA expression within the brain suggests possible roles for its involvement in proliferation, differentiation and migration of CNS neurons (Tecott *et al.*, 1995, Mol. Cell. Neurosci., 6: 43–55).

Therefore, we asked if 5-HT_3 receptors are also involved in the neurogenesis of olfactory sensory cells. Using RT-PCR, we demonstrated the expression of 5-HT_3 receptors in the olfactory mucosa of postnatal rats of both sexes and at different ages. Semiquantitative analysis of the expected PCR-product band on an agarose gel with ethidiumbromide revealed that there was a marked decrease in the expression of 5-HT_3 receptor mRNA with increasing age of the animals. Animals at postnatal day 10 (P10) had ~4 times the amount of P36, and P36 had ~4 times the concentration of P500. The results of the tested age groups P98 and P120 fit into this pattern. P900 had much less 5-HT_3 than all other age groups. In contrast, the expression of other serotonin receptor subunits, such as 5-HT_{2B}, did not change postnatally.

This decrease in 5-HT_3 receptor mRNA parallels the decrease in proliferation density in the OE of postnatal rats (Weiler and Farbman, 1997, J. Neurosci., 17: 3610–3622). This correlation suggests an involvement of the serotonin receptor 5-HT_3 in olfactory neurogenesis.

167. Distribution of *c-fos* in the accessory olfactory bulb of prepubertal female mice exposed to pheromonal cues

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The major urinary proteins (MUPs) are small proteins secreted by the urine of the adult male mouse that act as pheromones. Among their functions, they have been reported to accelerate the onset of

puberty in young female mice and this effect appears to be mediated by the vomeronasal organ (VNO). Recently, two different classes of G proteins ($G_{\alpha o}$ and $G_{\alpha i2}$) and two different families of putative pheromone receptors (V1R and V2R) have been identified in the VNO. The pattern of expression of the VNO receptors strongly suggests that V2Rs are coupled to $G_{\alpha o}$ whereas V1Rs to $G_{\alpha i2}$. V1R expressing neurons are present in the apical layer of the VNO and project to the anterior AOB whereas the posterior AOB receives inputs from the neurons located in the basal VNO which express V2Rs.

The different structure of the vomeronasal receptors and their laminar distribution suggest that two separate classes of pheromones may mediate behavioural responses. Recent biochemical experiments seem to indicate that the urinary proteins of the rat, stimulate the $G_{\alpha o}$-pathway while $G_{\alpha i2}$ is activated by the lipophilic molecules that are naturally bound to the Urinary Proteins.

We used the immediate early gene c-fos to assess the activation of neurons in both anterior and posterior AOB of prepubertal femal mice that were exposed to natural MUPs, i.e. MUPs with the naturally bound ligands.

In situ hybridization experiments indicate that natural MUPs induce c-fos activation in mitral cells of both anterior and posterior AOB.

Since some of the natural ligands of MUPs have been identified as the lipophilic molecules 2-s-butyl-4,5-dihydrothiazole and 2,3-dehydro-exo-brevicomin, we performed *in situ* experiments using these two ligands at different concentration.

In an independent set of experiments we also tested a MUP isoform which was cloned in *Pichia pastoris*.

Our preliminary data indicate that neither synthetic odorants nor recombinant MUP are able to induce c-fos expression in the anterior or posterior AOB. This suggests that MUPs require the naturally bound ligands to elicit neural activation of the AOB.

168. Monoclonal antibodies GBC-2 and GBC-3 label globose basal cells after methyl bromide exposure

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The mammalian olfactory epithelium has a capacity for producing new neurons throughout life. Damage to the olfactory sensory neurons in the epithelium, which can be accomplished by various experimental means (e.g. olfactory bulb ablation or methyl bromide gas exposure), greatly accelerates the rate of the renewal process of neurons. The production of new neurons and the consequent reconstitution of the olfactory epithelium after lesion are due to the presence of the globose basal cell population, which is the majority of proliferating cell population remaining after lesion.

In attempting to identify markers for globose basal cells, we have generated two monoclonal antibodies, named GBC-2 and GBC-3. At 2–4 days following methyl bromide exposure GBC-2 and GBC-3 more intensely label the basal cell population on the lesioned side of the olfactory epithelium compared with the control non-lesioned side. By 14 days after lesion, the intensity of the labeling by both GBC-2 and GBC-3 revert to near normal on the lesioned side. Western blots show that the antigens recognized by GBC-2 and GBC-3 are found in the membrane fraction, and are 30–35 kDa and 45 kDa in size, respectively. The partially purified antigens are being prepared for microsequencing. These antigens are also found in two different olfactory epithelium-derived cell lines, NIC (Goldstein *et al.*, 1997, J. Neurobiol., 33: 411–428) and odora (Murrell and Hunter, 1999, J. Neurosci., 19: 8260–8270), which are reported to be phenotypically similar to the globose basal cells. *In vitro* the antigens of both GBC-2 and GBC-3 appear to be down-regulated in densely populated areas, suggesting that these antigens are contact-regulated. We are currently investigating the effects of various growth factors on the expression of these antigens *in vitro*.

These monoclonal antibodies will be potentially useful in manipulating globose basal cells in studies of the basal cell population during the reconstitution of the olfactory epithelium.

169. Expression of lactoseries carbohydrates in the rat olfactory system reveals a glycocode

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Monoclonal antibodies LA4 and KH10 recognize distinct α-galactose extended lactoseries carbohydrate epitopes (Dodd and Jessel, 1985, J. Neurosci., 5: 3278–3294). We have used these antibodies to define a subpopulation of primary olfactory neurons in the rat from embryonic day 17 to adulthood. Both antibodies specifically labelled the perikarya and axons of distinct subpopulations of olfactory neurons, which terminate in discrete glomeruli in the olfactory bulb. Strong expression of both carbohydrate antigens is also seen on sensory neurons in the vomeronasal organ and their axons in the accessory olfactory bulb. At E17, low levels of expression were observed on olfactory neurons widely scattered throughout the olfactory neuroepithelium lining the nasal cavity and on their axons in the nerve fibre layer of the olfactory bulb. The number of positive neurons increases during the late embryonic and early postnatal period. Axons expressing these carbohydrate antigens self-fasiculate and form distinct bundles in the nerve fibre layer, terminating in clearly distinguishable glomeruli. In the postnatal animal axons positive for the KH10 antigen projected predominately to glomeruli in the ventromedial regions of the bulb, whereas axons positive for the LA4 antigen exhibited a wider distribution pattern. At all ages studied the KH10 antibody recognized neurons restricted to a smaller subpopulation than the LA4 antibody. The spatiotemporal expression patterns of positive glomeruli was mapped using an image analysis system. Use of the monoclonal antibodies has enabled us to generate a glycocode of olfactory glomeruli based on the specificity of targeting of chemically-identifiable axons expressing unique carbohydrates.

170. T2R1 as a candidate gene for the PTC/PROP locus: results of fine genetic mapping of 5p15

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Taste blindness for phenylthiocarbamide (PTC) and propylthiouracil (PROP) is the best characterized of sensory polymorphisms in humans but the gene or genes responsible for this trait have not been identified. One of the recently described mammalian taste receptors (T2R1) (Adler *et al.*, 2000, Cell, 100: 693–702) is within the genomic region (5p15.2) we previously reported as linked to PTC/PROP taste blindness (Reed *et al.*, 1999, Am. J. Hum. Genet., 64: 1478–1480). To examine whether T2R1 and the PTC/PROP locus are the same gene, we determined whether they mapped to the same genomic location. Caucasian families phenotyped for suprathreshold ratings of PROP and genotyped for three markers within 5p15.2 (D5S2488, D5S2088 and D5S807) were studied. Maximum Z scores using quantitative multipoint analysis methods demonstrated peak linkage nearest D5S2088 ($Z = 2.62$, $P = 0.004$). Analysis of the genomic clones containing T2R1 place it ~7 Mb centromeric to D5S2088. Although these results do not rule out T2R1 as a PTC/PROP gene, a more likely candidate is a centromeric gene or gene cluster.

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171. Evaluation of a novel family of mammalian taste receptors on human chromosome 7q31 as candidate genes for the PTC/PROP locus: results of fine genetic mapping

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Taste blindness for phenylthiocarbamide (PTC) and propylthiouracil (PROP) is the best characterized of sensory polymorphisms in humans but the gene or genes responsible for this trait have not been identified. Early linkage reports indicated that the KELL locus (7q31) was linked to taste blindness (Conneally *et al.*, 1976, Hum. Hered., 26: 267–271) but later reports were unable to replicate this result (Spence *et al.*, 1984, Hum. Genet., 67: 183–186; Reed *et al.*, 1999, Am. J. Hum. Genet., 64: 1478–1480). The reason for this discrepancy in results is unclear. Recently, a cluster of four taste receptors (T2R3, T2R4, T2R5 and T2R16) have been localized <2 Mb from the KELL locus and are candidates for the PTC/PROP gene (Adler *et al.*, 2000 Cell, 100: 693–702). To determine whether this genomic region co-segregates with the ability of subjects to taste a suprathreshold concentration of PROP, a linkage study was undertaken. Two markers were genotyped on either side of the taste cluster: D7S2202 is ~3 Mb centromeric and D7S794 is ~2 Mb telomeric to the candidate genes. Maximum t-scores using quantitative multipoint analysis methods demonstrated no linkage between this region and PROP taste intensity ratings ($P > 0.05$). When subjects were divided into taster and nontaster groups and the linkage analysis was conducted with parametric methods, there was a slight but non-significant trend for linkage when a variety of dominant, additive and recessive models were tested ($0.05 > P < 0.10$). If these genes (T2R3, T2R4, T2R5 and T2R16) are involved in the perception of PTC and PROP they may be more important for threshold detection than suprathreshold intensity ratings. Further analysis of these genes with comprehensive psychophysical measurements of threshold and suprathreshold ratings is needed to resolve the contribution of genes on 7q31 to PTC/PROP perception.

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172. BDNF and insulin modulate ion channel activity in the olfactory bulb linked with development, sensory experience and food intake

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Insulin and related receptor tyrosine kinases (RTKs) activate a number of signaling pathways that regulate cellular metabolism, growth and plasticity in the CNS. Most recently it has been demonstrated that these molecules acutely regulate function of voltage-activated and ligand-gated ion channels. Acute insulin or brain derived growth factor (BDNF) application to olfactory bulb neurons (OBNs) causes current suppression of a prominent potassium channel in these neurons, Kv1.3. The goal of this study was to identify RTKs present in the developing olfactory bulb that used ion channel proteins as substrates for tyrosine phosphorylation. It is hypothesized that RTKs may serve a dual role in the olfactory system: (i) these enzymes could maintain their traditional role as growth factors in the developing nervous system; and (ii) they could additionally be retained in adults to modulate electrical activity in a system that undergoes continual regeneration.

We now show that insulin application to HEK293 cells co-transfected with Kv1.3 and hIR cDNA causes an increase in tyrosine (Y)-specific phosphorylation. Kv1.3 site-directed mutagenesis revealed that current suppression and concomitant phosphorylation can be reversed by removal of the Y-phosphorylation recognition motifs in the ion channel—residues YYY111–113, Y137 and Y479 are all important targets. Interaction of the IR with the adaptor protein nShc decreased the total insulin-induced Y-phosphorylation of Kv1.3. Unilateral naris occlusion of P1 rats reduced IR but not Kv1.3 expression following 20–30 days of odor/sensory deprivation to the ipsilateral olfactory bulb. Sensory-deprived OBNs failed to demonstrate insulin-induced neuromodulation of Kv1.3 current. High levels of insulin were detected in the OB by ELISA and levels were up-regulated fourfold during periods of dietary fasting in adult rats.

We also show that several RTKs (Trk A, Trk B, Trk C and IR) are developmentally present (postnatal stages P1–P60) in the

OB via Western analysis of purified membrane preparations. Patch-clamped rat OB neurons acutely stimulated for 15 min with 50 ng/ml of bath applied NT3 ($n = 6$), BDNF ($n = 7$), NGF ($n = 4$), insulin ($n = 7$), IGF-I ($n = 8$) or IGF-II ($n = 2$) show a 19 ± 8% suppression of outward current with BDNF and a 24 ± 6% suppression with insulin. Other biophysical properties, such as $V_{1/2}$, inactivation kinetics and deactivation kinetics, were not significantly affected by acute stimulation. Tyrosine phosphorylation of Kv1.3 increased twofold when OBs were acutely stimulated with BDNF as demonstrated by Western analysis and quantitative densitometry. Insulin-induced tyrosine phosphorylation of the channel was time-dependent and rapid, demonstrating increases after only 30 s of stimulation. When OB neurons were chronically stimulated (24–216 h) with the same battery of RTK ligands, we found incremental increases in peak current amplitude through days *in vitro* (DIV) above that of time-matched controls ($n = 50$) for BDNF ($n = 44$). As found with the acute trials, other biophysical properties were not affected.

In recapitulation, Y-phosphorylation of Kv1.3 channels by IR kinase or Trk B occurs at discrete tyrosine recognition motifs in both the C and N terminal of the channel and may be involved in OBN excitability. Modulation of OBN current is dependent upon availability and expression level of the kinases and their respective ligands, which fluctuate with developmental postnatal stage, feeding, or sensory-deprivation.

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173. Cloning of pheromone-binding protein-related protein genes in taste tissue of the fleshfly

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To identify genes involved in taste reception of the fleshfly, *Boettcherisca peregrina*, the taste tissue (labellum or taste receptor cell-enriched tissue) cDNA library was screened with the subtracted cDNA probe which enriched taste receptor tissue-specific cDNA. To generate the subtracted probe, cDNAs from femur, antenna or eye, which do not contain taste receptor cells, were subtracted from cDNAs from tarsus or labellum, containing the taste receptor cells. The labellar cDNA library (20 000 pfu) and the taste receptor cell-enriched tissue cDNA library (total 80 000 pfu) were screened. A total of 418 cDNA clones which were expressed predominantly in taste tissue were cloned. In these clones, seven groups of cDNA were identified that showed sequence similarity to pheromone-binding protein (PBP) genes. The predicted amino acid sequences contain the putative signal peptide sequence at the N-terminal and the conserved six cysteines commonly shared with known insect PBPs. We named these gene products 'PBP-related protein of *Boettcherisca peregrina* (PBPRPbp) 1–7'. Seven PBPRPbp genes are divergent and exhibit 17–34% amino acids identity. Such divergence has been shown also in PBP-related protein (PBPRP) genes of *Drosophila melanogaster* (Pikielny *et al.*, 1994, Neuron, 12: 35–49). The PBPRPbp1 gene shows high sequence similarities to the chemical-sense-related lipophilic ligand-binding protein of *Phormia regina* (CRLBPpr) gene (Ozaki *et al.*, 1995, Eur. J. Biochem., 230: 298–308). RT-PCR analysis showed that all seven PBPRPbp genes were mainly expressed in taste tissue, labellum or tarsus. Some of them were also expressed weakly in antenna (PBPRPbp1,4 and 5) or gut (PBPRPbp4). In addition, there is no difference in the expression of these genes between male and female. Furthermore, PBPRPbp2, 4, 5 and 7 genes are present in larval head tissue that contain chemoreceptor cells. The unique expression pattern of PBPRPbps was different from that of known insect PBPs (Gyorgyi *et al.*, 1988, Proc. Natl Acad. Sci. USA, 85: 9851–9855) or general odorant-binding proteins (Vogt *et al.*, 1991, J. Neurosci., 11: 2972–2984), which were expressed predominantly in antenna. These findings may suggest some role of PBPRPbp in taste reception and feeding behaviour of the fly.

174. Uncaged Ca^{2+} activates a Ca^{2+}-activated Cl^- and a Ca^{2+}-activated K^+ conductance in toad olfactory receptor neurons

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In *Caudiverbera* olfactory receptor neurons, odorants may induce either excitatory and inhibitory responses. The inhibitory response involves a Ca^{2+}-activated K^+ conductance; this conductance is responsible for a hyperpolarizing receptor potential that results in a transitory decrease in action potential firing (Morales *et al.*, 1994, Proc. R. Soc. Lond. B., 257: 235). Our previous work showed that putrid odorants induce a small calcium current that leads to a rise in intracellular calcium, presumably allowing the activation of a ciliary Ca^{2+}-activated K^+ conductance (Morales *et al.*, 1997, FEBS Lett., 402: 259). However, there was no direct evidence connecting the calcium increase associated to the response to odors with the activation of the inhibitory potassium conductance. Using caged calcium in olfactory receptor neurons, we show that a rise in intracellular calcium activates a Cl^- conductance sensitive to micromolar DIDS and niflumic acid, as well as a K^+ conductance sensitive to nanomolar IBTX and CTX. In cells lacking the olfactory cilia we did not see activation of Ca^{2+}-dependent conductances, sensitive to these drugs, when uncaging calcium. This observation supports the notion that Ca^{2+}-activated Cl^- and Ca^{2+}-activated K^+ conductances reside in the cilia. The ciliary Ca^{2+}-activated K^+ conductance differs from the somatic Ca^{2+}-activated K^+ conductance present in the soma, which is insensitive to CTX (Morales *et al.*, 1995, FEBS Lett., 359: 41) and IBTX. Under I-clamp whole-cell conditions, uncaging calcium induced despolarization in some cells while in other cells it induced hiperpolarization, and the magnitude of these responses depends on membrane potential. Our results are consistent with the notion that both an excitatory Ca^{2+}-activated Cl^- conductance and an inhibitory Ca^{2+}-activated K^+ conductance, with opposite depolarizing an hyperpolarazing effects in physiological conditions, can be activated in response to calcium increases induced by odorants, depending on the cell. These results must be considered in order to understand the net response of olfactory receptor neurons to odors.

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175. Signaling mechanisms in the olfactory epithelium that regulate proliferation

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The two proliferative cell populations in the murine olfactory epithelium, horizontal basal cells (HBCs) and globose basal cells (GBCs), have distinctly different phenotypic characteristics and respond differently to olfactory bulbectomy (OBX)-induced neurotrauma (summarized in Getchell *et al.*, 2000, Cell Tissue Res., 299: 185–192). Based on these differences, we sought to investigate the signaling mechanisms that selectively regulate their proliferation *in vivo*. HBCs represent only 2.4% of the very small number of proliferative cells in the unstimulated olfactory epithelium, whereas GBCs account for 97.2% (Huard and Schwob, 1995, Devl Dynam., 203: 17–26); following OBX, GBC proliferation is up-regulated, but HBC proliferation is not (Schwartz Levey *et al.*, 1991, J. Neurosci., 11: 3556–3564). Based on the expression of epidermal growth factor receptor, the receptor for transforming growth factor (TGF)-α, by murine HBCs (Rama Krishna *et al.*, 1996, J. Comp. Neurol., 373: 297–307), we investigated the proliferation of olfactory epithelial basal cells in a transgenic mouse model in which overexpression of the TGF-α gene was driven by a keratin-14 promoter. The level of TGF-α protein was 73% greater in the nasal-olfactory epithelium of the transgenic mice than in that of nontransgenic littermate controls. Correspondingly, the number of proliferative HBCs was increased 5.8-fold in the transgenic mice compared with that in the controls; there was no difference in the numbers of proliferative GBCs between the transgenic and nontransgenic mice. These data indicate that TGF-α is an autocrine proliferation factor for HBCs but not GBCs. To investigate proliferation signaling mechanisms in GBCs, we analyzed the expression of members of the IL-6 family of neuropoietic cytokines and their receptors following OBX. Northern analysis demonstrated an early up-regulation of leukemia inhibitory factor (LIF) mRNA transcripts at 16 h post-OBX that was coincident with the infiltration of macrophages, a probable source of LIF, into the olfactory epithelium; LIF mRNA levels reached a maximum at 2 days post-OBX. The GBCs showed a selective and transient upregulation of expression of the receptor for LIF (LIFR) that was maximal at 2 days post-OBX, which preceded their peak proliferation at days 4–5 post-OBX. The combined data suggest a paracrine signaling mechanism, with macrophages as a source of LIF and the expression of LIFR on GBCs but not HBCs. Our data suggest that TGF-α acts as a proliferation factor for HBCs and LIF as a proliferation factor for GBCs, representing initial signals in the receptor-mediated transformation of quiescent progenitor cells leading to cell cycle progression and proliferation in the olfactory epithelium.

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176. Functional expression of members of a mouse olfactory receptor library responding to citronellal and derivatives

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A vast number of olfactory receptors (OR) have been cloned in the last decade, and sequence data have become publically available. The major obstacle of heterologous expression of recombinant OR in cell lines has been overcome by extending the N-terminal region of OR with a rhodopsin tag (rho-tag) (Krautwurst *et al.*, 1998, Cell, 95: 917–926). When co-expressing the G protein subunits α15,16, activation of rho-tag-OR by odorants leads to a transient increase in intracellular free Ca^{2+}, which can be monitored by the Ca^{2+}-imaging method. Cognate OR–ligand pairs were identified out of an expression plasmid library of mouse OR, one of which was I-C6, which at 10 μM concentration responds stereospecifically to citronellal (Krautwurst *et al.*, 1998). At the level of OR–ligand interaction, a combinatorial code (Malnic *et al.*, 1999, Cell, 96: 713–723) is commonly accepted, implying that an odorant can bind to different receptors and an OR can be activated by different odorants. Clustal alignments of all sequences from the OR library revealed a subgroup of seven structural closest homologs to the I-C6 OR. One goal of this study was to functionally characterize members of this subgroup, displaying 44–99% amino acid (aa) identity across transmembrane (TM) regions II–VII. For two OR of this subgroup with 50% aa identity to I-C6 we show their responsivness to citronellal and related substances, such as citral, citral-dimethylacetal, citronellol and citronellic acid. Another goal was to functionally identify other citronellal responders out of the OR library. Using the same co-expression strategy combined with Ca^{2+} imaging, we screened another eight pools, each with eight olfactory receptor chimeras, against (–)citronellal. One responsive pool was subdivided and screening was repeated with single receptor chimeras. An OR was identified (mV-D2) that responded specifically to (–)citronellal at concentrations as low as 0.1 μM, and also responds to the stereoisomer (+)citronellal and other citronellal-related substances. mV-D2 has 36% amino acid identity to mI-C6, while being a member of a homologous subfamily of eight ORs within the OR library, sharing 79–99% aa identity with each other. These experiments provide a basis for further studies on structure–function relations of OR subfamilies.

177. Cellular and molecular modifications in the olfactory after olfactory nerve lesion before and after birth

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After a lesion the olfactory neurons have a unique capability of regeneration from neuronal stem cells (for a review see Farbman, 1992, Cell Biology of Olfaction. Cambridge University Press, Cambridge). These processes have been characterized mainly in

postnatal mammals. We have previously shown (Leibovici *et al.*, 1996, Devl Biol., 175: 118–131) that in the chick embryo within 2days after the lesion of the olfactory nerve the expression of olfactory receptor genes and immunoreaction to CAM kinase II, a marker of mature olfactory neurons, drop rapidly while expression of the Cash1 gene, involved in olfactory neurogenesis, is increased.

This work was aimed at further characterizing the evolution of the different neuronal cell types in the olfactory epithelium after a unilateral nerve lesion and analysing the molecular processes involved in the concomitant cell death and regeneration under such experimental conditions.

Neuronal death was detected at different periods after the lesion by TUNEL detection of DNA fragmentation, on sections or after electrophoresis of DNA preparation from normal and lesioned olfactory epithelium. Mitosis was evidenced after BrdU injection in the animal and detection on tissue sections. This study revealed a striking and unexpected difference in the response to the lesion of the cells of olfactory neuronal lineage depending on the stage of the animal when the operation was performed: before or after hatching.

To further characterize the genes involved in these processes we have used the differential display technique. It was applied to mRNAs extracted from the olfactory epithelia of a lesioned animal either ipsilateral or contralateral to the lesion. Several cDNA fragments have thus been isolated. After sequencing and analysis in gene data banks they were used to analyse the pattern of expression of the corresponding genes by *in situ* hybridization on sections of the olfactory system and nervous system of lesioned and normal animals. Three fragments corresponding to genes activated after the lesion has been selected for cloning and complete sequencing of the corresponding gene. One of them would be a marker of neuronal stem cells or precursors in the olfactory epithelium but also in the central nervous system.

178. Chemoresponse in *Paramecium*: involvement of the calcium pump

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Paramecia are attracted to stimuli such as biotin, folate, acetate, cAMP and ammonium. There are at least three pathways for chemoresponse, at least two of which appear to involve the plasma membrane calcium pump for sustained hyperpolarization of the cell. In order to explore the role of the calcium pump in chemoresponse, we have used antisense technology to down-regulate levels of calmodulin, which is an activator of the pump. We found that reduced levels of calmodulin protein inhibited chemoresponse to glutamate and variably to acetate (Chem. Senses, 21: 55–58, 1996). To more effectively probe the regulation and role of the calcium pump, we have designed expression vectors to overexpress the calmodulin binding domain (CBD) in bacteria and transformed *Paramecium* cells. We know from our previous work that PKA activates the calcium pump ATPase *in vitro*. The CBD has potential PKA and PKC sites, and their phosphorylation should be comparable to calmodulin binding in activation of the pump. We have created mutants of these CBDs, with alanines or glutamates substituted for the serines of the putative PKA site. The wildtype CBD expressed protein is a substrate for heterologous PKA *in vitro*, and the mutant ala–ala and glut–glut forms are not, indicating that the PKA phosphorylation sites may be limited to the serines we identified. We have also found that there are multiple genes (minimally three) for plasma membrane calcium pumps in *Paramecium*. At least two are co-localized on the cell surface, and all bind calmodulin in overlays. We are testing the overexpression of individual CBDs of these pumps in transformed cells. We have found that overexpression inhibits chemoresponse to glutamate, folate and, to a variable extent, to acetate. We will test the overexpression of mutant CBDs next in transformed cells.

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179. Gene cloning of olfactory receptors recognized *R*-(–)- and/or *S*-(+)-carvone

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In rodents, olfactory receptors were estimated to consist of ~1000 members of G protein-coupled, seven-transmembrane domain proteins (Buck and Axel, 1991, Cell, 65: 175–187), whose transmembrane domains 3, 4 and 5 were proposed to be involved in odorant–receptor interactions, because of the sequence diversity discriminative among various structures of odorants (Buck and Axel, 1991; Lancet and Ben-Arie, 1993, Curr. Biol., 3: 668–674) and the analogy of the other members of the G protein-coupled receptor superfamily (Kobilka *et al.*, 1988, Science, 240: 1310–1316; Dohlman *et al.*, 1991, Annu. Rev. Biochem., 60: 653–688). One member of olfactory receptors is activated by multiple odorants and one kind of odorants is recognized by multiple receptors in olfactory system, where it was shown that the combinatorial receptors with the different odorant tuning specificity enabled to discriminate series of normal aliphatic odorants (Malnic *et al.*, 1999, Cell, 96: 713–723). In this paper, we examined how the olfactory receptor can discriminate optical isomers of odorants. The optical isomers of the carvone, the *R*-(–)- and *S*-(+)-forms, are recognized as different odors with some similarity. It is interesting how the olfactory neurons/receptors discriminate the molecules of the optical isomers. Only a receptor responsive to both of the isomeric carvones has been reported (Krautwurst *et al.*, 1998, Cell, 95: 917–926). Using a combination of the calcium imaging assay and single-cell RT-PCR (Malnic *et al.*, 1999), we cloned and sequenced the genes encoding the transmembrane domain 4 and 5 of the mouse olfactory receptors which obtained from isolated olfactory receptor neurons (ORNs) responsive preferentially to one of isomeric carvones or those equally to both of carvones. Among nine olfactory receptors identified in nine ORNs that responded to carvones, the amino acid sequences of loop domain were more conserved than the transmembrane domains as reported previously (Buck and Axel, 1991; Malnic *et al.*, 1999). The comparison of the amino acid sequences of the nine olfactory receptors with 88 receptor members which were previously identified in mouse showed that five and two of the receptors for the carvones were grouped in a cluster, respectively and the others were not. Furthermore, we discovered one pair of amino acid residues that is conserved at the special position in several carvone receptors but not in most of the other mouse olfactory receptors. The amino acid residues may play an important role to interact with carvones. This approach and

further analysis will reveal the specificities of the carvone receptors to recognize the optical-isomeric carvones by multiple receptors with different relative sensitivities to multiple odorants in the olfactory system.

180. Development of a method to assess implicit memory of food

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In eating and drinking, sensory memory is implicated in many forms and ways. It plays a role in sensory and liking expectations about food products, and in the development of personal preferences and aversions. Sensory memory is usually assessed by explicit tasks which required a conscious recollection of previously experienced events (recognition tasks; recall tasks). Nevertheless, in daily meals, sensory memory is usually incidentally acquired and implicitly and subconscious recollected.

The aim of the present work was to develop tasks allowing to assess implicit memory of food attributes in order to latter study the role of memory in the appreciation of food. (This study is part of the European program 'Healthy Ageing: How Changes in Sensory Physiology, Sensory Psychology and Socio-Cognitive Factors Influence Food Choice'.). Two types of implicit tasks were compared: two priming tasks and a conditioning task. Each task consisted of two stages: (i) implicit learning of target foods; and (ii) implicit recollection of target foods among distractor foods.

Target foods were presented during a meal in an accidental way, i.e. without any indications that something about them should be remembered (implicit learning). A few hours later, a first group of subjects received all possible pairs from the target and distractor foods, and indicated for each pair whether members were different or not. A second group rated liking or flavour intensity of each target and distractor food. Implicit memory was demonstrated for both groups by a change in performance compared with a control group, i.e. shorter latencies on trials involving target foods than on trials involving distractor foods (priming effect).

Subjects were given a stressful task (resolution of logical or mathematical problems) while they were eating target foods during a meal. A few hours later, subjects rated liking of each target and distractor foods. Implicit memory was demonstrated by a change in attitudes compared with a control group, i.e. lower liking scores for target foods than for distractor foods.

181. Lateralization of odor memory: effects of rhinal side of stimulation

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Results from behavioral and neural imaging studies suggest hemispheric lateralization for certain olfactory functions. Concerning the side of rhinal stimulation, there are findings, although somewhat conflicting, that indicate a right-nostril dominance for perceptual functions such as detection, discrimination and pleasantness. There are also data that suggest a left-nostril dominance for odor identification, but these results are mainly from patient studies (e.g. split-brain), which may not reflect normal populations. This study investigated whether there are differences in odor memory in healthy young subjects depending on the side of rhinal stimulation, as demonstrated in neuropathological patients. The functions targeted in this study were perceived familiarity, identification and episodic recognition (including measures of performance, experience, processes, and reaction time).

Forty right-handed, healthy subjects (20 men and 20 women, 20–30 years), who were screened for loss in odor sensitivity, participated. A set of 48 common odors, presented in opaque glass bottles, were used as stimuli. The procedure consisted of an encoding phase with familiarity ratings for 24 odors, and a retrieval phase including free identification for these odors and 24 new odors. All stimuli were presented monorhinally, alternating left and right nostril, and totally randomized, with the exception that the same odor always was presented to the same nostril in both the encoding and retrieval phase.

A difference in familiarity ratings was found between right- and left-nostril stimulation, with higher familiarity ratings for the right nostril. However, the results showed no side-related differences in free identification (naming) or in measures of episodic recognition.

The familiarity ratings suggest a right-nostril dominance for familiarity, which appears not to have been reported previously in the literature. Similar to these findings, previous research has indicated a right-nostril dominance for odor hedonicity. These observations may be related in the sense that odor hedonicity has been shown to be positively correlated with familiarity. The present lack of side-related effects on recognition and identification implies that, if any, such effects are rather marginal. This may be related to more extensive inter-hemisphere interaction in higher-order cognitive functions.

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182. Anesthetic infusion into the cortical taste area in one side reversibly alters response characteristics of cortical taste neurons in the other side in rats

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The cortical taste areas (CTA) of two hemispheres in rats are anatomically connected by the corpus callosum. We found that the fraction of taste neurons with bilateral receptive fields was larger in the CTA than in the thalamus in the previous study, which suggests a contribution of afferent inputs from the contralateral CTA. To confirm this electrophysiologically, we examined changes in taste responses of single CTA neurons in one side by blocking those in the other side with local anesthetic in urethane-anesthetized rats.

Adult female SD rats were used. To block the contralateral CTA, 10% procaine in 0.9% saline was infused through a canula which was inserted to the dorsum of the cortex toward the area. Taste stimuli used were 0.1 M NaCl, 0.5 M sucrose, 0.01 N HCl and 0.02 M quinine–HCl.

Taste responses of 68 taste neurons were recorded for up to 2 h until the recovery from the treatment, and those in 50 of them were affected significantly. No remarkable difference was noted in the spatial distribution of the affected and non-affected neurons in the CTA. Many affected neurons were located in layers IV and V of

area GI and in layer V of area DI. In most cases, the taste responses of single neurons were decreased. Several neurons totally lost taste responses. However, in several cases they were increased, and in a few cases they were decreased or increased depending on the stimulus. The response profile and best stimulus changed with the treatment.

The results suggested that the CTAs on one side receive excitatory or inhibitory inputs by callosal fibers from the other side. Since the CTA sends efferents to the contralateral NTS, the CTA blockade probably affects thalamic afferents to some extent. The present findings suggest that dysfunction in the CTA on one side causes disturbances of taste discrimination by changing taste profiles in many CTA neurons in other side.

183. Modified use of functional measurement: an examination of context effects on taste

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Context effects on taste indicate that taste is not invariant. There is some question as to whether sensory or judgement processes of taste are affected by context (e.g. skewness in the distribution of concentrations). In investigations using the difference judgement approach to functional measurement (De Graaf *et al.*, 1987, Percept. Psychophys., 41: 383–392; Anderson, 1990, A functional theory of cognition), it was concluded that context affects sensory processes but not the form of the judgement function. The psychophysical functions were not invariant, but the simple effects of judged differences were linear (i.e. parallel) across contexts (Schifferstein, 1995, Percept. Psychophys., 57: 56–70). Evidence from our laboratory, however, suggests that difference judgements may bias the data toward parallelism, so that effects of context on the judgement function are obscured (Blot and Stevens, 2000, AChemS XXII). This also has implications for the use of difference judgements in validating interval scaling in the psychophysical functions. In a current study, we investigate these issues more systematically using a modified approach to functional measurement that does not involve difference judgements. We report the results from an absolute judgement task where two tastants presented in close succession are considered a pair, so that an expected judgement function can be derived post hoc.

184. Inhibition of gastric motility induced by superior laryngeal afferents and their neural mechanism

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Receptive relaxation can occur prior to food reaching the stomach and facilitate reservoir function of stomach. Mechanical stimulation of the pharynx and distension of the esophagus had been assumed to induce such relaxation. The present study was undertaken to clarify the role of the superior laryngeal afferent signals elicited by water in gastric relaxation. Their neural mechanisms were also considered. All experiments were performed in urethane–chloralose-anaesthetized rats. Animal care was in accordance with the guidelines of the Physiological Society of Japan.

Gastric motility was measured by the intragastric balloon connected with a strain-gauge pressure meter. Administration of water into the larynx inhibited motility of the distal stomach but 0.15 M NaCl did not induce the inhibitory response. Electrical stimulation of the central cut end of the superior laryngeal nerve (SLN) inhibited motility. Bilateral sectioning of the SLN abolished the inhibitory response induced by water. Bilateral sectioning of the cervical vagus abolished the inhibitory response. In animals which underwent the sectioning of the right vagal nerve at the cervical level, chemical lesions of the dorsal motor nucleus of the vagus (DMV) were achieved by injecting kainic acid (4.7 mM, 30–60 nl) into the left DMV at the level 0.5 mm rostral to the obex. The lesion of the DMV completely abolished the inhibitory response of gastric motility induced by the administration of water into the larynx. Neural responses of the DMV neurons to the administration of water into the posterior oral cavity were recorded extracellularly in the animals which underwent the bilateral sectioning of the chorda tympani and glossopharyngeal nerves. Seventy-eight DMV neurons which showed antidromic response to the electrical stimulation of the anterior subdiaphragmatic vagus were recorded. Among them, 34 neurons showed a decrease in firing rate in response to the administration of water (water-responsive neurons). These neurons did not respond to the administration of 0.15 M NaCl. Electrical stimulation of the SLN elicited inhibition in all water-responsive neurons tested (10 neurons).

These results suggest that the SLN afferents appears to provoke inhibition of the stomach via a vagal excitatory pathway. Water fibers in the SLN might be a novel neuronal source for the receptive relaxation.

185. Pattern of oral and taste representation in the insular cortex in rats

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Gustatory cortex (GC) is located in the granular and dysgranular regions of the insular cortex in rats, though it is found that gustatory neurons are intermingled mainly with high-threshold mechanoreceptive ones. To clarify the microstructure of the GC, we aimed to examine the response features of both mechanoreceptive and taste neurons by recording them along the tracks made at various angles against the surface of the insular cortex.

Adult female SD rats were anaesthetized with urethane, immobilized and artificially ventilated. The bone covering the insular cortex was removed. A tungsten electrode was inserted through a hole in the dura into the cortex. Impulse discharges were amplified, and led to an FM tape recorder and/or a PC. Mechanical stimulation of the tissue was made manually by brushing, stroking with a glass rod or pinching with a pair of non-serrated forceps. Taste stimulation and data acquisitions were controlled by another PC. Taste solutions used were 0.1 M NaCl, 0.5 M sucrose, 0.01 N HCl and 0.02 M quinine–HCl.

Neurons at the border with SI had RFs on the contralateral lip; however, neurons encountered more laterally in the insula had RFs

on the bilateral side of the mouth. RFs localized to the intraoral tissue were small in fraction (~20% of the sample), but in a larger fraction the RFs were found outside the oral cavity as well as on its inside: extraoral RFs were found on the lips (~40%) or on both lips and other tissue, e.g. pinna (~40%). Most neurons were of high threshold; though low at the lips and/or intraoral tissue, the threshold was high at the external surface of the body. Taste neurons were encountered along the tracks. About half of them were activated by pinching, with the RFs at the extraoral and/or intraoral tissue. High-threshold inputs were not localized in the infragranular layers, in contrast to SI. Thus, the GC not only represents taste and intraoral mechanical information, but also integrates noxious information from the whole body as in the retroinsular area in primates.

186. Novel calcium binding proteins express at catfish (*Ictalurus punctatus*) taste epithelium

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Calpain, a calcium-activated neutral protease found in the cytoplasm, may play important roles in intracellular signal transduction cascades that are regulated by calcium. Here we characterized novel type calpains from catfish (*Ictalurus punctatus*) barbell epithelium. These calpains (IP-nCL4a and 4b) share ~60% identity with the human digestive tract calpain (human nCL4). Northern blot analysis revealed that IP-nCL4a was predominantly expressed in the barbell, but not expressed in the brain in catfish. IP-nCL4b was expressed in the barbel, intestine and skin. Antibodies against the N-terminal segment of IP-nCL4a recognized the taste pore region in catfish barbells (Ookura *et al.*, 1997, Chem. Senses, 22: 764; Ookura *et al.*, 1999, AchemS XXI, 64). These results suggest that IP-nCL4a may be a taste-cell-specific calpain. We also characterized other calcium-binding proteins (Ictacalcins) (Porta *et al.*, 1996, Brain Res. Mol. Brain Res., 41: 81–89) isolated from barbell epithelium. Antibodies against an internal segment of Ictacalcins (2 and/or 3) recognized cells in catfish taste buds. These calcium-binding proteins, specifically expressed in taste cells, might play regulatory roles in taste signal transduction.

187. Responses of pharyngeal taste nerve fibers to fatty acids in rats

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Fat in food is an important constituent that affects the food's palatability. Although it is generally accepted that the texture of fat is the most prominent feature for the palatability, the mechanisms by which fat is detected in the mouth have not been clarified. Recent behavioral studies suggest the existence of chemosensory mechanisms in the oral cavity for detection of fat. However, there are few studies that have examined directly whether fat influences the activities of the taste nerves. This study was therefore designed to investigate the responsiveness of the glossopharyngeal nerve to fat or fatty acids. In particular, we studied the response properties of pharyngeal branch of the glossopharyngeal nerve because, in recent years, there has been increasing attention on taste reception in the pharynx. Wistar rats were anesthetized with urethane and placed in the supine position. The pharynx and larynx were surgically opened to expose the posterior pharyngeal wall, posterior pillars and soft palate. Fat or fatty acids were applied to these regions of the pharynx. Oleic acid and linoleic acid were used as stimuli of fatty acids because these long-chain fatty acids were preferred by rats (Tsuruta *et al.*, 1999, Physiol. Behav., 66: 285–288). The nerve activities were recorded from the whole bundle or pauci-fiber bundles of the pharyngeal branch of the glossopharyngeal nerve. Oleic acid elicited vigorous discharges in the pharyngeal nerve. The response activities immediately increased after the application and continued for >10 s. Linoleic acid also elicited an excitatory response, but the magnitude of the response was smaller than that for oleic acid. Triolein, which was used as a pure fat, had no effect on the nerve activity. Vegetable oil (safflower oil) also had no effect. Paraffin oil and mineral oil, non-fat substances with a fat-like texture, did not evoke any response. These results indicate that only fatty acids had potent excitatory effects on the pharyngeal branch of the glossopharyngeal nerve. The present findings provide the first evidence for the existence of taste nerve fibers which respond to long-chain fatty acids.

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188. HCN1 channels in taste receptor cells

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HCN channels form a small family of cyclic-nucleotide-gated, hyperpolarization-activated, non-selective cation channels. Members of this family are well known to occur in the heart and brain. In the heart they underlie the I_f current.

In order to demonstrate whether or not HCN channels are also present in taste receptor cells, RT-PCR using degenerated primers from conserved regions of these channels was carried out. cDNA fragments of the expected size were amplified using RNA from vallate papilla. Sequence analysis of five cloned fragments showed that all of them represent HCN1 channel sequences. RT-PCR with primers specific for the HCN1 channel revealed that low levels of HCN1 channel mRNA are present in the vallate papilla. No cDNA fragments were amplified using RNA from the epithelium surrounding the vallate papilla. This observation suggested that HCN1 is expressed in the taste buds. This assumption lent further support from RT-PCR experiments using cDNA from isolated taste buds which actually proved the presence of HCN1 channel mRNA in taste buds.

In situ hybridization experiments using the standard alkaline phosphatase protocol clearly showed the presence of HCN1 channel mRNA in taste buds of the vallate papilla. Further *in situ* hybridization experiments using a signal amplification protocol and fluorescence-dye detection methods identified HCN1 mRNA

in a subset of cells within the taste buds that show the typical elongated shape of taste receptor cells.

189. Age-related alteration of taste bud distribution in the common marmoset

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Age-related alterations of taste buds distribution are different among subpopulations within the soft palate (SP), fungiform (FF), circumvallate (CV) and foliate (FL) papillae. Maturation of taste buds on the rat SP precedes that of taste buds in other areas within the oral cavity, suggesting that the palatal taste buds is functionally important for suckling behavior in neonates (Harada *et al.*, 2000, Physiol. Behav., 68: 333–339). Although a few studies were reported for the FF, CV and FL papillae of monkey and human (Bradley *et al.*, 1985, Anat. Rec., 212: 246–249; Mochizuki, 1937, Okajimas Folia. Anat. Jap., 15: 595–608; Mochizuki, 1939, Okajimas Folia. Anat. Jap., 18: 337–354), age-related differences in taste buds distribution within the SP have not been investigated. To clarify the functional importance of taste buds distribution in the marmoset, the present report investigates age-related alteration in the number, size, shape and maturation of their taste buds, and compares these with similar information from the rat.

The development of taste buds on the SP, FF, FL and CV papillae at different postnatal ages was examined histologically in the marmoset. After paraffin embedding, complete serial sections at 10 μm thickness were made and stained by hematoxylin & eosin. Digitized images for each section were examined carefully. The number of FF taste buds at day 1 was 313. While only 20% of all taste buds at birth possessed a taste pore, 39% of 174 taste buds on the SP at day 1 possessed one. The number of taste buds with pores at day 1 was small for the CV (22/58, center; 7/22, one side) and FF (2/13, one side). The total number of taste buds increased with increasing age and reached a maximum at 2 months: FF, 1022; SP, 544; CV-center, 545; CV-side, 344; FF-side, 159. Virtually all taste buds possessed a taste pore at 2 months of age. After 2 months, the total number of taste buds tended to decrease with increasing age. These results suggest that the functional maturation of SP taste buds might precede maturation in other areas of the tongue, and that the decrease in the number of taste buds in the oral cavity with increasing age might result in a change in taste sensitivity.

190. Leptin suppresses sweet taste sensitivities in mice

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Leptin, which is released from adipocytes, acts as a potent inhibitory factor against obesity by regulating energy expenditure and food intake (Friedman and Halaas, 1998, Nature, 395: 763–770). It has been reported that the obese diabetic *db*/*db* mouse has defects in the leptin receptor (Ob-R) and displays enhanced neural responses and elevated behavioral preference to sweet stimuli (Ninomiya *et al.*, 1995, Am. J. Physiol., 269: R930–R937; Sako *et al.*, 1996, Chem. Senses, 21: 59–63; Ninomiya *et al.*, 1998, Am. J. Physiol., 274: R1324–R1330). This line of study has raised the possibility that taste sensitivity to sweeteners might be regulated by leptin. To clarify this point, we investigated the effects of leptin on the peripheral taste system in control lean (C57BL/KsJ and BALB/c) mice and *db*/*db* mice, and the expression of Ob-Rs in circumvallate papillae (innervated by the glossopharyngeal nerve) of control lean mice. An intraperitoneal injection of leptin suppressed the responses of peripheral taste nerves (chorda tympani and glossopharyngeal) to sweeteners (sucrose and saccharin) without affecting the responses to sour, salty and bitter substances in control lean mice. Whole-cell patch-clamp recordings from taste cells isolated from circumvallate papillae of control lean mice demonstrated that bath-applied leptin activated K^+ conductances in a subset of taste cells, which resulted in hyperpolarization of the cells, and, moreover, almost all leptin-sensitive cells responded to saccharin with decrease in K^+ conductances. The *db*/*db* mouse with impaired Ob-R showed no such effects of leptin. Immunohistochemical study showed that some taste bud cells were immunoreactive to antibodies against the Ob-Rs. Taken together, these observations suggest that leptin may suppress sweet sensitivities of peripheral taste system through the activation of K^+ conductances in taste cells mediated by Ob-Rb in control lean mice. On the other hand, defects in this leptin suppression system in *db*/*db* mice may lead to their enhanced peripheral neural responses and enhanced behavioral preferences to sweet stimuli.

191. Invalid sensory comparisons across groups: examples from prop research

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Comparisons of perceived sensory intensities across groups are valid only if ratings are made relative to a standard equally intense, on average, to each group. The standard can be a stimulus (actual or imagined) or a label (eg, 'strong'). Commonly used techniques include magnitude estimation with a standard stimulus, labeled category scales and labeled lines (i.e. visual analogue scales). Because we cannot share experiences, we cannot prove a standard equivalent to all. However, we can identify cases where the (often implicit) assumption of equivalence is likely false. Such false assumptions not only prevent experimenters from seeing effects that are real but also produce apparent effects in the wrong direction. Genetic variation in perceived bitterness of 6-*n*-propylthiouracil (PROP) provides examples. We initially used a nine-point scale ('very strong' at the top) for a spatial test to assess each of the cranial nerves mediating taste but ultimately switched to a line scale ('strongest imaginable oral sensation' at the top) developed by Green and his colleagues (1993, Chem. Senses, 18: 683–702). Since oral stimulus intensities vary across nontasters, medium tasters and supertasters, this is an invalid standard for PROP studies. We used 'strongest imaginable sensation of any kind' (more likely to be equivalent across PROP groups). Comparison of the two methods showed dramatic differences.

Using the Green scale, saltiness of NaCl correlated positively with PROP bitterness for all spatial loci, including the palate. The only significant correlation obtained with the nine-point scale was a negative correlation on the palate. We conclude that since supertasters cannot rate their strongest taste sensations greater than 'very strong' on the nine-point scale they must proportionately reduce ratings of weaker tastes. Some are reduced so far that they are lower than the ratings given by nontasters. The wide usage of sensory scales means that invalid standards may have grave consequences in a variety of fields. We note that the logic of the psychophysical errors we have highlighted in a sensory context also applies to the scaling of preference and emotional states which extends the impact even further.

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192. Transcellular and paracellular pathways for salt taste transduction in the ventral skin of desert toads

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Transduction of salt taste is in part mediated by the epithelial Na^+ channel (ENaC) in the taste cell membrane. Another part is possibly mediated by a flow of small ions through a tight junction in the gustatory epithelium (Ye *et al.*, 1991, Science, 254: 724–726). The ENaC as a selective route for Na^+-influx was first shown in frog skin (Lindemann and Van Driessche, 1977, Science, 195: 292–294), where the physiological role of ENaC is for the homeostasis of body fluids, not for a taste transduction. However, we recently discovered that Na^+-influx through the ventral skin of desert toads triggers salt taste transduction of the spinal nerves and allows them to discriminate hypertonic salt solutions (Nagai *et al.*, 1999, J. Comp. Neurol., 408: 125–136). Interestingly, amiloride, a blocker for ENaC, partly reduced the spinal nerve responses that occurred in taste transduction. Amiloride-blockable and non-blockable pathways in the toad skin were studied with neural recordings, antibody and electron microscopy.

Activities of the fifth and sixth spinal nerves innervating the ventral skin of the desert toads (*Bufo alvarius*) were extracellularly recorded, when the receptive filed of the nerves was stimulated with salt solutions. The nerve responses to NaCl solutions were significantly reduced by pre-exposure of the field to amiloride (10 μM, 5 min). The rabbit polyclonal antibody against the 19-amino-acid sequence in the extracellular loop of the α subunit of *Xenopus* ENaC (Zuckerman *et al.*, 1999, J. Biol. Chem., 274: 23286–23295) was generated and used to visualize ENaC in the toad skin. Positive immunoreactivity for ENaC occurred in the apical cell membrane of the first cell layer in the stratum granulosum. The ventral skin was exposed to $LaCl_3$ (5 mM, 30 s) to occlude intercellular junctions of the epithelium, resulting in profound reduction of the neural responses to NaCl. The skin was further exposed to 0.1 M Na cacodylate containing 10 mM Na_2CO_3 for 30 min to make lanthanum precipitate and subsequently processed by the fixation protocol for electron microscopy (Zampighi *et al.*, 1988, J. Cell Biol., 107: 1667–1678). Precipitates of lanthanum were seen in the intercellular spaces of the str. granulosum. These results suggest that the spinal nerve responses were induced by sodium ions flowing into the cell layers of the skin through ENaC in the first layer (transcellular pathway) and intercellar spaces in the str. granulosum (paracellular pathway).

193. Electrogustometric taste detection thresholds imply a right cerebral hemisphere advantage in gustatory processing

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In this paper we review published evidence, and present new evidence, that aspects of gustatory function may be preferentially processed in the right hemisphere of the human brain. When automated electrogustometry was used to measure taste detection thresholds from the left and right sides of the tongues of 97 normal volunteers, a small but statistically significant advantage was obtained for the right side of the tongue ($P < 0.01$) (Stillman *et al.*, 2000, Clin. Otolaryngol., 25: 120–125). A similar advantage was obtained in two volunteers who undertook to monitor their taste detection thresholds daily for ~80 days. Furthermore the thresholds of both volunteers were more variable on the left than on the right. Neural pathways for taste project ipsilaterally to the cortex (Pritchard *et al.*, 1999, Behav. Neurosci., 113: 663–671), thus these behavioural outcomes are in keeping with recent evidence from imaging studies suggesting a functional asymmetry favouring the right hemisphere of the brain in the processing of taste information (Zatorre *et al.*, 1992, Nature, 360: 339–340; Small *et al.*, 1997, J. Neurosci., 17: 5136–5142). A growing body of evidence also supports the notion of a functional asymmetry favouring the right hemisphere of the brain in the processing of olfactory information (Zatorre and Jones-Gotman, 1990, Percept. Psychophys., 47: 526–531). However, there is uncertainty over which aspects of gustatory and olfactory processing are lateralized, and whether any are related to handedness (Zatorre and Jones-Gotman, 1990; Cer *et al.*, 1998, Ann. N.Y. Acad. Sci., 855: 575–578; Hummel *et al.*, 1998, Chem. Senses, 23: 541–544). Taste and olfaction each combine with other sensations from the oral cavity to produce the experience of flavour, and both are emotionally evocative senses. Thus it might be expected that patterns of hemispheric asymmetry should apply to both, and are likely to favour the right hemisphere.

194. Acetylcholine may modulate taste transduction via muscarinic receptor in taste receptor cells

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Neuroactive compounds have been proposed to modulate taste responses in taste receptor cells. Previous studies suggest that acetylcholine (ACh) is a transmitter released from taste cells as well as a transmitter in cholinergic efferent autonomic neurons innervating taste buds (Nagai *et al.*, 1996, Chem. Senses., 21: 353–365). However, the physiological effects of ACh on taste cells

and their possible modulatory effects on taste transduction have not been established. I examined ACh responses by monitoring $[Ca^{2+}]_i$ using Ca^{2+}-imaging with fura-2. ACh increased $[Ca^{2+}]_i$ in taste cells of rat circumvallate papillae as well as in taste cells of the mudpuppy *Necturus maculosus*. A muscarinic ACh anatagonist blocked the ACh response, but the nicotinic inhibitor D-tubocurarine did not. U73122, a phospholipase C inhibitor, and thapsigargin, a Ca^{2+} ATPase inhibitor that depletes intracellular Ca^{2+} stores, blocked the ACh response. These results suggest that ACh binds to muscarinic receptors, causing an increase in IP_3 and subsequent release of Ca^{2+} from intracellular stores. In non-gustatory mammalian cells, M_1, M_3 and M_5 receptor subtypes stimulate PI metabolism (Bonner, 1989, Trends Neurosci., 12: 148–151). These data suggest that the ACh-induced $[Ca^{2+}]_i$ increase is via $M_1/M_3/M_5$-like receptors. In the presence of extracellular Ca^{2+}, a long incubation with ACh induced a transient response followed by a sustained phase of $[Ca^{2+}]_i$ increase. In Ca^{2+}-free saline, only transient responses persisted and sustained phases disappeared, suggesting that Ca^{2+} influx is involved in the sustained phase. To determine if muscarinic receptor protein is present in taste receptor cells, I examined immunoreactivity for M1subtype of muscarinic ACh receptors. Immunoreactivity for the muscarinic ACh receptors was present in both rat and mudpuppy. In rat, immunoreactivity appeared to be present in both apical and basolateral membrane compartments. We found previously that the bitter compound denatonium increases $[Ca^{2+}]_i$ via the IP3 pathway in mudpuppy taste cells (Ogura *et al.*, 1997, J. Neurosci., 17: 3580–3587). Thus, I tested whether ACh modulates the response to denatonium. Incubation with ACh suppressed the Ca^{2+} response to denatonium, and the effects of ACh were inhibited by atropine. These results suggest that ACh may modulate responses to taste stimuli by affecting intracellular Ca^{2+} levels via a muscarinic ACh receptor in taste cells.

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195. Are apical membrane receptor sites responsible for taste responses of the frog glossopharyngeal nerve to electrolytes?

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The frog glossopharyngeal nerve (GL) responds to low $CaCl_2$ (>0.01 mM) and relatively high NaCl (>100 mM). The NaCl response of the frog GL was not affected by addition of amiloride, an epithelial Na^+ channel blocker, to NaCl solution (Kitada and Mitoh, 1998, Chem. Senses, 23: 222), but it was enhanced following application of 1-anilinonaphthalene-8-sulfonate (ANS) to the tongue (Kashiwagura *et al.*, 1977, J. Membr. Biol., 35: 205–217). Since amiloride and ANS are large molecules, they are unable to pass through tight junctions. Thus, the enhancement of the NaCl response by ANS suggests that ANS affects the NaCl response via the apical membrane. However, there is a possibility that ANS enters taste cells and affects the NaCl response internally. In the present study, in order to determine whether apical membrane receptor sites are responsible for the responses to electrolytes, we investigated whether ANS affects the NaCl response of the frog GL externally or internally and whether the $CaCl_2$ response is affected by amiloride or ANS. The summated taste responses from the whole GL were recorded in anesthetized frogs. The tongue surface was treated with 1 mM ANS for 4 min and then rinsed with 10 mM NaCl for 1 min. Subsequent application of a solution of 100 mM NaCl (threshold concentration) to the tongue led to a greatly enhanced response. However, the enhanced response was observed only within 2 min after ANS application. The ANS effect was reversible and depended on the species of salt (NaCl, KCl, NH_4Cl, $CaCl_2$). The results do not appear to explain the effect of ANS in terms of an internal action of ANS. Amiloride at 0.5 mM had no effect on the enhanced NaCl response by ANS. Therefore, it appears that amiloride-insensitive Na^+ receptor sites may reside in the apical membrane of taste cells. Amiloride at 0.5 mM strongly reduced the response to 1 mM $CaCl_2$. The inhibition of the $CaCl_2$ response by amiloride was not due to a specific action of amiloride but to competition between Ca^{2+} and amiloride$^+$ ions. We conclude that apical membrane receptor sites are responsible for the responses of the frog GL to electrolytes.

196. Rapid transient increase in cgmp level in intact rat circumvallate taste-bud cells in response to sucrose stimulation

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The cellular transduction of sweet tastants is believed to involve G protein-coupled receptors. A 5 min incubation of rat circumvallate (CV) taste-bud sheets with sucrose increased cAMP (Striem *et al.*, 1991, Cell. Physiol. Biochem., 1: 46–54), a 10 s stimulation of a crude preparation of mouse fungiform papillae by sucrose increased cGMP (Miwa *et al.*, 1997, J. Vet. Med. Sci., 59: 81–83), and intracellular administration of cGMP or cAMP produced membrane depolarization in sweet-responsive cells (Cummings *et al.*, 1993, J. Neurophysiol., 70: 2326–2336). Such signal messengers can now be monitored biochemically in real time, in the sub-second range following taste stimulation.

CV and nonsensory epithelium (EP) sheets were isolated (Striem *et al.*, 1991) and incubated with and without sucrose using a computerized fast-pipetting system (FPS) which delivers 'start' and 'stop' solutions consecutively into an Eppendorf test tube at predetermined, short time intervals (from 75 ms). Following cell permeabilization, the intracellular content of cGMP and cAMP was determined by RIA.

We demonstrate a rapid and transient (75–250 ms) increase of cGMP (but not cAMP) level in intact rat CV taste cells following stimulation by sucrose. This rapid increase did not occur in nonsensory EP cells. Pretreatment with a nonspecific phosphodiesterase (PDE) inhibitor (IBMX), a specific cAMP-PDE4 inhibitor (denbufylline) or an adenylyl cyclase activator (forskolin) increased basal cAMP and abolished the sucrose-stimulated cGMP increase at 150 ms. Pretreatment with a soluble guanylyl cyclase inhibitor (ODQ) reduced, whereas a specific cGMP-PDE inhibitor (zaprinast) abolished the sucrose-stimulated cGMP increase.

It is proposed that cGMP is involved in the initial stage of sugar-taste transduction and that cGMP is more important than cAMP at this stage. Activation of soluble guanylyl cyclase and inhibition of cGMP-PDE may be involved in the transient elevation of cGMP in response to sucrose stimulation.

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197. Neuropeptides (CCK and NPY) and catecholamines in the vagal gustatory lobe of goldfish

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The vagal lobe of goldfish is the medullary primary gustatory nucleus homologous to part of the nucleus of the solitary tract (NST) of mammals. The vagal lobe, unlike the NST, receives only vagal gustatory and not general visceral inputs. Further, the vagal lobe is a laminated, large structure which facilitates anatomical investigations. Since the vagal lobe is concerned with gustatory-mediated food-sorting behavior, we have undertaken an immunocytochemical study of the distribution of various neuropeptides and neurotransmitters known to be involved in regulation of food intake: cholecystokinin (CCK), neuropeptide Y (NPY) and catecholamines (studied by examination of tyrosine hydroxylase, TH).

The outermost layers of the vagal lobe include a capsular fiber system which includes some of the primary afferent fibers of the vagus nerve. The capsular fiber system contains numerous fibers immunoreactive for either NPY or CCK. Following lesion of the vagus nerve root, the majority of CCK but not NPY fibers are lost. Thus primary afferents to the lobe include some fibers immunoreactive for CCK.

Both peptidergic fiber populations terminate in the vicinity of small TH-immunoreactive neurons lying within superficial layers of the lobe. The majority of the vagal gustatory fibers, which terminate in the deeper layers (vi and viii) of the lobe, are not immunoreactive for either peptide. However, numerous varicose axons immunoreactive for CCK, NPY or TH are present in the deepest portions of the sensory layers of the lobe. Their continued presence in lesioned animals indicates that these fibers are intrinsic to the CNS.

The vagal lobe motor layers, equivalent to portions of the nuc. ambiguus, include primary motoneurons that innervate the palatal musculature important for food selection behaviors. The superficial portion of the motor layer contains small GABAergic interneurons. These interneurons appear to receive input from a peptidergic stream of fibers which course along the outer margin of the motor layer. These fibers are not primary afferent fibers as they are not lost following nerve transection.

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198. Analysis of a rise in cytosolic calcium elicited by tastants in gerbil and human taste cells

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Gustatory transduction mechanisms for taste stimuli seem to differ among species of animals (Lindemann, 1996, Physiol. Rev., 76: 719–766). In rat taste cells, sugars increase the concentration of cyclic adenosine monophosphate (cAMP), but synthetic sweeteners elevate the concentration of inositol 1,4,5-trisphosphate (IP_3) (Bernhardt *et al.*, 1996, J. Physiol., 490: 325–336). On the other hand, in hamster taste cells, both sugars and synthetic sweeteners may induce a cAMP-PKA cascade that blocks a resting K^+ conductance, resulting in a depolarization of the taste cell (Cummings *et al.*, 1996, J. Neurophysiol., 75: 1256–1263).

To elucidate the physiological cytosolic Ca^{2+} dynamics in taste buds in response to taste stimuli, we used Fura-2 imaging of the epithelial sheets containing some fungiform papillae in gerbil and human. The lingual epithelial sheet was located upside up and only the apical receptive membrane of taste cells was stimulated chemically. We confirmed histologically the presence of the taste buds in the papilla after the measurement of the cytosolic Ca^{2+}. In the gerbil, sodium saccharin (60 mM), sucrose (0.5 M) and NaCl (0.5 M) increased the cytosolic Ca^{2+} concentration in 41% ($n = 46$), 45% ($n = 22$) and 31% ($n = 13$) of the taste buds tested, respectively. In the human, sodium saccharin (60 mM), sucrose (0.5 M) and NaCl (0.5 M) increased the cytosolic Ca^{2+} concentration in 23% ($n = 7$), 10% ($n = 10$) and 33% ($n = 9$), respectively. These results suggest that sensitivity for sweeteners in the gerbil is much higher than that in the human, and that sensitivity for NaCl in the gerbil is similar to that in the human.

199. Single taste fiber response in m. mulatta and b. taurus to derivatives of denatonium benzoate—which is the critical moiety for its bitterness?

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Denatonium benzoate (DB) is one of the most bitter compounds known to humans and is strongly rejected. While humans reject it at micromolar concentrations, a 10 times higher concentration is needed in the chimpanzee and a 100–1000 times higher concentration is needed in the rhesus monkey, marmoset, pig and calf to elicit a taste nerve response. In all these species DB elicited responses in Q fibers which are predominantly responsive to bitter compounds. The purpose of our study was to elucidate which part of the denatonium (D) molecule is responsible for its bitter taste.

We synthesized seven derivatives of D and then used them as taste stimuli on the tongue of rhesus monkey, *Macaca mulatta*, and calf, *Bovis taurus*, while we recorded from single Q fibers of chorda tympani nerve. Five of these novel derivatives were modified by substitution of the *N*-benzyl moiety on the quaternary nitrogen to produce: *N*-methyl, *N*-(4-nitrobenzyl), *N*-(3-nitrobenzyl), *N*-allyl and *N*-*t*-butyl. In two additional derivatives the anilino portion of the molecule was replaced at the quaternary nitrogen with *N*-ethyl alone to produce *N*,*N*′,*N*′-triethylbenzyl ammonium and the anilino ring was modified to produce 2-desmethyl-3-(*N*-*t*-Boc) denatonium. The concentrations of these stimuli were adjusted to elicit measurable response: 0.5 or 1 mM in the monkey and 5 mM in the calf.

Our results showed that although we had to use a >10 times higher concentration in cattle than in the monkey to obtain a nerve response, the effects of the modifications on the Q fiber response were the same in both species. Relative to DB, the compounds

showed variable potency. The results indicated several features of the DB molecule which are required to elicit response in the Q fibers: (i) the complete D molecule is essential; (ii) the phenyl ring on the *N*-benzyl substitution is critical and cannot be replaced by a single double bond substitution (*N*-allyl) or a bulky alkyl group (*N*-*t*-butyl); and (iii) positions 3 or 4 for nitro substitutions on the phenyl ring of the benzyl moiety do not drastically alter the bitter properties of denatonium. The most dramatic consequence of derivatization of the D molecule was the removal of the phenyl group on the quaternized nitrogen, indicating that the agonist properties of D require an unsaturated benzenoid ring for proper interaction in the receptor binding site.

200. Establishment of the primary culture of rat taste bud cells and gene transfer for investigation of intracellular taste signal transduction

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Mammalian taste buds consist of morphologically and structurally distinct cells with different physiological responses and gene expression patterns. Conventionally, some of these multiple and heterogeneous cells are thought to be sensory cells, the so-called taste cells, the function of which still remains unclear at the molecular level. To investigate some characteristics of taste bud cells in detail, we established a primary culture system and attempted exogenous gene transfer to the resulting cultured cells.

Taste buds were isolated from rat circumvallate papillae and then cultured on a dish with coated Matrigel. The cells took on a round shape and, 1 h later, began to adhere to the bottom of dish. After ~8 h, they became spindle-shaped and their number increased gradually with time. For a viability test, we stained the 3-day-cultured cells with trypan blue and neutral red, and found that ~90% of adhering cells were vivid. Our confirmatory experiments showed that these cells expressed CK8, TR2, gustducin and PLCβ2 mRNAs, all of which are known as functional molecules occurring in taste bud cells. Next, we transferred the GFP gene to the cells by an adenovirus vector and found that, 24 h after infection, GFP was expressed in almost all the cells. We also transferred the myc-tagged α1-adrenergic receptor gene and carried out both immunostaining and intracellular calcium measurement, with the result that this gene is functionally expressed on the cell surface.

It should be noted that in our primary culture system the taste bud cells maintain their original state of animation and also that the use of the adenovirus vector facilitates the transfer and expression of exogenous genes. The success of the present work will contribute to characterizing the multiple taste bud cells and to disclosing molecular mechanisms of taste signal transduction that takes place in each of them.

201. Taste sensitivity to 6-*n*-propylthiouracil (PROP): a possible genetic marker for liking for cigarettes?

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Research into the genetics of taste perception has established that sensitivity to the bitter taste of two chemically similar compounds, phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP), is a heritable trait (Tepper, 1998, Am. J. Hum. Genet., 63: 1271–1276). Linda Bartoshuk has distinguished between non-tasters, regular tasters and 'supertasters' of PROP on the basis of PROP detection thresholds and the mean ratios of intensity ratings of suprathreshold PROP solutions relative to solutions of sodium chloride (Bartoshuk, 1993, Food Qual. Pref., 4: 21–32). The distinction was also supported by anatomical studies, which showed that the density of taste receptors on the anterior tongue correlates significantly with the perceived bitterness of PROP. In addition, supertasters have the most fungiform papillae, the largest number of taste buds and the highest density of taste buds per papilla (Bartoshuk *et al.*, 1994, Physiol. Behav., 56: 1165–1171). Genetically mediated sensitivity to the taste of PROP has been identified as a potential marker for unhealthy dietary habits (Drewnowski and Rock, 1995, Am. J. Clin. Nutr., 62: 506–511), and may also be associated with a predisposition towards alcoholism (Pelchat and Danowski, 1992, Physiol. Behav., 51: 1261–1266).

Self-report data of liking for cigarettes were collected from 16- and 17-year-old females (n = 323) who were categorized as non-tasters, medium tasters and supertasters of PROP, based on their responses to filter-papers previously soaked in PROP. The supertasters' rated liking for cigarettes (median = 9) was significantly lower than the non-tasters (median = 50) (W = 5874.5, P = 0.023). Coding the top 10% of the preference scale as representing liking and the bottom 10% of the scale as disliking also illustrated the dislike of cigarettes by supertasters [$\chi^2(3)$ = 11.70, P = 0.009]. This pattern is highlighted by considering only the most extreme responses (within 1 mm of the ends of the line) as extreme like and dislike [$\chi^2(3)$ = 10.37, P = 0.016]. These results suggest that taste sensitivity to PROP could be useful in identifying young people with the greatest predisposition to liking the taste of cigarettes and may assist in the development of appropriate cessation strategies.

202. Effect of dental deafferentation on gustatory sensitivity

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An epidemiologic study was undertaken in order to evaluate taste impairment after dental avulsion or root canal treatment (both referred to as dental deafferentation: DD).

Two hundred and four subjects (78 males, 126 females, aged 18–78 years, mean ± SD = 31 ± 13) under treatment at the Dental Faculty, University Paris 7, were volunteers for taste sensitivity

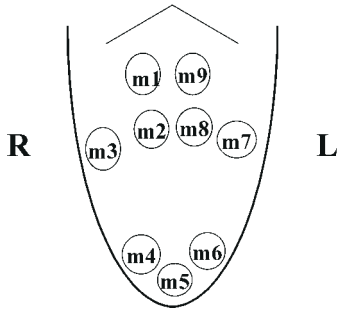

Figure 1

evaluation. A laboratory-built electrogustometer fitted with a removable 5 mm diameter spherical stainless steel cathode delivered a limited range from 0 to 200 μA. Detection thresholds were measured for each subject on nine tongue loci as well as on the soft palate. This method enables measuring the chemical sensitivity of taste cells in response to iontophoretical stimulation with salivary cations. Subjects were divided into several groups. Of the subjects, 166 were healthy non-smokers without history of complication (e.g. paresthesia) following dental treatments and who were not receiving any medication. Results of another group of 38 subjects who were either smokers and/or under pharmacological treatment will be presented separately. Among the 166 non-smoking, non-medicated subjects, a group of 32 without DD was used as a control group. The others were further divided according to the number of DD and their location, i.e. anterior (incisors and canine: three teeth per quadrant) or posterior teeth (bicuspids and molars: five teeth per quadrant).

Results showed that thresholds (i) depended on the locus tested, from 10 ± 5 to 38 ± 36 μA in controls, according to the density of papillae (Miller *et al.*, 1990, Physiol. Behav., 47: 1213–1219) and (ii) increased depending on the number of DD. Thirty-three subjects with only one or two posterior DD exhibited no significantly different thresholds compared with controls. Thirty-five subjects with three or four posterior DD exhibited significantly increased thresholds at posterior loci m1 and m9 (48 ± 58 versus 25 ± 19 μA in controls: 92% increase, $P = 0.03$, Student's *t*-test) (see Figure 1). Twenty-three subjects who had seven or more posterior DD exhibited significantly higher thresholds for posterior loci m1 and m9, and m2 and m8 (208 and 134%, $P < 0.001$ and $P < 0.01$, respectively). Eleven subjects with one or two anterior and 0–6 posterior DD (light anterior and posterior DD) did not exhibit significantly different thresholds, whereas nine subjects with 1–6 anterior and 7–16 posterior DD (heavy anterior and posterior DD) exhibited significantly higher thresholds both in posterior loci (m1 and m9, and m3 and m7: 168 and 194%, respectively, $P < 0.01$) and anterior loci (m4 and m6: 140%, $P < 0.001$). Threshold at palate, possibly measured in 87% of subjects, was not significantly increased in any of these groups.

In conclusion, not only the number but also the localization of the DD is important for the localization of the increased taste threshold on the tongue. Results suggest that taste thresholds are locally increased when a topographically corresponding dental branch of the Vth nerve is injured. Gustatory and somatosensory functional convergence in central relays might be responsible for such an effect.

203. Effect of ethanol on gustatory and non-gustatory receptors in tongue of rhesus monkey, *Macaca mulatta*

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Responses to ethanol were recorded in single taste fibers of chorda-tympani (CT) and glossopharyngeal (NG) nerves and in non-taste fibers oflingual nerve (LN). First we characterized each fiber with its adequate stimuli: gustatory fibers were characterized with 30 tastants and non-gustatory fibers with touch and temperature. Then we stimulated the taste fibers with 1 and 3 M ethanol, 3 M methanol and 0.5 M butanol. The non-taste fibers were tested with 0.3–12 M ethanol, 1 and 3 M methanol, 0.5 and 1 M propanol, and 0.5 and 1 M butanol. Finally we recorded the responses to simultaneous application of ethanol and the adequate stimulus (taste, touch or temperature).

We found that in CT, ethanol stimulated different types of the tastes fibers differentially. One molar ethanol stimulated only S fibers and 3 M stimulated all S and some N and Q fibers, while there was no response to ethanol in the H fibers. 3 M methanol elicited the same response profile as ethanol. In contrast, during stimulation with 0.5 M butanol there was little S fiber activity. In NG 1 and 3 M ethanol stimulated fibers from every clusters.

In both nerves the taste responses were modulated by ethanol and, what is more important, modulated differentially depending on the fiber type. In H fibers, addition of 1 or 3 M ethanol significantly inhibited the responses to citric and ascorbic acids. In Q fibers, the responses to QHCl, denatonium benzoate, brucine, aristolochic acid and naringin were suppressed when mixed with ethanol. However, the responses to caffeine and SOA were not affected. The responses in S fibers to mixtures of ethanol and all non-alcoholic stimuli were larger than the responses to the stimuli alone.

Ethanol stimulated ~70% of the non-gustatory LN fibers. Some fibers responded already to 0.3 M methanol. The response grew with increase of concentration. In 56% of the fibers responding to ethanol, the response to 5, 8, and 12 M ceased before the end of stimulation. Methanol and propanol gave similar results to ethanol, while butanol never stimulated.

Ethanol and methanol modulated the responses in mechano-receptors. Thus in 70% of the mechanoreceptors the response to mechanical stimulation was potentiated by low ethanol concentrations and in 50% it was inhibited by high concentrations. In contrast, butanol did not potentiate, but only suppressed the response to mechanical stimulation.

204. Calcium activated potassium channels in the taste cells of mudpuppies (*Necturus maculosis*)

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Previous electrophysiological work (Cummings and Kinnamon, 1992, J. Gen. Physiol., 99: 591–613) identified a large-conductance calcium-activated potassium (BK) channel in the apical membrane

of mudpuppy (*Necturus maculosus*) taste cells. This conductance, which is partially activated at resting potentials, is directly blocked by acids and several bitter compounds. When blocked, the channel causes the taste cells to depolarize and is likely involved in detecting both sour and bitter taste stimuli. The current study utilized molecular and immunocytochemical techniques to identify and characterize this channel in the taste cells of mudpuppies. Using RT-PCR, we found at least three isotypes of this channel present in the lingual epithelium of the mudpuppy. Of 17 PCR products sequenced, 11 products were 97% identical at the amino acid level to mslo, the BK channel found in mice. Four PCR products were 99% identical to mslo, while two products were 88% identical at the amino acid level. Further analysis will determine if these products are splice variants of a single gene or represent multiple genes. Immunocytochemical analysis using an antibody to the C-terminal end of the mslo alpha subunit found labelling within the majority of taste cells of all taste buds examined. Negative controls showed no specific labelling of any taste cells. Immunocytochemical data found this channel at the apical end of taste cells, the site of taste transduction. These data support earlier electrophysiological data indicating the presence of a BK channel in mudpuppy taste cells at the site of signal transduction.

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205. Gustatory-evoked responding in the nucleus of the solitary tract of the rat is altered by calcium deprivation

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When rats are fed a diet low in calcium, they develop a compensatory appetite for calcium-containing substances (Coldwell and Tordoff, 1996, Am. J. Physiol., 271, R1–R10). Recent evidence indicates that this appetite does not depend on learning and is expressed during sham-feeding, when primarily gustatory factors are involved (McCaughey and Tordoff, 2000, Appetite, 34: 305–311). For reasons that are not clear, calcium-deprived rats also demonstrate increased acceptance of some non-calcium minerals, including sodium (Tordoff *et al.*, 1990, Am. J. Physiol., 259: R411–R419). To investigate this further, we recorded the activity of single neurons in the nucleus of the solitary tract (NST) of calcium-deprived and replete rats. Calcium deprivation was induced by maintenance on a low calcium diet for 6–11 weeks. In the calcium-deprived group, NST neurons with salt-sensitive response profiles gave larger responses to $CaCl_2$ and NaCl than did corresponding cells in the replete group. These differences in taste-evoked responding may play a role in directing the appetites for $CaCl_2$ and NaCl that accompany calcium depletion.

206. Quantification of taste disturbance in burning mouth syndrome and oral dysgeusia: preliminary results

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Burning mouth syndrome (BMS) and oral dysgeusia (OD) are perception abnormalities that affect the lips and oral cavity and which are unaccompanied by clinical signs (Hay and Large, 1995, New Ethicals, July: 1–6). BMS is generally characterized by a severe burning sensation of the oral cavity, commonly experienced on the anterior tongue (Bartoshuk *et al.*, 1998, Am. Pain Soc., Oct–Nov: 138), although it may affect the lips, buccal mucosa, floor of mouth and throat (Lamey, 1996, Dermatol. Clin., 14: 339–354). OD includes a variety of disorders of taste and common sensation which generally conform to aberrations of sensations derived in the oral cavity, i.e. sweet, sour, salt, bitter and possibly metallic (Hay and Large, 1995). These two disorders are generally considered equivalent, with similar treatment regimes and outcomes.

Eight patients with either BMS or OD, or with both of these conditions, were tested on an automated electrogustometer (Loudon and Stillman, Behav. Res. Methods, Inst. Comp., 29: 358–363). Four patients were also given taste solutions (sweet, sour, salty and bitter) to measure quality of perception, and differing concentrations of these tastants to measure intensity perceptions.

Three patients with BMS and one with both BMS and OD (no OD symptoms at time of testing) had normal taste thresholds on electrogustometry, and normal results in their Quality and Intensity measures. One patient with OD but no symptoms at time of testing was also normal on electrogustometry (no Quality or Intensity measures taken). The remaining patients with OD ($n = 3$) or both OD and BMS ($n = 1$) and symptoms at time of testing all had significantly abnormal taste thresholds (>60 μA). Two of these patients had Quality and Intensity measures taken, and both identified errors of perception in both taste quality and intensity, relating to their individual symptoms.

BMS has no measurable effect on taste threshold and perception. Conversely OD has measurable effects on threshold, quality and intensity perceptions. This is the first clinical indication of a measurable difference between these two conditions.

207. P2y receptor-mediated calcium release and ion channel modulation in mouse taste receptor cells

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Purinoceptors are ubiquitously involved in the regulation of different cellular functions (North and Barnard, 1997, Curr. Opin. Neurobiol., 7: 346–357). We monitored cytosolic Ca^{2+} ($[Ca^{2+}]_{in}$) in mouse TRCs and found it to be transiently increased from nearly 70 nM at rest up to 300 nM in response to the bath application of micromolar ATP. The ATP responses were impaired dramatically by U–73122, a phospholipase C (PLC) inhibitor. The specific P_{2X} agonist ,-Methylene-D-ATP affected $[Ca^{2+}]_{in}$ negligibly and vice versa for the P_{2Y} agonist 2-methylthio-ATP (2MeSATP), favouring PLC-coupled P_{2Y} receptors as the main contributors to the nucleotide effects. The P_{2Y} agonists mobilized cytosolic Ca^{2+} in the order of potencies: ATP ≥ 2MeSATP > UTP > ADP > UDP. Notably, we found ATP-responsive TRCs in all circumvallate, foliate and fungiform papilla. To elucidate possible mechanisms of nucleotide-response coupling, we recorded from circumvallate and foliate TRCs using the patch clamp technique. Distinctive TRC subpopulations with the characteristic sets of ionic currents were sensitive to ATP. In TRCs exhibiting VG Na^+ currents and Ca^{2+} currents, 100 μM ATP inhibited the last by 30–50%. Perhaps the

TRC Ca^{2+} channels are directly regulated by the G proteins linked to the P_{2Y} receptors, as documented in neurons (Dolphin, 1998, J. Physiol., 506: 3–11). Given that ionotropic P_{2X} receptors have been found in nerve fibres innervating taste buds, ATP may be an afferent neurotransmitter in the taste transduction process (Bo, *et al.*, 1999, NeuroReport, 10: 1107–1111). If so, the P_{2Y}-mediated inhibition of the TRC Ca^{2+} channels may provide the negative feedback regulation of tastant-dependent neurotransmitter (i.e. ATP) release, thus facilitating taste adaptation. By mobilizing intracellular Ca^{2+}, ATP gated indirectly Ca^{2+}-activated Cl^- currents in TRCs that showed merely VG K^+ currents. ATP released from damaged cells activates P_{2X} receptors in sensory endings, contributing to pain caused by tissue injury (McCleskey and Gold, 1999, Annu. Rev. Physiol., 61: 835–865). For the taste system, ATP content may characterize food quality to some extent. Therefore, activation of the Cl^- current by ATP in a subpopulation of TRCs may be a taste-related signal, which is also generated in response to the tastants of different modalities that trigger IP_3 formation and Ca^{2+} release in the TRC cytoplasm (Bernhard *et al.*, 1996, J. Physiol., 490: 325–336). Collectively, our findings point to the integral role of purinergic signalling in TRC physiology.

208. Membrane properties of taste cells in mouse vallate papilla during postnatal development

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Transduction mechanisms in taste buds undergo significant changes during postnatal development (Stewart *et al.*, 1997, Am. J. Physiol., 272: C1–C26). These changes may be related to corresponding age-dependent alterations in the membrane properties of taste cells (TC), which use ion channels and receptors for chemotransduction (2,3). Yet, scarce information is available on the membrane events in developing TCs. We have addressed this issue by studying voltage-gated Na^+, K^+, and Cl^- currents (i_{Na}, i_K, i_{Cl}, respectively) in TCs from vallate papilla in mouse pups and adults. These currents play a key role in TCs during taste transduction, especially the generation of action potentials (Lindemann, 1996, Physiol. Rev., 76: 719–766; Herness and Gilbertson, 1999, Annu. Rev. Physiol., 61: 873–900). We have also used immunocytochemistry to study the expression of gustducin, a taste-specific G protein involved in sweet and bitter transduction mechanisms (Wong *et al.*, 1996, Nature, 381: 796–800), as a morphological marker for the functional maturation of TCs.

The analysis of voltage-gated currents in TCs from adult mice revealed a significant diversity. In particular, the relative proportion of i_K and i_{Cl} to the outward currents was highly variable. Thus, we grouped i_K and i_{Cl} as i_{OUT}. Both i_{Na} and i_{OUT} were detectable as early as postnatal day (PD) 4. i_{Na} did not show any significant variation from first postnatal week to adulthood. Unlike i_{Na}, the amplitude of i_{OUT} increased continuously throughout the first three postnatal weeks, reaching the adult level just after weaning. Membrane capacitance of TCs (an estimation of cell surface area) did not change over the same period, suggesting that the increase in i_{OUT} was due to an increase in channel density. Gustducin-positive cells could be detected in taste tissue as early as PD1. These cells increased in number during the first 2 weeks and reached the adult average number (~10 cells per bud) in the third week. Thus, the expression of specific components of taste transduction mechanisms, such as voltage-gated ion channels and G proteins, seems to be developmentally regulated in mouse TCs. Changes in membrane excitability and signalling pathways may have profound effects on the chemotransduction activity of TCs during postnatal development.

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209. Intracellular pH regulation in taste receptor cells by basolateral membrane Na^+/H^+ exchanger, type 3

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From earlier studies on isolated taste receptor cells (TRCs) we have determined that TRCs regulate intracellular pH (pH_i) when acid is loaded at constant external pH (pH_o) with pulses of pCO_2, NH_4Cl, or sodium acetate (Lyall *et al.*, 1997, Am. J. Physiol., 273: C1008–C1019). However, TRCs *in vivo* are polarized epithelial cells, so to determine the site of pH regulation, pH_i was monitored in polarized fungiform papillae loaded with the pH-sensitive fluoroprobe, BCECF. The polarity was maintained by mounting a piece of isolated rat lingual epithelium containing a single fungiform papilla in a special microscopy chamber (Chu *et al.*, 1995, Am. J. Physiol., 269: C1557–C1564). Apical and basolateral sides of the papilla were perfused independently with HCO_3^--free HEPES-buffered media (pH 7.4; 22 ± 1°C). The cells were imaged from the basolateral side through a ×40 objective at 510 nm with an intensified CCD camera as they were excited alternately at 490 and 440 nm. pH_i was monitored with the fluorescence emission ratio (F_{490}/F_{440}).

Several lines of evidence indicated that Na^+–H^+ exchange (NHE) activity is present in the basolateral membrane of TRCs. (i) Removing Na^+ from the basolateral perfusate by substituting NaCl with equimolar NMDG-Cl decreased pH_i, and amiloride, an NHE inhibitor, attenuated that decrease in pH_i. (ii) TRC pH_i also decreased when amiloride was added to Na^+ containing perfusate. (iii) Acid loading of TRCs by pulsing with 15 mM NH_4Cl or by exposing to 15 mM sodium acetate induced transient decreases in TRC pH_i that recovered spontaneously to baseline values. The spontaneous recovery of TRC pH_i was blocked by the addition of amiloride to the basolateral perfusate, and was blocked by the removal of Na^+ from the basolateral perfusate. To characterize the NHE more fully we monitored the rate of pH_i recovery following acid loading with NH_4Cl in the presence of amiloride and of the NHE3 blockers, S3226 and S1611. The respective K_i values were: 33.2, 1.9 and 0.138 M. These results suggest that the observed NHE activity is most likely due to NHE3 located in the basolateral membrane of the TRCs. We conclude that at constant extracellular pH intracellular acid–base balance of TRCs is maintained by NHE3 activity in the basolateral membranes.

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210. A case of agusia and loss of 'electric taste'

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A 70-year-old woman, complaining of 'complete loss' of taste, was examined for olfactory and gustatory sensitivity and for 'electric taste' sensations. Olfaction was measured by the University of Pennsylvania Smell Identification Test (UPSIT) and gustatory sensitivity in three tests. There was no evidence for olfactory deficit. There were clear gustatory deficits. In the first test, using sucrose, sodium chloride, citric acid and quinine sulphate, no discrimination was found between the sweet and sour tastants; for both, 'no taste' was reported. Citric acid did not have its typical sour taste, but was sensed as 'sharp sensation' and having a 'dry' feeling. The quinine samples had no taste, but on some trials produced a sensation felt on the back of the tongue. In the second test, using sucrose and the intensive sweeteners sodium saccharin and aspartame, no taste sensations were reported. For the third test, glucono-1,5-1actone was used. For people with normal gustation, this chemical changes in taste from principally sweet to principally bitter and sour over time, as the solute autohydrolyses in water (Parke *et al.*, 1997, Chem. Senses, 22: 53–65). Here, the reports were consistent with those found in the first test; no sweet taste was reported, and the sensations previously reported for bitter and sour were given increasingly over time. The woman reported no sensations when the electrodes of a fresh 9 V battery were applied to the tip and sides of her tongue.

Visually, her tongue was slightly fissured but there were no indications of abnormality. Her condition occurred suddenly in 1995 without any obvious cause, several months before she presented herself for testing. She remains the same today.

211. No NHDC-taste enhancement in aqueous sucrose solutions

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Neohesperidin dihydrochalcone (NHDC) is an intensive sweetener, obtained by alkaline hydrogenation of neohesperidin. In this investigation a supposed taste-enhancing effect of this substance was tested. A three-step procedure was used. In the first experiment, using a pool of 31 subjects, NHDC- and sucrose detection thresholds were measured. In the second experiment psychophysical functions for both tastants were determined. Then, 15 participants closest to the group threshold, who in addition had produced monotonic psychophysical taste functions, were selected to participate in the next two experiments. In the third experiment taste enhancement was tested. Three psychophysical sucrose functions were constructed, one with a near-threshold amount of NHDC added to each of seven sucrose concentrations, one with a near-threshold amount of sucrose added (control 1) and one without any addition (control 2). No difference was found between the NHDC-enriched sucrose function and the sucrose-enriched sucrose function. Finally, in experiment 4, differential threshold functions were constructed with either NHDC or sucrose added. Neither the overall shape of the functions nor a comparison of the points of subjective equality showed enhancement. It was concluded that weak NHDC does not enhance the taste of aqueous sucrose solutions.

212. Taste interaction between various amino acids and IMP

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It is known that there is strong synergistic taste interaction between L-amino acids with acidic side chain, such as L-Glu or L-Asp and 5′-mononucleotides, such as disodium 5′-inosinate (IMP) or disodium 5′-guanylate. However, it has not been clarified whether there is synergistic taste interaction between other amino acids and IMP.

In our psychophysical study, we firstly examined the change of taste intensity of amino acids at the threshold concentration (Yoshida *et al.*, 1966, Nippon Nogeik. Kaishi, 40: 295–299) when 1 mM IMP was added via a two-alternative forced choice test. It was found that the taste intensity of several L-amino acids, such as L-Ala, L-Ser and Gly, which have a dominant taste of sweetness, was enhanced by adding IMP significantly. Enhanced taste was recognized as umami, which was not blocked by the sweetness inhibitor +/–2-(4-methoxyphenoxy) propanoic acid (Schiffman *et al.*, 1999, Chem. Senses, 24: 439–447). Furthermore, total taste intensities of amino acid and IMP mixture solutions at various concentrations were measured by magnitude estimation [amino acids (L-Ala/L-Ser/GLy/D-Ala), 0–200 mM; IMP, 0–2.0 mM]. The results showed that potentiation ratios (= taste intensity of mixture solution/arithmetic sum of those individual components in the mixture) were >1 in the cases of L-Ala, L-Ser and Gly. However, it was ~1 in the case of D-Ala, which had an enhanced taste of sweetness. Thus, umami taste enhancement of sweet LD-amino acids by IMP is synergistic rather than additive like that of acidic amino acids.

Not only acidic L-amino acids but also several sweet L-amino acids (e.g. L-Ala, L-Ser and Gly) showed synergistic enhancement of umami taste when IMP was added.

213. Design and synthesis of new monatin derivatives

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Monatin (Figure 1) is a high-intensity natural sweet compound isolated from the roots of *Schlerochiton ilicifolius*, a spiny-leafed hardwood shrub growing in the rocky hills of north western Transvaal (South Africa). Its chemical structure has been elucidated by Ackerman and co-workers (Vleggaar *et al.*, 1992,

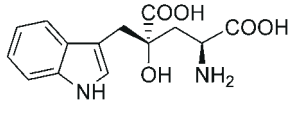

Figure 1

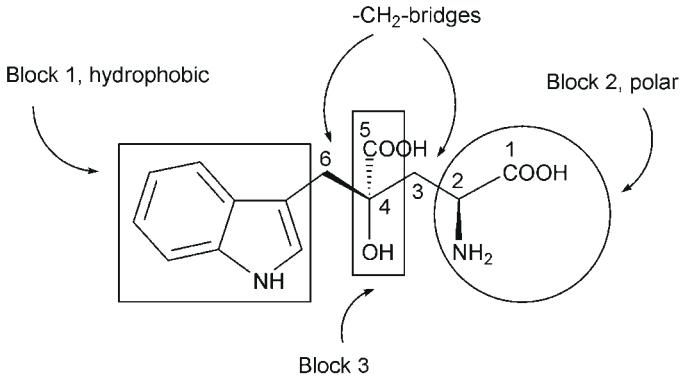

Figure 2

J.C.S. Perkin Trans., 1: 3095–3098) and corresponds to 2*S*,4*S*-4-hydroxy-4-(indol-3-yl-methyl)glutamic acid.

The recognition threshold of monatin salt was estimated 2.75×10^{-4}%. Thus, the relative sweetness was 1400 and 1200 times that of 5 and 10% (w/v) sucrose solutions, respectively.

Approaches to the synthesis of monatin as a racemate have been previously described in the literature (Holzapfel *et al.*, 1994, Synth. Commun., 24: 3197–3211) (1999, US Patent 5994559), but the asymmetric synthesis of the optically active compound has not yet been obtained, and no monatin analogues have been prepared and tasted until now. Structure–taste relationships for this new potential sweetener are lacking and the interaction of this compound with its possible taste receptor has not been studied; this molecule is thus an interesting starting point for the development of a new class of taste active ligands. A rational disconnection of the monatin molecule is represented in Figure 2.

We will report the synthetic strategies for new monatin derivatives, obtained by modifications of some of these blocks, and the first results of the tasting trials. We will also discuss the possible role of each block in the interaction with taste receptor by means of comparison with known receptor models.

214. Brain magnetic fields measured by two smells comparison method

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Physiological experiments on monkeys have previously suggested that the primary activation area of olfaction existed in orbito-frontal fields (Tanabe *et al.*, 1975, J. Neurophysiol., 38: 1269–1283). We showed that the location of the primary activation area of human olfaction also existed in the orbito-frontal cortex area in both hemispheres with a magnetoencephalography device (Yamaguchi, 1998, Brain Topogr. Today, 1147: 740–743). Differing from primary olfactory activation, a few later responses due to smell recognition were obtained by the use of the two smells comparison method.

We detected brain magnetic responses with the use of the oddball paradigm using two smells with a whole-head-type magnetometer with 122 channels and analyzed the data. To a subject, an exhibition probability of two smells was compared with the ratio of 1:3 (target smell:non-target smell) at random, and he was requested to pay attention to a smell with low exhibition probability. The same period pulses (300 ms) of odour to either nasal cavity of right or left were given in the synchronization with subject's breathing repeatedly at random. Though we blasted the odorant gas into the nasal cavity of another side after an interval for recovery, the somatic response was not detected. Our experiments were tried to exchange the smells, and target and non-target smells were also exchanged each other. White noise sound was used to reject the sound noises.

In this experiment, by using a new breathing mask we obtained better efficiency for spraying smell molecules on the mucosa in the nasal cavity. In addition to the fast neuromagnetic response by single smell stimulation, the late neuromagnetic responses observed with the two smells comparison method were apparent at the lateral side of the superior temporal fields or insula in both hemispheres.

The results are regarded to be due to olfactory recognition without contributions from hearing or somatic sense, and in these new findings, responses appeared in relation to attention to a smell.

215. *In situ* imaging of taste receptor potentials and cytosolic Ca^{2+} in the mice

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Taste substances induce the depolarization and the increase of cytosolic calcium concentration ($[Ca^{2+}]_{in}$) in isolated taste bud cells (TBCs). Since taste substances would reach the basolateral membranes of isolated TBCs, the depolarization and/or the increase in $[Ca^{2+}]_{in}$ may involve responses other than taste responses. In order to record the taste response free from other responses, we developed a method to stimulate receptor membranes only with mouse TBCs embedded in peeled lingual epithelia, and recorded their membrane potentials with a voltage-sensitive dye and $[Ca^{2+}]_{in}$ with a calcium indicator.

The application of 0.5 M NaCl on receptor membranes depolarized ~30% of TBCs and hyperpolarized ~30% of them in the same taste buds. Depolarized cells were clustered together. Hyperpolarized cells were also clustered. The application increased ,0$[Ca^{2+}]_{in}$ in 27–33% of TBCs. These results were consistent with the results of our voltage-calmp experiments on the number and distribution of functional HVA-Ca^{2+} channels in single taste buds. The application of 1 mM $CdCl_2$ on the basolateral membranes of TBCs blocked the NaCl-induced increase in $[Ca^{2+}]_{in}$, and the application of 10 μM ryanodine had no effect.

The application of 1 mM denatonium on receptor membranes increased $[Ca^{2+}]_{in}$ in 17–79% of TBCs, indicating that the number and distribution of TBCs responding to denatonium in single taste buds was different from those eliciting HVA-Ca^{2+} channel currents. The application of 1 mM $CdCl_2$ on basolateral membranes had no effect on the denatonium-induced increase in $[Ca^{2+}]_{in}$, and

the application of 10 μM ryanodine on basolateral membranes blocked it.

These results suggest that the elevation of $[Ca^{2+}]_{in}$ in response to NaCl is caused by the influx of the extracellular Ca^{2+} via voltage-dependent Ca^{2+} channels while that of $[Ca^{2+}]_{in}$ in response to denatonium is induced by the release of Ca^{2+} from internal stores. Our data also showed that the number of taste bud cells that increased $[Ca^{2+}]_{in}$ in response to taste substances was larger than that having synaptic connections with taste nerves.

216. Taste spatio-temporal pattern depicted by fMRI and MEG

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This study aimed at establishing the temporal sequencing of activation of areas involved in cortical taste representation in humans by comparing fMRI and MEG results.

Twelve right-handed subjects participated in the fMRI study carried out in France with a 3 T MR scanner (Bruker, Germany). Each run (22 slices, EPI 64 × 64 vox, 5 mm thick, TR = 5 s, NR = 62) consisted of three ON–OFF cycles (18 s ON with stimulus and 75 s OFF with water). Stimuli were NaCl, aspartame, HCl, pH 2.4, or quinine. Images were superimposed on a high-resolution anatomical image (resolution: $1 \times 1 \times 1$ mm^3) and processed using spm96 (Friston *et al.*, 1995, NeuroImage, 2: 45–53), by correlation to a mean time–intensity profile (Van de Moortele *et al.*, 1997, NMR Biomed., 10: 230–236).

Seven right-handed healthy volunteers participated in 23 MEG sessions, in Japan, using a 64-channel whole-head SQUID system (CTF Systems Inc., Canada). Each session included 40 trials for one single stimulus—either 1 M NaCl or 3 mM saccharin (sampling rate: 250 Hz, low-pass filter: 40 Hz, stimulus duration: 400 ms, ISI: 30 s, flow rate: 200 ml/min) (Kobayakawa *et al.*, 1996, Neurosci. Lett., 212: 155–158). Coordinates of the centers of equivalent current dipoles were overlaid on individual MRI slices (Siemens 1.5 T) to allow anatomical localization of activations. Experiments were analyzed individually and results expressed in Talairach coordinates for both sets of data.

Coronal sections and distribution histograms of x, y and z coordinates showed that activated areas were localized in comparable regions in the insula, rolandic, frontal and temporal opercula, for both studies, although MEG activation tended to spread more posterior than fMRI activation, including all G area found in primates (Ogawa, 1994, Neurosci. Res., 20: 1–13). Combining all MEG dipoles from all subjects for saccharin showed that the left posterior insula, rolandic operculum and right median insula were first activated at 130 ms. The bilateral posterior insula, right temporal and rolandic opercula were activated between 216 and 250 ms. Activation was seen in bilateral insula between 300 and 400 ms, and at ~700 ms. In the meantime, the hippocampus and posterior cingulum (300–400 ms) were activated, then the superior frontal F1 (460 ms). The right temporal operculum was reactivated at 750 and 950 ms. No region was activated later (800 ms, 1316 ms) than the lower left insula. This area corresponded to the localization of the insular area found activated in the dominant hemisphere in left and right-handed subjects in a previous fMRI study (Cerf *et al.*, 1997, NeuroImage, 5: 200; Faurion *et al.*, 1999, Neurosci. Lett., 277:189–192). The present sequence of activation reinforces the notion of early projection in superior insula, rolandic operculum and late integration in the lower insula. Work is in progress to examine the temporal relationship between this lower left insular and the angular gyrus activation.

217. fMRI activation in response to odorants orally delivered in aqueous solutions

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During food intake, the global flavor perception results from simultaneous stimulation of gustatory, olfactory and trigeminal systems. The olfactory perception occurs then mainly through the retronasal pathway and is often interpreted as a taste perception, thus leading to well known sensory confusions between taste and olfaction (Murphy *et al.*, 1977, Sensory Process., 1: 204–211). The present experiment was designed to study the cortical representation in humans of the olfactory perception related to a retronasal stimulation.

Six young, healthy and right-handed subjects participated in this study (three men and three women, aged 24–32 years). fMRI experiments were conducted with a 1.5 T whole-body MR scanner (functional echoplanar images: 32 sagittal slices, TR: 4 s, resolution: $4 \times 4 \times 4$ mm^3; anatomical images: 180 sagittal slices, resolution: $1 \times 1 \times 1$ mm^3). Each subject performed six runs of five minutes each and tested two stimuli among amyl acetate, citral and ethyl butyrate. Each run was composed of a first OFF period with water followed by three ON–OFF cycles including 18 s ON with stimulus and 75s OFF with water. Stimuli and water were presented directly to the subject's mouth through plastic tubes, as boluses of 50 μl every 3 s. Subjects were trained to regularly swallow the small amounts of liquids in the horizontal position prior to the scanning session in order to limit motion artifacts. Functional data were processed with AFNI software (Cox, 1996, Comput. Biomed. Res., 29: 162–173) by correlation to a post-hoc averaged perception profile unique for every subject and every stimulus (Van de Moortele *et al.*, 1997, NMP Biomed., 10: 230–236).

The results showed activation in regions previously found with direct stimulation through odorized airflow (Zatorre *et al.*, 1992, Nature, 360: 339–340; Yousem *et al.*, 1997, Radiology, 204: 833–838; Sobel *et al.*, 1998, Nature, 392: 282–286; O'Doherty *et al.*, 2000, NeuroReport, 11: 399–403; Kobal and Kettenmann, 2000, Int. J. Psychophysiol., 36: 157–163) especially including orbitofrontal gyri, piriform cortex, and insular cortices, which were found activated in all subjects. These results demonstrate the feasibility of efficiently stimulating the olfactory system through the retronasal pathway in an fMRI scanner. The retronasal presentation of olfactory stimuli through liquids in the mouth is a realistic model for the study of food-related flavor perception. This presentation mode furthermore allows presenting taste and olfactory stimuli separately or combined, thus allowing direct

comparisons between single modality representation—taste or olfaction—and representation of mixtures.

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218. Imaging olfactory bulb activity as a probe for selective olfactory receptor inhibition by sequence-selective antibodies

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Site directed anti-peptide sera have been employed successfully as specific tools to explore subunit structure (Stevenson, 1992, Biochem. Soc. Trans., 20: 853–856), topology (Weiss, 1987, J. Biol. Chem., 262: 4319–4323), tissue distribution (Wanaka, 1989, Brain Res., 485: 125–140) and function (Weiss *et al.*, 1988, J. Biol. Chem., 263: 6150–6154) of transmembrane signalling proteins. We have raised olfactory receptor-specific polyclonal rabbit antibodies from a chemically synthesized peptide that correspond to a predicted extracellular domain of the second extracellular loop between the 4th and 5th transmembrane loops of the encoded proteins from *Xenopus* OR, XR42 (Freitag *et al.*, 1995, Neuron, 15: 1383–1392). The olfactory bulb (OB) was impregnated with a voltage-sensitive dye (RH 414), and antibodies were applied to olfactory mucosa.

Spatial and temporal patterns of odour responses were measured by changes in dye fluorescence that occur when OB neurons fire. This allowed imaging over an area of the OB glomerular layer to high resolution. A CCD camera was used to capture image sequences following changes in OB fluorescence in response to olfactory receptor activity (Shah *et al.*, 1999, Cell. Mol. Biol., 45: 339–345).

When the OM was exposed to 1,8-cineole vapour the odour response was inhibited while little effect was observed with the responses to a number of odorants such as isoamyl acetate. The effect on the 1,8-cineole response was reversible, and a Ringer's wash of the OM after antibody treatment partially restored the OB response to 1,8-cineole. The evidence indicates that the polyclonal antibody selectively prevents 1,8-cineole sensitive olfactory receptor neurons from firing, without interfering with other odour responses. Chemical modification of the OM and their effect on OB response patterns may provide a useful approach to investigate olfactory quality coding.

219. Human gustatory cortices with passive perception by fMRI and MEG

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We have located the primary gustatory area (area G) at the transition between the inner face of the parietal operculum and the insula in the human cerebral cortex, by means of MEG (Kobayakawa *et al.*, 1999, Chem. Senses, 24: 201–209). A region at the central sulcus was activated with almost the same latency in cases of NaCl stimulation, but was less frequently observed than area G. Following area G, we found activation in several cortical regions, e.g. the frontal operculum, the anterior part of the insula, the angular gyrus, the hippocampus and the intraparietal sulcus.

MEG has a good temporal resolution and can give a good estimation of the location of the activity because the magnetic field generated from living brain is free from distortion by the skull. When more than three regions are activated simultaneously, however, the authenticity of the equivalent current dipoles would decrease. fMRI, on the other hand, has good authenticity for many activated regions, but a very poor temporal resolution. In this study, we tried to measure changes in the regional cerebral blood flow (rCBF) by gustatory stimulation, using the fMRI technique, and compared them with the cortical areas estimated by the previous MEG study to examine the authenticity of the latter.

Multi-slice fMRI data were acquired on a 1 T SIEMENS Expert Magnetom Impact with an RF whole-head coil. A gradient EPI (echo planar imaging) with 10 slices was used for imaging. The effect of the condition (target versus control) at each voxel was compared using liner contrasts. The contrast produced a *t*-statistic for each voxel, which was subsequently transformed to be unit normal *z*-distribution.

Participants were presented with taste stimuli passively, i.e. without any task. The duration of the target was 30 s, and that of the reference was 18 s. In the target condition, a gustatory stimulus with a short duration (500 ms) was given and followed by 1 s deionized water. A 1 M concentration of NaCl was used as a gustatory stimulus and applied to the tongue of the participants by using a taste stimulator with a rapid-rise time. The gustatory stimulus and deionized water were separated by air to avoid mixing. The stimulation of this short duration was repeated 15 times. In the reference condition, the gustatory stimuli were replaced by deionized water. Five pairs of the target and the reference conditions were repeated in one session, and three sessions were repeated in one experiment. Eight healthy adults participated in the experiments.

A change in the rCBF was observed in area G. This is coincident with previous findings, based on the magnetic field changes in a short latency. The changes were also observed at the pre-central sulcus, the central sulcus, post-central sulcus, the frontal operculum, the anterior part of the insula, the angular gyrus and the intraparietal sulcus, though they are among the regions activated in a long latency in the MEG study. The present study proved the validity of location of equivalent current dipoles by MEG study. We could obtain more reliable spatial and temporal information about cortical activities in taste stimulation by combining fMRI and MEG.

220. 4-D Imaging of the neuromagnetic activity in human olfaction

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The objective of this study was to investigate how brain activities are visualized non-invasively in human olfaction. Although PET and fMRI studies have often been used recently to measure human olfactory responses (Zatorre *et al.*, 1992, Nature, 360: 339–340; Sobel *et al.*, 2000, J. Neurophsiol., 83: 537–551), the time resolution on these methods is not very good. We first tried to visualize neuromagnetic brain activity by 4-D imaging using a 122-channel

biomagnetometer, with time changing in the order of milliseconds and the spatial source moving in the order of millimeters in human olfaction.

In these olfactory neuromagnetic experiments, two methods were used: the 'blast method', stimulating by odorant pulses administered into the subject's nasal cavity; and a new 'sniffing method' on the state of human active olfaction. Data analyses were done using a signal space projection (SSP) method (Ilmoniemi, 1992, Finnish patent application no. 925541, November 30) which was applied to the rejection for many kinds of magnetic noises.

The characteristics of the olfactory evoked fields were analyzed from the experiments using the 'blast method' which was passively given under the synchronization with the subject's respiration. From the next experiments of olfactory oddball paradigm we obtained a few more late components on the olfactory recognition at the different regions in the brain than a single odorant stimulation. On the other hand, the 'sniffing method' was analyzed by SSP processing and it was found that a large pre–stimulus changing before sniffing might be the early movement-related response.

As the results of the application of the SSP method for the noisy experimental data in our olfaction, a few equivalent current dipoles were precisely estimated with two kinds of olfactory experiments (both the 'blast method' and the 'sniffing method'). These results suggest the possibility of visualization of a spatio-temporal changing by 4-D imaging and the effectiveness of the neuromagnetic activities non-invasively in human olfaction.

221. Effects of wine aroma on relaxation. I. Subjective scores analysis

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The aroma of wine can induce relaxation in human brain activity. The aroma effects of white and red wines were evaluated using the parameters preference to aroma, degree of wakefulness and degree of mood state in normal female subjects.

Twenty healthy right-handed female volunteers were recruited from the local community. The average age of the subjects was 34.4 years, and the study focused on female wine users. All subjects usually drink wine more than two or three times a month. The subjects were exposed eight aroma samples: three red wines (Cabernet Sauvignon, Merlot, Concord), three white wines (Chardonnnay, Sauvignon Blanc, Muscat), 12% (w/w) ethanol solution and distilled water. Preference to aroma, degree of awakeness and degree of mood state were measured on a 9-point scale after each aroma exposure (Nagai *et al.*, 1996, Appl. Hum. Sci., 15: 281–286).

Subjective scores for relaxation and mood scale indicated that almost all subjects felt better with the wine aroma compared with the ethanol solution exposure. These results suggested that the aroma components of wine samples can affect the brain mechanism for relaxation, and that these effects are different between each wine (Ribereau-Gayon *et al.*, 1998, Traite d'Oenologie, Tome 2, Chimie Du Vin, Stabilisation et Traitements. Dunod, Paris, pp. 239–257). In a further data analysis, we will try to find a relationship between EEG activity and subjective scores.

222. Electroencephalogram response in venous olfaction

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The objective olfaction test is not generally used in the clinical field. To attempt a new type of objective olfactometry, we recorded an electroencephalogram (EEG) during venous olfaction test. When thiamine propyldisulphide (Alinamine, Takeda Pharmaceutical Company, Osaka, Japan) is injected into a vein, the subject experiences a garlic smell. The latency and duration time of the garlic smell are then measured. This test is called the venous olfaction test and is widely used in Japan (Takagi, 1989, Human Olfaction. University of Tokyo Press., Tokyo, pp.35–69), and we found that the EEG changed during this test.

Subjects, young MDs and medical students of our school, who were healthy and had no olfactory dysfunction, were tested. Two electrodes were located on the bilateral frontal head and a parietal electrode was used as a ground. EEGs were amplified, filtered (2–1000 Hz) and recorded on a digital audiotape recorder (DAT). Subjects had their eyes and ears masked to avoid any influences from the visual and auditory senses. The recorded EEGs were analysed by a personal computer.

When venous olfaction, the garlic smell, occurred, the amplitude of the spontaneous EEGs increased. In the frequency spectrum, this increase is well observed in the 30–200 Hz band, but is not observed at <30 Hz. Injection of physiological saline did not increase the EEGs.

It is generally known that the olfactory bulb fires a sinusoidal wave when the olfactory mucosa is stimulated by an odorant (Adrian, 1942, J. Physiol., 100: 459–473; Sem-Jacobesen *et al.*, 1950, Staff Meetings of the Mayo Clinic, 28: 166–170; Dorries and Kauer, 2000, J. Neurophysiol., 83: 754–765). Because, in the frequency spectrums, EEGs produced by venous olfaction were similar to the EEGs of the human olfactory bulb (Sem-Jacobesen *et al.*, 1950), we think the EEGs during venous olfaction are originated in the olfactory bulbs. Measuring EEGs when venous olfaction occurs is may be applicable to objective olfaction test.

223. Neuromagnetic response to sniffing in human subjects

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An fMRI study (Sobel *et al.*, 1998, Nature, 392: 282–286) has shown activities in the frontal cortices due to sniffing odor. We tried to elucidate the temporal properties, including oscillation, of these activities with MEG (magnetoencephalography).

Two healthy subjects participated in this study. Wearing a MEG helmet (NeuroMag0122), the subject sniffed air or the odor of either lemon, lavender or soy sauce through both nostrils at his natural inhalation rate of sniffing at a sampling frequency of approximately once during three consecutive inhalations. Otherwise he inhales air and exhales slowly through his mouth. After 2 months of practice, MEG signals were acquired between 0.03 and 100 Hz at the sampling frequency of 400 Hz while the subject

sniffed air or one of the odors for 10 min. First, the data were simply averaged with respect of the onsets of sniffing. Then, for extracting oscillations, we modified the TSE method (Salmelin and Hari, 1994, Neuroscience, 60: 537–550). Considering impulsive noises to have a wide spectrum, the data were fed to three band-pass filters (size: 128, pass-band: 6 Hz), one for extracting the oscillation and the others for lateral inhibition in the frequency domain; they were then rectified, laterally inhibited and averaged with respect of sniffing.

Sniffing odor, but not air, induced transient oscillations in the frontal part of the right cortex in 21 out of 26 experiments, irrespective of the odors and the subjects, with a peak latency of 1032 ± 255 ms, a duration of 466 ± 150 ms and at a central frequency of 49 ± 5 Hz. The signals simply averaged also yielded odor-specific peaks at almost the same locations, but with shorter latencies of between 300 and 800 ms. These findings imply that the oscillations represent a later stage for processing sniffed odors.

224. Evidence for oxidative stress-induced damage in the human olfactory epithelium: age and Alzheimer's disease

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Key theories of the mechanisms related to the neurodegeneration that accompanies both aging (Shigenaga *et al.*, 1993, Proc. Natl Acad. Sci. USA, 91: 10771–10778) and Alzheimer's disease (AD) (Harman, 1993, Age, 16: 23–30) postulate the involvement of oxidative stress that results from the interaction of reactive oxygen species (ROS) with cellular lipids, proteins and nucleic acids. We have previously demonstrated that immunoreactivity for superoxide dismutases (SODs) in the olfactory epithelium (OE) from patients with AD was significantly greater than that in age-matched non-demented elderly controls (Kulkarni-Narla *et al.*, 1996, Exp. Neurol., 140: 115–125). SODs scavenge superoxide anion radicals, catalyzing their conversion to hydrogen peroxide, which minimizes damage from the superoxide anion radical itself as well as minimizing the formation of the more damaging hydroxyl radicals and peroxynitrite anions. In the OE of AD patients, SODs may be induced by ROS generated either by β-amyloid protein, which has been localized in the OE of AD patients (Crino *et al.*, 1995, Ann. Otol. Rhinol. Laryngol., 104: 655–661), or by olfactory receptor neurons due to the loss of neurotrophic support from their synaptic targets in the olfactory bulb (Struble and Clark, 1992, Neurobiol. Aging, 13: 469–473). The high levels of SODs in the OE of AD patients may contribute to additional damage by ROS as a result of their production of hydrogen peroxide. The aim of these studies was to determine if evidence of ROS-induced cellular damage was detectable in the lipids and proteins of the OE of non-demented elderly subjects and AD patients. The interaction of ROS with polyunsaturated fatty acids results in membrane lipid peroxidation, a product of which is 4-hydroxynonenal (HNE), a potent neurotoxin that forms adducts that accumulate in neuronal membranes (Mark *et al.*, 1997, J. Neurochem., 68: 255–264). Intense HNE immunoreactivity was observed in the olfactory receptor neurons and olfactory nerves as well as in the endothelium of many blood vessels in the olfactory mucosa from AD patients. The interaction of superoxide anion radicals with nitric oxide, another ROS, results in the formation of peroxynitrite, a neurotoxin that modifies protein tyrosine residues to form 3-nitrotyrosine (Lipton *et al.*, 1993, Nature, 364: 626–632). Immunoreactivity for 3-nitrotyrosine was more intense and widespread in the OE of AD patients than in that from non-demented elderly controls. These initial studies provide the first insight into molecular mechanisms that may underlie age- and AD-related neurodegeneration in the human OE.

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225. Loss of olfactory function before and after transplantation of the liver

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Only few data on chronic liver disease and olfactory function have been published, but nevertheless it has been reported that cirrhosis of the liver is occasionally accompanied by a reduced chemosensory ability. In an earlier study we reported on the correlation of olfactory performance and liver function.

In the present study we examined the olfactory threshold (T), odor discrimination (D) and odor identification (I) in patients with chronic cirrhosis of the liver before (n = 50) and after (n = 15) undergoing a liver transplant. The aim of the study was to assess the impact of the liver transplant on (i) the olfactory sensitivity and (ii) the performance in global psychometric measurements (Reitan-A, Mini Mental Health), and (iii) to see whether a relationship between the level of zinc or bilirubin and olfactory function can be revealed.

The vast majority of both patients before and patients after transplantation of the liver performed worse than the healthy controls: 4.1% of the patients with their own insufficient liver were anosmic, 65% hyposmic and only 30.9% were normosmic. None of the transplanted patients was anosmic, but 72.7% were hyposmic and only 27.3% were normosmic. Both groups did not perform better than the 30th percentile in any of the three (T, D, I) olfactory tests (all data were age and gender adjusted).

The Reitan-A test, representing the data for the hepatic encephalopathy, correlated significantly with the TDI-score of the olfactory test, the most significant correlation was found for the identification test (P = 0.01) with the non-transplanted patients and for the discrimination test (P < 0.05) with the transplanted patients.

Before transplantation of the liver and also afterwards, zinc levels were generally much beyond normal values and correlated significantly with each other (P < 0.05), but they did not correlate with the degree of olfactory loss in one of the two groups.

Due to the small number of transplanted patients, a relationship between the severity of the liver damage and zinc levels can be revealed (P < 0.05) only before transplantation. No correlation between the two groups could be found for the bilirubin levels and the Reitan-A testing, nor for testing of threshold, discrimination or identification.

226. Daily life impact of olfactory loss

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Taste and smell are fundamental sensory systems essential in nutrition and food selection, for efficient metabolism, for the hedonic and sensory experience of the environment, and, in general, for the maintenance of a good quality of life.

A sample of 278 consecutive patients with hyposmia or anosmia were examined by ENT specialists, aCT scan of the paranasal sinus and a psychophysiological olfactory test. Additionally, they were interviewed using a standardized self-reporting questionnaire. The causes of the chemosensory impairment were trauma (17%), viral infections (39%), inflammatory/congestive nasal disorders (21%), congenital anosmia (3%), other causes (3%) or idiopathic (18%).

Dividing the sample into anosmic and hyposmic patients, no major difference of subjective estimation of quality of life (QoL) could be detected. Depressive patients rated their QoL lower than patients without mood disorder, although their olfactory performance was about the same. No difference between pre- and postmenopausal women could be found. Unchanged taste perception was reported in 27% of hyposmic patients, compared with 21% of anosmic patients. Respectively difficulties in cooking, eating of spoiled food less appetite and too little perception of their body odor was reported by nearly half of our sample. Patients under the age of 55 years suffered more from the lower performance (reduction of QoL 40 versus 33%). The most striking age specific problem seems to be body odor perception.

With reference to the etiology of the chemosensory disorder, at least one-third of the patients (conducive disorders) could probably be helped, and they could be relieved of their reduced quality of life and change in mood by surgical or medical treatment.

227. MR-imaging of patients with primary anosmia: volume measurements of hypoplastic olfactory bulbs and measurements of olfactory sulci in comparison to normosmic individuals

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The purpose of this study was to evaluate the appearance of frontal skullbase structures in patients with isolated congenital ansomia, and to compare volume measurements of hypoplastic olfactory bulbs and measurements of olfcactory sulci in patients with anosmia and normosmic volunteers.

Fifteen patients with isolated congenital anosmia verified by anamnestic data and electrophysiological and electropsychological tests were included. For comparison, data of an age- and gender-matched group of 10 normosmic and otherwise healthy subjects were acquired. Imaging was performed in a 1.5 T system (Magnetom Vision, Siemens) with a standard quadrature head coil. T1 weighted spin echo sequences in a coronal plane perpendicular to the frontal skullbase with a slice thickness of 3–4 mm were used. Pixel size was 0.43 × 0.39 mm. Additionally a sagittal T1 weighted MP-Rage gradient echo sequence with isometric voxels (1.0 × 1.0 × 1.0 mm^3) was performed. Data were transferred to a workstation to calculate volumes of normal and hypoplastic olfactory bulbs (OB), and to measure lengths and depths of olfactory sulci (OS).

Volumes of OBs in probands were 125 ± 17 mm^3. Eight patients had hypoplastic OBs with volumes of 21.1 ± 2 mm^3 or aplastic OBs (seven patients). Neither absolute length nor maximal depth of OS showed any significant difference in anosmic and normosmic people. If related to head size, length of OS was shorter in patients than in probands; depth did not show any differences. In six patients with aplasia of OB the OS began behind a coronal plane through the dorsal edge of the eyeballs.

T1 weighted spin echo and gradient echo sequences are methods of choice to visualize structures of the frontal skullbase in patients with isolated congenital anosmia. Aplasia or hypoplasia of olfactory bulbs as well as changes of morphology of the olfactory sulci are clearly depicted.

228. Olfactory deficits in different diseases: a new olfactory test

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Many studies have used various olfactory tasks to rate performance in patients with Alzheimer's disease (AD), Parkinson's disease (PD), schizophrenia (SC) or epilepsy (EP) (e.g. Doty *et al.*, 1984, Science, 226: 1441–1443; Harrison and Pearson, 1989, Br. J. Psychiat., 155: 822–828; Doty *et al.*, 1991, In T.V. Getchell *et al.*, eds, Smell and Taste in Health and Diseases, Vol. 26. Raven Press, New York, pp. 449–462; West and Doty, 1995, Epilepsia, 36: 531–542; Martzke *et al.*, 1997, Biol. Psychiat., 42: 721–732). Olfactory deficits have been reported with respect to odor detection, discrimination, recognition memory, identification and naming. However, these tests do not necessarily discriminate between diseases. Thus, no significant difference between the UPSIT scores of PD and AD patients (Doty *et al.*, 1988, Neurology, 38: 1237–1244), or between those of AD and SC patients (Moberg *et al.*, 1997, Neurobiol. Aging, 18: 163–167), has been found. Rather than using these tasks, we proposed a test allowing subjects to rate successively different olfactory judgements. Schab (1991, Psychol. Bull., 109: 242–251) suggested that the process of olfactory identification includes different levels of analysis with performance ranging from non-verbal feelings of familiarity to specific object names. We think that intensity, familiarity, hedonicity and edibility judgements can represent different olfactory judgements performed by subjects before identifying odors.

The test is divided into two sessions: in the first session, intensity, hedonicity, familiarity and edibility judgements are successively assessed using a linear rating scale, and in the second the subjects

are asked to identify the odorants. For the identification task, a list of four or five alternative names for the odor are proposed as a function of the difficulty of patients to perform the task. Patients with different pathologies were tested in different studies. Control groups matched in age and in gender with patients were systematically investigated. For epileptic patients, groups were also distinguished as a function of the localization of the epileptogenic site.

The results highlight the influence of pathologies and age on different odor processing. They show that intensity, hedonicity, familiarity, edibility judgements and also odor identification were differentially affected in these various patients when compared with control subjects. Patients with SC, EP and PD, but not AD, exhibited clear deficits in hedonicity judgement. Patients with PD presented a higher deficit in odor identification than patients with other pathologies, and only the PD patients exhibited a deficit in the intensity judgement. In conclusion, our test could easily discriminate patients with different diseases in terms of olfactory deficits.

229. Taste and smell function in chronic renal failure

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It has been noted that chronic renal failure (CRF) is often accompanied by an impaired chemosensory perception. Considering the importance of the chemosensory senses for nutrition, and the high prevalence of a reduced nutritional state in patients suffering from CRF, the major aims of this study, based on preliminary data, were to explore whether there is a relationship (i) between chemosensory perception and renal function, (ii) the global psychometric measurements (Reitan-A, MMSE-Folstein) and (iii) how various parameters as nutritional state, subjective oral dryness and laboratory findings influence the chemosensory abilities.

Twenty-nine patients with CRF of various degrees participated in the study and were compared with age- and sex-matched healthy control subjects. Patients with cognitive deficits, oral inflammation, upper respiratory airways diseases, antibiotic or immunsuppressive treatment were excluded. The 'Three Drops Method' was used to measure the taste perception, by determining the detection (dtt) and the recognition threshold (rtt) of solutions of the four different taste qualities sweet (glucose), sour (citric acid), bitter (quinine) and salty (sodium chloride). For evaluating odour perception, the 'Sniffin' Sticks' test was used to determine the detection threshold (T), the discrimination (Ds) and the identification (I) ability.

Two interesting results could be gained by these priliminary data. Regarding gustation, the patients had a significantly higher dtt of three taste qualities: sweet ($P = 0.02$), sour ($P = 0.048$) and bitter ($P = 0.026$). No significant difference was observed for the dtt of salty ($P = 0.449$) and the rtt of all four qualities.

With reference to the olfactory performance, we could demonstrate that healthy controls had significantly higher scores in D ($P < 0.02$) and I ($P < 0.001$) compared with patients. No significant difference was observed in T ($P = 0.988$). We also noticed that patients had a significantly higher rate of complaining about a dry mouth ($P < 0.001$) and a feeling of thirst ($P < 0.001$). The patients group reached significantly higher scores in the Reitan-A test ($P < 0.001$); no significant differences were found in the MMSE-Folstein test ($P = 0.655$)

Our results show an impairment of the chemosensory abilities in patients suffering from CRF: a higher detection threshold for sweet, sour and salty. They do not show a reduction of the detection threshold of olfaction in CRF. The impairment of the discrimination and identification might be related to an impairment of higher cognitive functions due to CRF.

230. Olfactory acuity in patients with one or more seasonal allergies

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Patients with seasonal nasal allergies often report hyposmia during the season when the allergy reaches its peak. It has been reported that patients with seasonal allergies exhibit olfactory loss when compared with healthy subjects, even when this is measured out of season. Thus, it has been concluded that the pathophysiology of the allergic reaction is similar to the reaction of an inflammatory disease resulting in a decreasing number of olfactory receptor neurons. Regarding the priming effect reported in nasal allergies, we hypothized that a patient suffering from two or more allergies would have a longer duration and greater severity of symptoms, which should result in an even lower olfactory sensitivity compared with patients with only one allergy.

We examined 112 patients divided into three groups: group 1, patients with one seasonal allergy: 28 patients were allergic to birch pollen and 34 patients to grass pollen; group 2, 42 patients with birch and grass pollen allergy; and group 3, eight patients with birch and/or grass pollen and a third allergy. All patients were desensitized. Olfactory testing was performed using the 'Sniffin' Sticks' test, which includes assessment of odour threshold (T), odour discrimination (D) and odour identification (I). Results were compared with the age- and sex-matched normative data of healthy subjects.

In contrast to previous reports, the mean olfactory sensitivity of patients with one or two allergies was no different from that of healthy subjects. Patients with three allergies showed a significant loss of olfactory acuity.

This indicates that there is no statistically significant impairment of the sense of smell in allergic patients with one or two seasonal allergies during the season without allergen exposure. As all of these patients were desensitized, there might be an alteration of the allergic reaction. Patients with three allergies had a significant olfactory loss which indicates cumulative impairment.

231. The relationship between olfactory acuity and chronic sinusitis

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Nasal and sinus disease is one of the most common causes of olfactory loss, accounting for 15–27% of patients presenting to

taste and smell centers (Deems *et al.*, 1991, Arch. Otolaryngol. Head Neck Surg., 117: 519–528). Contrary to a sensory or neural loss, a loss secondarily due to nasal and sinus disease is thought to be conductive, which means the odorant can not reach the olfactory epithelium and stimulate the appropriate receptors. Such patients often present because of impaired nasal obstruction, discharge or headache, and recognize the loss of smell to be a predictable consequence. It has been shown that patients suffering from pathologies of the osteomeatal complex may suffer from olfactory disorders, but do not complain about nasal obstruction (Doty, 1997, Chem. Senses, 22: 565–586). Whereas no specific therapies have been found to be effective in the case of sensori-neural loss, inflammatory or obstructive abnormalities in the nose impeding olfactory transport should certainly be amenable to further treatment (Rowe-Jones, 1997, Clin. Otolaryngol., 22: 377–381; Lund and Scadding, 1991, J. Laryngol. Otol., 105: 823–835).Twenty consecutive patients suffering from chronic sinusitis and olfactory disturbances were evaluated before and after surgical treatment. Olfactory function testing was performed by means of a psychophysiological examination (using Sniffin' Sticks). All patients reported about a gradual onset of hyposmia (which was revealed in 18 patients; only two were completely anosmic). Patients were tested 3–6 weeks after surgery, complete recovery of the olfactory function (age and gender adjusted data) being observed in 75% of our sample. Fifteen percent improved in their performance of the olfactory function test, although subjectively they did not recognize a change in daily life. Finally, two patients failed to show any improvement due to surgery. There was no prognostic parameter detected that could predict the effect of surgery on olfaction. Therefore we suggest olfactory function testing should be performed prior to nasal surgery in the same way as audiometry precedes any sort of ear surgery.

232. Bradyosmia: the olfactory deficit in Parkinson's disease may be due in part to a deficit in sniffing

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Parkinson's disease (PD) is accompanied by an olfactory deficit. It has been suggested that this deficit is related to specific neuro-pathophysiological and/or neuropathochemical profiles of PD as they are expressed in the olfactory system. Here we suggest that PD patients have an impairment in sniffing. To measure olfactory abilities in PD patients and healthy controls, we used a standard olfactory identification test (UPSIT), and ascending staircase threshold tests for the odorants vanillin and propionic acid. To measure sniffing abilities, we used a pneomatotachograph-coupled spirometer that recorded during a two-alternative forced-choice odorant detection task. We have currently tested 13 PD and five control subjects. PD UPSIT scores ranged from 12 to 28 (mean = 18.7), which is significantly lower than the expected age-matched normative score of 34 ($P < 0.0001$). Mean detection thresholds for both odorants were three log-steps higher for the PD than for the control subjects. All sniff measures were diminished in PD in comparison with controls (peak airflow: PD = 0.41 l/s, control = 0.52 l/s; mean airflow: PD = 0.25 l/s, control = 0.33 l/s; volume: PD = 0.4 l, control = 0.6 l; mean sniff duration: PD = 1.6 s, control = 1.8 s). There was a correlation of 0.76 within the PD patients between their ability to sniff and their ability to smell. Patients who sniffed at a higher mean airflow rate performed better on the UPSIT [$F(12) = 15.3$, $P < 0.003$]. These findings suggest that the olfactory impairment in PD may be due in part to an impairment in sniffing.

233. Effects of diurnal rhythm and adaptation/habituation on olfactory and chemo-somatosensory event-related potentials

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The aims of the present study were to investigate (i) diurnal variations in, and effects of adaptation/habituation on, olfactory event-related potentials (OERPs) and chemo-somatosensory event-related potentials (CSSERPs); (ii) diurnal variations in odor and pain thresholds; and (iii) relations between OERP/CSSERP measures and perceived intensity.

Five men (23–34 years) took part in four sessions at each test hour of 04:00, 08:00, 12:00, 16:00, 20:00 and 24:00. Each session included assessment of OERPs (H_2S), CSSERPs (CO_2), psychophysical odor (H_2S) and pain (CO_2) thresholds, oral temperature, blood pressure, heart rate, nasal volume and tiredness. For the OERP and CSSERP recordings, at Fz, C3, Cz, C4 and Pz, and for ratings of perceived intensity, the stimuli were presented in 15 series of five stimuli with a 5 s ISI within series and a 30 s ISI between series.

The P2 and N1P2 amplitudes of the OERPs and CSSERPs were found to follow a diurnal rhythm with its peak at 16:00, which corresponds well with oral temperature, blood pressure, heart rate, nasal volume and tiredness, but not with odor or pain thresholds. However, the variability of the odor thresholds followed this rhythm. The N1, P2, P1N1 and N1P2 amplitudes of the OERPs and CSSERPs as well as the perceived-intensity ratings decreased over the stimulus series, and the P1 latency increased. Interestingly, the P2 latency decreased over the series. Based on the diurnal-induced variation in electrophysiological amplitude and perceived intensity, the relation between these measures improved from the relatively early (P1) to later (P2) component.

The results suggest that the OERPs and CSSERPs, but not odor or pain thresholds, follow a diurnal rhythm similar to the autonomic nervous system, and that they are affected by adaptation/habituation and, possibly, by expectations (P2 latency). The findings do also imply that the relatively cognitive P2 amplitude, better than the P1 amplitude, reflects perceived intensity.

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234. Vocs in urine detected by gc-ms and electronic nose

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Volatile organic compounds (VOCs) of human urine represent an important source of information of metabolic processes inside the organism and can potentially be used as a rapid method for diagnosis purposes (Wahl *et al.*, 1999, J. Chromatogr., 847: 117–125). In the last few years we have been involved in a study that has the aim of finding out if it is possible to use an 'electronic nose' as a diagnostic method. We are investigating urine odour from people affected by different myopathies (Darras and Friedman, 2000, Pediatr. Neurol., 22:171–181), which are inherited diseases related with enzymatic deficiency. In our previous work (Pisanelli *et al.*, 1999, Chem. Senses, 24: 103) we presented the results obtained from a research carried out by using an 'electronic nose' device for diagnosing myopathies. The results were promising and we were able to differentiate not only patients from healthy population but also patients affected by different myopathies. In order to find out how we can use the 'electronic nose' in the medical diagnosis field, we performed a GC-MS study on the urine head space. The aim of this work is to select volatiles that can be used as target for some myopathies and to build gas sensor arrays selective for these compounds. We focused our attention on ketones, aldheydes and organic acids that seem to have a stronger correlation with myopathies and urine head space.

Urine samples from 20 healthy people and 12 patients affected by different muscle diseases were collected for a period of 2 weeks and frozen. Aliquots of 2 ml were used for the head space analysis with a 32 conducting polymer sensor array (Osmetech plc UK). This size of aliquot has been used for GC-MS analysis (HP 5890, HP 5971, Hewlett-Packard). Head space was extracted before and after acidification by SPME and the separation was performed using a 30 m DB5 column.

Patient affected by myopathies have enzymatic defects that induce abnormal excretion of organic acids, ketones and aldhehydes. The presence of such compounds leads the production of a peculiar head space, that can be easily detected with the electronic nose (Pisanelli *et al.*, 1999).

The GC-MS analysis of urinary organic acids showed excess excretion of dicarboxylic acids such as adipic, sebacic and malonic acids. The information obtained from the GC-MS will be used to build a specific gas sensor array that will be used as a rapid diagnostic method.

235. Comparison of acceptability of polimaltose ferric iron complex with ferrous sulfate in infants

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Adherence to oral supplementation regimens can be compromised by poor acceptability of the preparation.

The aim of this study was to evaluate the acceptability of oral administration of a ferrous sulfate compound and compare it with polimaltose ferric iron complex in infants.

Seventy-one infants, 6–23 months of age, were recruited from six day-care facilities in the urban periphery of Santiago. These infants were healthy, with normal anthropometric measures. They were fed a standardized diet sufficient in macronutrients, but were not given any vitamin–mineral supplements. The acceptance of the diet was measured with a facial hedonic scale of five levels of acceptability. They were rated by two investigators during administration of the products in an open label design. Each infant was randomly given one product during 5 days and then crossed over to the alternative product for another 5 day period. The infants had a daily photographic record that was evaluated offline by a third, blind investigator. At the end of the 2 weeks hemoglobin levels were measured. Anemia was treated when present.

The average acceptability (mean ± SD) of polimaltose ferric iron complex (2.81 ± 0.08) was significantly higher (paired t-test < 0.001) than for ferrous sulphate (2.03 ± 0.09). The 2.81 value is closer to 3.0, which means that the answers of the children were mostly 'neither like nor dislike it', while the 2.03 value is closer to 2.0, which means 'dislike it quite a bit'. Using the Wilcoxon Signed Rank Test to analyse the same data, 63 answers favoured polimaltose ferric iron complex over ferrous sulphate, seven preferred ferrous sulphate and three answers were tied, from a total of 73 ($P < 0.001$).

We conclude that the polimaltose ferric iron complex (Maltofer®) was significantly more accepted by children than ferrous sulphate (Fer-in-sol®).

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236. Evidence for inborn olfactory preferences in human neonates: the case of conspecific milk

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Newborns of various mammalian species have been demonstrated to be especially reactive to the chemosensory cues of the milk produced by females of their own species. The expression of this spontaneous attraction is not conditional upon postnatal exposure to conspecific milk, as it is noted in naive newborns prior to any ingestion of milk or in older newborns fed a replacement milk of synthetic origin. This study aimed at commecing the examination of the existence of inborn attraction or appetitive responses to the odour of homospecific milk in newborns of our own species.

Four-day-old infants were submitted to two-choice tests in which the odour of human milk (HM) was pitted against the odour of cow-based formula milk (FM). They were exclusively breast- (Brf) or bottle-fed (Bof) prior to the test. In experiment 1, the infants were exposed to a paired-choice between the odours of HM and FM, both of them being unfamiliar (i.e. collected from an unfamiliar mother and from an unfamiliar FM brand). Both Brf and Bof infants oriented their nose longer to the sample of unfamiliar HM than to unfamilar FM, indicating that (i) HM contained an olfactory factor that is generally attractive to newborns; and (ii) the attractive power of HM is not dependent on postnatal exposure to it. Experiment 2 compared in Bof infants the relative strengths of the attractive factor carried in HM and of familiarity-related attraction to the FM consumed since birth. These infants did not differentiate either odour substrate in terms of head orientation, suggesting that unfamiliar HM is as

reinforcing as the familiar FM with which they were satiated during 15–24 feeding episodes. However, another behavioural marker, duration of mouthing, indicated that the HM odour still remained more appetitizing than the FM odour. Finally, experiment 3 controlled the effect of an intensity imbalance between HM and FM on the relative preference of Bof infants. Both stimuli were matched on subjective intensity by a panel of adults. A test opposing undiluted HM with diluted familiar FM resulted in the reinstatement of the head-turning preference to the odour of HM relative to FM.

These results clearly demonstrate that the odours of HM and FM have differential reinforcing properties for human newborns. In terms of attractive strength, unfamiliar HM compares with FM that has repeatedly been associated with satiety.

237. Experimental study of neuronal differentiation and synapse formation in the olfactory system

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For the formation of a functional olfactory system, the key processes are the neuronal differentiation, including the expression of one or the other olfactory receptor, and the correct organization of nerve terminals and synapses in the olfactory bulb. These processes take place during embryonic development starting from early stages of development. We have used a model of avian embryos for an experimental study of these processes.

Taking advantage of the species-specific equipment of olfactory receptor genes in different bird species, interspecific avian chimeras were designed. The results of early olfactory placode grafting were analysed with different complementary approaches. *In situ* hybridization using probes to different chick olfactory receptor genes indicated that the choice of expression of an olfactory receptor by a neuron is independent of the environment of the olfactory placode or epithelium, notably of the centro-periphal synaptic connections.

The olfactory performances of normal embryos and of such chimeras are presently being analysed.

The fibre convergence and target connections in such chimeras provided a good experimental model to check the factors involved in these processes.

238. Focal denervation and the developing rat olfactory bulb

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Contact between the olfactory nerve and the forebrain is critical for normal olfactory bulb development. For example, several studies have demonstrated that the removal of the embryonic olfactory placode results in a failure of the olfactory bulb to form, as well as causing other forebrain malformations. The current study explores a technique that permits removal of contact between the olfactory nerve and specific regions of the bulb early in development, without causing damage to other brain regions and without removing the peripheral olfactory organ. The manipulation, which involves insertion of a small Teflon chip between the cribriform plate and the bulb, prohibits growth of new axons into the 'shadow' region behind the implant. In the present work, surgery was done on the day after the day of birth, and the consequences were examined either 10, 20 or 30 days later. Focal denervation causes a decrease in bulb and constituent layer sizes, a reduction in mitral cell number, and changes in bulb architecture. Using a battery of antibodies (OMP, MAP2, TuJ1, calretinin, calbindin, parvalbumin, TH and GAD), we further demonstrate that: (i) focal denervation alters the relationship between the olfactory nerve and the bulb; (ii) the fine structure of cells in denervated regions is disrupted; and (iii) cellular phenotypes change in response to loss of afferent contact. These results suggest that contact between the olfactory nerve and the bulb is important for maintaining bulb architecture and cell survival, structure, and phenotype.

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239. The importance of neuron–glia interaction in the formation of an olfactory memory in mice

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Female mice form an olfactory memory of the pheromones of the male with which they mate. The pheromonal memory is of critical biological importance, because it prevents any subsequent exposure to the mating male's pheromones from initiating neuroendocrine mechanisms that would terminate pregnancy. Pheromones from strange males, for which no memory has been formed, activate the vomeronasal system, thereby causing pregnancy block. The synaptic changes underlying this memory occur in the accessory olfactory bulb (AOB) (Brennan *et al.*, 1990, Science., 250: 1223–1226). Memory formation requires glutamatergic neurotransmission from mitral to granule cells via both ionotropic and metabotropic receptors (Kaba and Nakanishi, 1997, Rev. Neurosci., 6: 125–141; Brennan and Keverne, 1997, Prog. Neurobiol., 51: 457–481). In addition, memory formation is associated with an increase in length of the mitral-to-granule glutamatergic synapses and an increase in GABAergic neurotransmission (Brennan and Keverne, 1997; Matuoka *et al.*, 1997, NeuroReport, 8: 2501–2504). It is facilitated by nitric oxide signalling (Okere *et al.*, 1996, Neuroscience, 71: 349–354). The so-called GABA–glutamine–glutamate cycle is of importance in normal glutamate and GABA neurotransmitter activity, and is dependent almost exclusively on astroglial cells. L-Arginine, the nitric oxide (NO) precursor, is predominantly localized in glial cells. The purpose of this study was twofold: (i) to assess neuronal nitric oxide synthase (NOS) gene expression during the critical period for memory formation; and (ii) to investigate a possible involvement of glial cells in the formation of the pheromonal memory.

A significant increase in neuronal NOS mRNA expression in the AOB was observed 120 min after mating, extending our previous findings on the role of NO in the pheromonal memory. The NOS gene expression was characteristically more predominant in the

anterior–middle than in the middle–posterior portions of the AOB. An immunohistochemical analysis revealed that there was an increased expression of glial fibrillary acidic protein in the external plexiform and glomerular layers of the AOB during the critical period for memory formation. Functional analysis in the context of pregnancy block was performed using L-methionine sulfoximine (MSO) and fluorocitric acid (FCA). MSO inhibits conversion of glutamate to glutamine; FCA inhibits *de novo* formation of glutamine from glucose in astrocytes. Both drugs, when infused into the AOB 0 and 1.5 h after mating, produced a memory deficit, as revealed from the mating male being able to cause his own pregnancy block. These results demonstrate a role for glial cells in the formation of a pheromonal memory.

240. Predator odor: what it is signalling about?

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The behavior of prey can be changed dramatically in the presence of a predator. Chemosensory detection may be an important aspect of predator avoidance strategy for many mammals. In our earlier studies we examined the influence of predator chemical cues derived from geographically sympatric and allopatric predators (from urine of the coyote *Canis latrans*, and anal sac secretions from the mink *Mustela vison* and the feral cat *Felis catus*) on reproductive output of rodents. Laboratory naive animals responded to chemical cues from all three sources with reduced litter size and skewed sex ratio, though the urine of feral cats geographically sympatric to the prey tended to be more effective. This indicates the innate nature of the response. A further set of experiments was performed to determine physiological mechanisms underlying this phenomenon. We used Norwegian rats as a model for potential prey and the urine of a feral domestic cat maintained on a wild mouse diet as the test stimulus. Exposure to predator urine maximally affected implantation and maintenance of implantation when predator urine was applied to the bedding of rodents during the first third of gestation. We monitored progesterone levels in female Norwegian rats during early gestation because this is a key ovarian hormone responsible for the maintenance of the fertilized egg, preparation of the endometrium and maintenance of pregnancy. At the same time, corticosterone patterns were recorded for the same animals. Additionally, a rough handling group was used as a control for stress-induced changes of plasma corticosterone level. Female rats exposed to cat urine had smaller litter sizes. Based on the physical appearance of corpora leuteal scarring, it appeared that the reduction in litter size was owing to resorption of the embryos during the early part of gestation. Consistent with the morphological evidence was the observation that plasma progesterone levels were dramatically suppressed in rats exposed to cat urine relative to levels observed in the water control group and for rats exposed to guinea pig urine. We did not observed robust differences in plasma corticosterone levels for rats exposed to predator and non-predator urine, while rough handling of animals caused clear elevation of corticosterone. At the same time, rough handling did not cause reductions in litter size, indicating the specific nature of the response. Manipulations with the diet of the predator and non-predator urine donors revealed the key role of sulfur-containing compounds. Removal of sulfur-containing compounds virtually eliminated the activity of urine. These findings indicate that predator odors may work as specific reproductive disrupters.

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241. Coding alarm in the olfactory bulb of Crucian carp

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Extract of carp fish skin induces via the olfactory organ a characteristic alarm reaction in conspecific individuals (von Frisch, 1941, Z. Vergl. Physiol., 29: 46–145). We have shown that the medial part of the medial olfactory tract (mMOT) in the Crucian carp is necessary and sufficient to mediate the alarm reaction (Hamdani, El-H. *et al.*, 2000, Chem. Senses, 25: 103–109). Electron microscopy of the mMOT reveals the presence of both myelinated and unmyelinated fibres. To understand how the olfactory system transforms the information from the sensory neurons dealing with a feeding substance (L-alanine) and the alarm substance(s), we made recordings of the nervous activity from the olfactory bulb in the region where the mMOT originate. Two types of spontaneous nervous activity were recorded simultaneously. One type of unit was characterized by spikes of large amplitudes that were always followed by slow potential changes at a fixed interval. These units were presumably discharges from the 'ruffed' cells (Kosaka and Hama, 1979, J. Comp. Neurol., 186: 301–320; Zippel *et al.*, 1999, Cell. Mol. Biol., 45: 327–337). Another type of unit, presumably mitral cells, displayed small amplitude spikes only. Electrical stimulation of the mMOT induced antidromic discharge of the mitral cells at a long latency, but did not induced activity in the ruffed cells. Stimulation of the olfactory organ with extract from the skin of the Crucian carp induced increased activity in the mitral cells. During this stimulation period the ruffed cell activity was suppressed. Stimulation of the olfactory organ with L-alanine caused a cessation of spike activity in the mitral cells and induced an increased spike activity in the ruffed cells.

In summary, skin extract containing alarm substance(s) induces activity of receptor neurons that connect to unmyelinated mitral cells. The axons of these cells project to the mMOT of the olfactory tract and mediate the information of alarm by an increase discharge of nerve impulses. The feeding substance L-alanine induces activity in the ruffed cells and this activity probably causes inhibition of the adjacent mitral cells that project to the mMOT.

242. Chemical communication of the typical feline: Eurasian lynx (*Lynx lynx* L.) as an example

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Felines are mainly solitary-living animals (excluding lions and partly cheetahs and domestic cats) and the meetings of individuals in nature are quite rare. The overlapping of home ranges and the use of the same ways for movements by different individuals allow them to detect odor marks of conspecifics very easy.

We studied marking behavior, the possible role of excretes and

olfactory behavior of the Eurasian lynx in semi-natural conditions at the biological station 'Tchernogolovka' in 1989–1998. The method of continuous data recording was used. More than 2200 h of observations were analyzed. The pattern of lynx marking behavior was similar with that of other felines. Urine marking of vertical objects was the main type of lynx marking behavior. We found significant sexual, age, seasonal and individual differences in lynx marking behavior (urine marking, scratching and rubbing). The reaction of lynx to conspecific odors was studied by the presentation of pairs of the odor samples. The recipients recognized the species, sex and age of conspecifics, familiar and unfamiliar lynx. Lynx olfactory behavior during the contact with the conspecifics was closely related to the social status of the individual. Chemical communication plays an important role in lynx social behavior. The regular distribution of the odor marks of the lynx promotes a constant exchange of diverse information between conspecifics in nature.

243. The interaction between pheromone-elicited and odour-elicited behaviour in the newborn rabbit

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To locate a maternal nipple, the rabbit newborn depends on a signal emitted both on the female's abdominal surface (Hudson and Distel, 1983, Behaviour, 8Z: 260–275; Coureaud and Schaal, 2000, Devl Psychobiol., in press) and in milk (Keil *et al.*, 1990, Physiol. Behav., 47: 525–529; Coureaud *et al.*, 2001, Anim. Behav., 61: 153–162). We have recently chemically identified a mixture from these substrates and characterized its truly pheromonal properties (labelled P thereafter). Pups exposed to a glass rod carrying P exhibit a very high level (>90%) of stereotyped search/grasping response (SGR). Beside what appears to be an unconditional response to P, rabbit pups are also able to learn any odour associated with sucking (Hudson, 1985, Devl Psychobiol., 18: 575–585). The present study aimed to assess the stability of the response to P during the first postnatal week and to determine whether it is mitigated by the acquisition of a novel odorant in association with satiation.

Experiment 1 examined the developmental course of the SGR to P in the complete absence of postnatal exposure to it. Pups (n = 24) were separated from the female right at birth (d0). They were then bottle-fed a formula without P. At d6, they were assayed with the oral grasping test. The initial high level of SGR to P was maintained, demonstrating that P remains a stable releaser of SGR despite the absence of postnatal exposure. In experiment 2, we determined: (i) whether SGR could come under the control of any odorant associated with satiation, and how far this odorant would bear a releasing power similar to P; and (ii) whether the engagement of the SGR in a new learned response would interact with the level of SGR to P. Additional pups (n = 14) were isolated from their mothers and bottle-fed once-per-day with the formula to which was added furaneol. On d0, 2, 4 and 6, these pups were exposed to the oral grasping test with either (i) furaneol, (ii) P, (ii) a control odorant and (iv) a blank stimulus. Repeated testing with either the control odorant or the blank did not elicit SGR at any testing time. However, the association of furaneol with bottle-feeding induced the progressive release of the SGR by pure furaneol from 0% on d0 to 50% on d6. Further, the associative establishment of the link between the novel odour and SGR did not interact with the releasing potency of P: the SGR rate to P was maintained at its initial high level between d0 and d6, and remained always higher than that to furaneol.

This study reveals the very strong behavioural significance of the pheromonal compound we have isolated for the rabbit pup, as well as the independent plasticity of learning of novel odorants. The conjugated operation of both pathways may reinforce the adaptive responses of the newly born pups facing the essentially new and unpredictable postnatal environment.

244. Patterns of expression of the immediate early gene *egr-1* in the accessory olfactory bulb of female mice mated with males from different inbred strains

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In addition to gender information, mouse urinary pheromones also convey information regarding individual identity. Both gender information and the information required to distinguish among individuals are conveyed by the vomeronasal system. Two main classes of receptor have been discovered in the vomeronasal organ, which are likely to respond to different types of vomeronasal stimuli. Furthermore, vomeronasal receptor neurons expressing the different classes of receptor project separately to the anterior and posterior sub-regions of the accessory olfactory bulb (AOB). The nature of the stimuli that activate these sub-regions of the AOB is still unclear. However, one possibility is that the information about the individuality of the pheromonal signal is handled exclusively by either the anterior or the posterior sub-region. Therefore, analysis of differences in the pattern of activity, in response to urinary stimuli from different inbred strains, might reveal whether the anterior or posterior sub-region is more involved in the representation of information regarding individual identity.

We investigated the patterns of expression of the immediate early gene *egr-l* in the AOB of female mice that mated with males of different strains. Different groups of females were mated with males of either the DBA strain or the BALB/c strain. Other females were exposed to males of both strains at mating (DBA+BALB/c group), a procedure that results in the formation of a pheromonal memory for both strains. A control group of females was also used that had not received any male exposure. Females that had been exposed to both DBA and BALB/c males had a significantly higher density of egr-positive mitral/tufted (MIT) cells than females that had mated with either the BALB/c or DBA males alone. All three of these groups had a significantly higher density of egr-positive MIT cells than control females. The anterior–posterior distribution of these cells differed across the groups. The anterior sub-region of the AOB had a pattern of results similar to that observed overall. The density of egr-positive MIT cells in the DBA+BALB/c group was significantly higher than both the BALB/c and DBA groups. However, in the posterior AOB the density of egr-positive MIT cells did not differ significantly between the DBA+BALB/c group and the BALB/c group. Both of these were significantly higher than the DBA group. These findings imply that MIT cells in the anterior sub-region of the AOB respond differentially to males of different strains and therefore convey

some information about individuality. The situation is less clear-cut for the posterior sub-region, but it is possible that it too conveys information regarding individuality of the phermonal signal.

The density of stained granule cells was significantly increased in females that had mated, but was not significantly different among the three groups. There was an uneven distribution of egr-positive granule cells across the AOB, with the highest densities laterally and the lowest medially. However, this pattern of staining was not significantly different in mated versus control females, indicating that it was intrinsic to the AOB.

245. The unique nasal epithelium and TGF-α positive olfactory cells: a comparative study

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The nasal epithelium exclusively consisting of supporting cells and horizontal basal cells was found in the dorsal fossa (roof) of nasal turbinates and corresponding septum, as several epithelial patches in mice (BALB/c, DBA/2), rats (Wistar, SD) and hamsters (Syrian). The localization of the epithelial patches in the posterior nasal cavity, and their ultrastructure, were essentially similar to that of ddY mice (Suzuki *et al.*, 2000). In guinea pigs, no specialized epithelium was observed. In mice and rats, distinct populations of transforming growth factor (TGF)-α immunoreactive olfactory cells occupied the boundary between the epithelial patches and surrounding olfactory epithelium. In the electron micrograph, the cytoplasm of olfactory cells, including the knob and cilia, was intensely labeled by anti-TGF-α antibody (MAb213, Oncogene). At this region, TGF-α-positive olfactory cells were devoid of labelings for olfactory marker protein, protein gene product (PGP) 9.5 and β-tubulin, suggesting different processing of the signal in the olfactory bulb. Moreover, three-dimensional reconstruction from double-labeled (TGF-α and PGP9.5) sections revealed a unique relation between the epithelial patch, TGF-α-positive cells and olfactory epithelium. In the posterior part of the turbinate, many TGF-α-positive cells occupied the dorsal fossa. From posterior to anterior, the number of TGF-αΦ255–ποσιτιῶε χελλσ ωασ δεχρεασινγ, ανδ α γρουπ οφ ΤΓΦ–α-positive cells surrounded the epithelial patch. In the anterior part, normal olfactory epithelium (PGP9.5 positive) surrounded the epithelial patch. Although this unique epithelium does not participate in odor reception, it is related to the functionally distinct subsystem (TGF-α-positive olfactory neurons) within the olfactory system.

246. Nasal and olfactory function in hog confinement workers

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Published reports and survey data suggest that farm workers in hog confinement environments have an increased prevalence of rhinosinusitis symptoms and decreased olfactory ability. We have measured pulmonary, nasal and olfactory function in 11 hog confinement workers and in 16 non-farmworker controls. The workers range in age from 21 to 49 years (mean = 34.8), with two being female. All work for >8 h each day in the hog facility. They were all tested after being in the hog environment for >6 h. Four of these workers have a history of fever, aches and chills after previous exposure to organic (grain) dusts. The control subjects range in age from 22 to 43 years (mean = 28.6), with nine being female. The tests administered are: spirometry, saccharin nasal transit time and the 12-item scratch-and-sniff 'Cross-Cultural' Smell Identification Test (Sensonics, Inc., Hadden Heights, NJ).

The hog confinement farm worker's lung function was not significantly different from that of the controls for the percent predicted FEV1/FVC (workers 96.5 ± 2.0 versus controls 100.2 ± 1.5, $P = 0.16$). The saccharin transit times have a mean of 6.6 ± 1.4 min in the workers and 9.1 ± 0.9 min in the controls ($P = 0.14$). The olfactory tests, expressed as percentiles because of the differences in age and gender of the controls, show that the mean worker's score is 32.9 ± 9.0, while the control's is 54.5 ± 9.6 ($P = 0.085$). Six (55%) of the workers and three (19%) of the controls complained of chronic nasal/sinus problems and allergic rhinitis. Six (55%) of the workers and one (6%) of the controls also complained of occasional loss of the sense of smell.

These results suggest that the hog confinement environment is associated with abnormal function in the nasal airway. The trend toward increased saccharin transit times and the increased nasal/sinus symptoms in the worker group suggest that there is irritation at the mucosal level. The decreased olfactory ability seen in the hog workers and the history of occasional olfactory loss and nasal/sinus symptoms suggests that the hog confinement environment is at least associated with a conductive loss of olfactory ability. Whether there is also a neural loss is unclear.

247. Sweet–sour mixture suppression in the elderly

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It has been reported that taste, specifically sour taste, may actually improve swallowing physiology in subjects with neurogenic dysphagia (Logemann *et al.*, 1995, J. Speech Hearing Res., 38: 556–563). However, subjects reported the sour stimulus as unpalatable. Sweet–sour mixtures may be more palatable and yet still elicit improved swallowing response since there is some evidence that mixture suppression mechanisms are central rather than peripheral receptor phenomena ((Lawless, 1979, J. Comp. Physiol. Psychol., 93: 538–547; Kroeze and Bartoshuk, 1985, Physiol. Behav., 35: 779–783). Prior to undertaking a study of swallowing physiology, the objective of this study was to examine whether the pattern of sweet–sour mixture suppression was similar in healthy elderly ($n = 19$) and young subjects ($n = 21$). It has been reported that the elderly demonstrate higher detection thresholds of sucrose in citric acid (Stevens and Cain, 1993, Crit. Rev. Food Sci. Nutr., 33: 27–37). In this study, a flavour profiling technique was used. All subjects passed a screening test that demonstrated their understanding of the rating technique. Subjects rated the sweetness and sourness of nine mixtures in replicate, using separate rating scales of 15 horizontal boxes with written phrase anchors. The mixtures were deionized water, sucrose (4 and 8% w/v), citric acid (0.1 and 0.4% w/v), and all four possible combinations of sucrose and citric acid concentrations. Lemon flavouring (0.2% v/v) was added to all mixtures except water.

Mixture suppression occurred in elderly and young subjects, and it is level dependent ($p < 0.05$). The pattern of mixture suppression is similar in both groups; however, the elderly showed increased

suppression of sweet by acid. The palatability of sweet–sour mixtures may be similar in both groups if appropriate concentration levels are provided. Further research is planned to investigate whether sweet–sour mixtures will also provide improved swallowing physiology in subjects with neurogenic dysphagia.

248. Olfactory discrimination learning at the piriform cortex level in the rat

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In rodents, olfactory stimuli play a major role in behaviour and are important cues for learning. Learning is known to induce brain plasticity. One can wonder if brain plasticity at the primary olfactory cortex level would interfere with sensory coding of odours. In order to understand how odour-specific patterns in neuronal activity are established during odour discrimination learning we have used an odour-discrimination go/no go task with water reinforcement for studying extracellular single cell activity in piriform cortex in freely moving rats. Nose pokes to odour and water ports situated on the opposite walls of the experimental wooden chamber (75 × 85 cm) induced odour and water delivery respectively. All events were controlled by computer. The odour port is connected with an olfactometer capable to deliver eight odours. A driveable bundle of eight nichrome wire electrodes was implanted to the anterior piriform cortex. All movements of the rat are monitored by video tracking system. Spike activity is collected by computer. Rats (Long Evans, $n = 4$ and Wistar, $n = 4$) were trained to discriminate between the two odours of the first pair, one odour being rewarded by water, the other one not. The learning took 16–20 days for Wistar and 8–11 days for Long Evans rats (100 trials per day). After 80% of correct responses was reached a new pair was introduced. Learning time decreased with the successive pairs: 10/5 days for pair 2, 2 days for pairs 3 and 4. Until now, only spike activity recorded from Long Evans rats has been processed. From 31 recordings (28 cells) in 53.6% of cases cells had reaction connected with odour presentation, 21.4% responded for the water reinforcement. The inhibitory reaction for one or both odours was observed in 28.6% of cases, activation in 25%. Long-term recording from the same cell (up to 5 days) showed that the cell performs the same pattern of activity irrespective of the day of learning.

249. Toward a metric of similarity of odor quality

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Assessments of odor quality based on reports of mental content (e.g. profiling, direct ratings of similarity) provide negotiable answers. Rate of discriminative errors constitutes one performance-based definition of similarity. Unfortunately, since error-rate falls to an asymptote once odors differ to a certain extent, the use of discrimination has been limited to studies of fairly similar-smelling odors (Laska and Freyer, 1997, Chem. Senses, 22: 457–465). Since latency continues to decrease beyond the point of asymptotic error-rate (Luce, 1986, Response Times. Oxford University Press, Oxford), latency should extend the range of differences in odor quality that discrimination can resolve.

Research should establish that: (i) latency reflects accuracy. Study 1 showed that (difficult) discriminations between binary mixtures and their unmixed components required more time than (less difficult) discriminations between unmixed components. (ii) Latency provides better resolution of differences between odor-pairs than errors of discrimination. Study 1 showed that latency does provide superior resolution. (iii) Latency-based estimates of similarity among odors tested previously predict similarities among odors not yet tested. Study 2 showed that estimates of similarity tested previously did predict similarities not yet tested. These findings demonstrate validity and potential utility of latency-based measures of similarity.

Signal detection theory could provide ratio-level measurement through the index d', but data must satisfy the assumptions of the model. Since forced-choice designs are difficult to apply to odor quality, same–different and ABX designs are common. In these designs, one must take account of decision-strategy to model data, since different models will provide different estimates of sensitivity given the same data (Macmillan and Creelman, 1991, Detection Theory: A User's Guide. Cambridge University Press, Cambridge). In Study 2, models assuming both independent observations and differencing strategies were fitted to ROC curves based on rated confidence in same–different discriminations. Under the current conditions, subjects seemed to employ a differencing strategy.

These studies represent progress toward a performance-based metric of similarity of odor quality. This technique could provide the non-negotiable, quantitative measurements needed to generate and test quantitative models of structure–activity. Such measurements could also guide neurobiologists toward selection of stimuli with quantitatively specified relationships for humans.

250. Effect of labels on odor perception

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Labeling has a top-down effect that can alter learning and memory. The effect of labeling has been found in olfaction, for instance, with recognition performance for labeled odors being higher (Rabin *et al.*, 1984, J. Exp. Psychol.: Learn. Mem. Cogn., 10: 316–325). And false labeling causes more errors in memory (Cain *et al.*, 1996, Chem. Senses, 21: 35–44). In the present study, we manipulated labeling to investigate its effect on odor perception. The labeling effect may alter representation of odor.

Sixty participants smelled 10 everyday odors, judged their perceived intensity and pleasantness, and rated them according to 18 adjectival words. The participants were divided into two groups: presentation-with-labels (40 participants) and presentation-without-labels (20 participants). In the presentation-with-labels condition, the labels were either true or false; for instance, the odor of raisins was either 'raisins' or 'sweaty shirts'. The false labels were based on false responses given in a free identification study, and all labels were counterbalanced for the 10 odors.

The results in intensity indicated a significant effect of labeling, with the participants in the without-label condition judging the odors to be weaker than those in the with-label condition [$F(2,513) = 8.76$, $P < 0.01$]. In order to examine the stability of odor evaluation, the same experiment was conducted 1 week later with the same participants. An analysis of the pleasantness and adjectival words ratings indicated that the judgements of the participants in the without-label condition were more likely to change. The type of labeling has remarkable effect for almost all odors in terms of both the pleasantness and adjectival word ratings. For example, the participants tended to rate the raisins odor as being more pleasant when labeled as 'raisins' compared with when labeled as 'sweaty shirts' [$F(2,54) = 21.31$, $P < 0.01$], with marked differences in the adjectival word ratings, particularly for the items 'tasty', 'sweet' and 'pungent'. These results suggests that the representation for an odor is more stable when associated with a label, and that labels have a top-down effect on the perception of odors.

251. Olfactory stimulus concept and the discriminability of nearly identical apple aromas

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The headspace of the juice of an apple was analysed instrumentally to obtain an ecologically relevant stimulus-model mixture of apple volatiles. This model was used to test two hypotheses: (i) adding sub-threshold components to a familiar smelling mixture of odorants can change the perceived quality of this mixture; and (ii) subjects with elaborated stimulus concepts are better able to discriminate between slightly different stimuli than subjects with poorly refined stimulus concepts.

Two sets of volatiles were composed: a set of eight supra-threshold volatiles (MIX) and a set of three sub-threshold volatiles. In order to test the first hypothesis, three successive dilutions of the sub-threshold volatiles were prepared in such a way that the strongest was at threshold concentration and the two lower concentrations were below threshold. The detectability of the sub-threshold components in a blank stimulus was compared with the detectability in MIX.

It was observed that the sub- and peri-threshold volatiles were not detected better in MIX than in a blank. On the contrary, sub- and peri-threshold volatiles were better detected alone than when added to MIX. However, when the group of subjects was subdivided into two groups, employing either a poorly refined or a highly refined concept of the target stimuli, the subjects with highly refined concepts were significantly better able to detect the presence of sub-threshold volatiles in MIX than those with poorly refined stimulus concepts. The effect of stimulus concept occurred independently of the actual proportions of correct detections of sub-threshold volatiles in a blank.

252. The neural correlates of pleasant (edible) and disgusting odours

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Few studies have investigated the neural correlates of odour perception. Most have reported orbitofrontal cortex and entorhinal cortex activation in response to odours. A previous study (Simmons *et al.*, 1997, NeuroImage, 5: S196; Williams *et al.*, 1997, NeuroImage, 5: S197) examined the neural correlates of pleasant and unpleasant odours and found increased activation in response to unpleasant odours as compared with pleasant odours, supposedly mainly due to trigeminal activation during the 'unpleasant' condition. Odours have also been linked to emotions and have been shown to elicit basic emotions (Vernet-Maury *et al.*, 1999, J. Autonom. Nerv. Syst., 75, 176–183). In this study we want to determine the neural correlates of pleasant edible odours and disgusting odours; this forms part of a project to determine whether the neural correlates for the perception of a specific emotion (in this case disgust) depend on the sensory modality of stimulus presentation.

We expect activation of orbitofrontal cortex and entorhinal cortex in response to all odours. On the basis of previous results in the visual and auditory modality (Phillips *et al.*, 1998, Proc. R. Soc. Lond. B, 265: 1809–1817), we expect activation of the insular cortex in response to disgusting odours.

Eight right-handed, healthy, non-smoking male subjects were exposed to two pleasant and two disgusting odours during an AB design contrasting each odour with neutral (fresh air). An olfactometer, which had been designed for use in the MRI scanner, delivered the odours via a facemask. The odours were chosen on the basis of prior rating by 20 volunteers. Both pleasant and unpleasant odours can cause trigeminal stimulation, but were diluted to minimize this.

With this study we contribute to the investigation of the neural correlates of odour perception *per se*. The innovative aspect is the link to the neural for the perception of specific emotions, e.g. disgust. We hypothesize that the perception of a specific emotion is independent of the sensory modality of stimulus presentation. The insula is activated by both visual and auditory stimuli of disgust and we expect it to also be activated by olfactory stimuli of disgust, but not by pleasant olfactory stimuli.

253. Perceptual separability and taste–odour interactions

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Many studies have shown taste–odour interactions. In these studies, assessors are usually asked to estimate the overall taste intensity of solutions of tastant, alone or in mixture with odour compounds. The problem with this methodology is to dissociate a real perceptual effect from a response bias, the halo-dumping effect

(Clark and Lawless, 1994, Chem. Senses, 19: 538–594). We propose another task to study taste–odour interactions that eliminates the halo-dumping effect problem. This task is based on the filtering paradigm proposed by Gardner (1974, The Processing of Information and Structure. Wiley, New York), in which assessors are asked to categorize the stimuli rather than to judge their intensity. This paradigm is based on the following principle: if two dimensions (e.g. shape and colour) are separable, the performance at a categorization task based on one dimension (e.g. shape) is not affected by variations on the other dimension (colour).

Four solutions, prepared in water, were made by a factorial combination of two sucrose concentrations (S1 and S2) and two concentrations of vanilla (V1 and V2). In the filtering condition, assessors received a series of these solutions (S1V1, S1V2, S2V1, S2V2) and were asked to categorize each one according to its sweetness (low or high intensity). In the two control conditions, the vanilla concentration remained constant over the test: a series of S1V1/S2V1 solutions were tested in one session and a series of S1V2/S2V2 solutions in another one. Fourteen assessors performed 60 judgements based on sweetness and 60 judgements based on vanilla odour, in both experimental conditions.

For both vanilla and sweetness categorizations, the proportion of correct responses is lower in the filtering condition than in the control conditions, indicating that these two dimensions are not perceptually independent. A replication of the experiment with acid citric/citrus flavour mixtures, yields similar results. Results gave evidences of a real perceptual taste–odour interaction for these couples of stimuli.

254. Lateralization in olfaction: affection and cognition

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In the literature on olfaction an effect of which nostril is used to smell an odour is sometimes reported (Herz *et al.*, 1999, Chem. Senses, 24: 691–695). A lateralization effect could be due to a difference between the two brain halves, a prevailing hypothesis being that the left one is predominantly active during analytical tasks, here naming and recognizing odours, whereas the right one is more involved with affective processing (Schwarz *et al.*, 1975, Science, 190: 286–288), in our case assessing the pleasantness of odours. We wanted to study if the potential lateralization of affective and cognitive processing in olfaction can be studied by psychophysical means.

We collected data on 52 subjects and 16 odours spanning the whole hedonic range from very unpleasant to very pleasant. Eight of our odours were the same as those used elsewhere (Herz *et al.*, 1999). For each of the 16 odours, the experimenter presented a jar containing the odour to the subject for ~2 s. Subjects then scored the hedonic value, the perceived intensity and the familiarity of the odour, as well as providing a name for the odour. This procedure was administered for each of the 16 odours and each nostril twice over four sessions on four different days. At the last session we also asked subjects to report on the name of the odour in a 4AFC task containing the correct odour as one of the alternatives. Finally, we assessed the handedness of the subjects by means of the Edinburgh Inventory (Oldfield, 1971, Neuropsychologia, 9: 97–113).

We found results similar to those published by Herz *et al.* (1999), who suggested that odours are rated as more pleasant when sniffed through the right nostril, potentially indicating laterality in the processing of affective information. Our data suggest that this might be the case, but an ANOVA only shows significance at the 0.10 level. For both left-handers and right-handers, the right nostril rates odours as more pleasant than the left nostril, but again at a weak level of significance. Taken together, the data on the right and left nostrils demonstrate significantly that right-handers rate odours as more pleasant than left-handers. Right-handers also significantly perceive the odours as more intense than left-handers.

Our data might suggest that the laterality effect proposed be Herz *et al.* might be a consequence of differences in perceived intensity. This in turn could be a laterality effect or could be a result of the physical intensity provided to the nostrils. This question can only be settled by the use of carefully controlled olfactometers. Finally, the lack of any lateralization effect between affective and cognitive olfactory tasks does not necessarily imply that the brain is not functionally organized in this fashion. Signals might be passed over the corpus callosum before the task at hand is performed. We are at present performing human imaging experiments to further elucidate this problem.

255. Influence of match of name and odour on perception

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We increasingly believe that to understand olfactory function it is necessary to understand the contribution of experience to odour perception. Here we present two sets of findings which demonstrate that in humans knowledge of the odour source may have profound effects on judgements of intensity, pleasantness, and familiarity.

In the first experiment, subjects ($n = 2 \times 36$) were presented in a cross-over design with 2×12 everyday odorants in sniff bottles. For half of the odorants no information was provided, and for the other half the name of the odorant was given. Providing the name significantly enhanced rating scores for intensity, pleasantness and familiarity ($P \leq 0.0025$). Subjects were also asked to identify the odour when no name was given, or in the second condition, to rate how well the odour fitted to the name provided. If subjects were able to spontaneously identify the odour source (41% of presentations without the name), increased ratings were also found ($P \leq 0.001$). When odours were judged not to fit the name provided (59% of presentations with name), they were judged to be less intense, less pleasant and less familiar than when name and odour were perceived to match ($P \leq 0.0001$).

In the second experiment, currently in progress, subjects ($n = 48$) were presented with 3×4 pairs of related fruit juices in sniff bottles such that each subject received two of the stimuli labelled with the correct name, two with the name of the related juice and two

with the name of a quite different juice. In a first round of testing, subjects had to rate the intensity of the odours, and in a second round, how well the odours fitted to the names provided. Not surprisingly, when the correct or related name was provided almost 60% of the subjects thought it to fit to the odour, in contrast to 25% of subjects when the names of unrelated juices were provided. As in the experiment above, rating of intensity was correlated significantly with the match between odour name and perception, i.e. the better the match the higher intensity rating. Thus, deliberately introducing a mismatch confirmed the importance of sensory and cognitive interactions in odor perception.

256. Association of sweet, salty, sour and bitter tastes with autonomic estimated basic emotions

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The affective-emotional dimension of the gustative sensation plays a crucial role in the control of many taste-mediated responses related to food ingestion or rejection. Subjective methods have been developed in attempts to estimate the hedonic component of taste, but they imply a cognitive analysis which cannot faithfully translate the spontaneous emotional response preceding cortical integration. The purpose of this study was to present an original objective method of evaluation of the emotional gustative sensation component through the association of each primary taste (sweet, salty, sour and bitter) with autonomic estimated basic emotions. Thirty-four healthy non-smoker volunteer subjects (17 males, 17 females, mean age = 28 years) participated in the experiment. Taste stimuli were solutions of 0.3 M sucrose (sweet), 0.15 M NaCl (salty), 0.02 M citric acid (sour) and 0.00015 M quinine sulfate (bitter). Evian mineral water served as the diluent and control (neutral taste). Five autonomic parameters (skin potential and resistance, skin blood flow and temperature, instantaneous heart rate) were simultaneously and continuously recorded when subjects tasted the five solutions. The patterns of autonomic responses, obtained for each primary taste and each subject, were transcribed into one of the six basic emotions defined by Ekman *et al.* (1983, Science, 221: 1208–1210) (happiness, surprise, sadness, fear, anger and disgust), according to a method previously described with odorants (Alaoui-Ismaïli *et al.*, 1997, Physiol. Behav., 62: 713–720; Vernet-Maury *et al.*, 1999, J. Autonom. Nerv. Syst., 75: 176–183). The results showed a significant effect of taste solutions on the distribution of the basic emotions ($P < 0.0001$), which can be related to taste preferences: the innate-accepted sweet solution was mainly associated with happiness and surprise and the innate-rejected bitter solution with anger and disgust. Salty and sour solutions were associated with more various basic emotions, with a majority of disgust for salty and anger for sour. In conclusion, the use of this objective method allowed to associate each primary taste with autonomic estimated basic emotions. This procedure could provide references for the hedonic analysis of the more complex flavors of foods.

257. Are pleasant and unpleasant odours processed in the same way?

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The study is focused on the hedonic aspect of odour processing. Our hypotheses were: (i) that the hedonic judgement of odours differs from the intensity judgement, the former requiring a more cognitive involvement than the latter perceptual task; (ii) that the cognitive system would be more efficient in responding to bad odours than to neutral or pleasant ones; and (iii) the right nostril would manifest an advantage.

Sixty-four subjects (volunteers) participated in the experiment. They were divided in two groups. Odours were presented in the left nostril for the first group, and in the right nostril for the second group. The subjects had to perform four tasks: detection, intensity, hedonic and familiarity judgements. For each subject, the experimenter presented 12 odours (four pleasant, four neutral and four unpleasant). The order of presentation of odours and tasks were counterbalanced according to a Latin square.

A three-way ANOVA showed an effect of the task ($P < 0.05$) and an effect of the affective tone (pleasant, neutral or unpleasant) of odours ($P < 0.05$). However, no effect of the side stimulated was shown ($P > 0.05$). Comparison between means, using Student's *t*-test, showed that detection and intensity did not differ. Moreover, reaction times for detection and intensity were shorter than for hedonic and familiarity judgement ($P < 0.05$). Hedonic and familiarity judgements were also different: the first required less time than the last ($P < 0.05$).

Finally, pair comparisons showed that responses for unpleasant odours on the one hand and neutral or pleasant odours on the other hand were different.

Thus, the hedonic and intensity judgements should be considered as different, and do not differ according to the side stimulated. Furthermore, it seems that unpleasant odours on the one hand, and pleasant or neutral odours on the other hand are processed differently.

258. Olfactory lateralization based upon intensity cues

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When an odorant is being administered into one nostril and a blank into the other simultaneously, one detects the odor, but cannot accurately indicate the side. Subjects were unable to learn the side of odor stimulation when provided feedback information on actual lateralization. One subject, however, could fulfil the task due to a deviation of his nasal septum, which induced a difference in the subjective intensity of equal concentrations of stimuli acting through both nostrils. We have attempted to reproduce the underlying principle in experiments on healthy subjects. We have used phenyl ethyl alcohol (PEA, a 'pure' odorant acting through the olfactory epithelium only, and not stimulating trigeminal endings) at two concentrations—strong and weak. Either one was administered into one nostril, with an odorless blank administered

into the other simultaneously. Both the strong PEA versus blank and the weak PEA versus blank combinations were presented repetitively, with the side of odor administration and the sequence of different stimuli randomized. We confirmed that lateralization of simultaneous stimuli was impossible. In the next step, a similar series of stimulus combinations was administered with the only difference being that the higher concentration of PEA was always administered on the same side (e.g. right), whereas the lower concentration of PEA was given to the opposite side. The subjects were not informed about this. They were, however, told that they do have a chance to discover the 'trick' underlying lateralization. Few subjects were successful in this task. However, after being told about the relationship between odor intensity and lateralization, all the subjects were able to learn to lateralize the pure odorant by making use of the intensity clue. Measurements concerning the minimal intensity difference allowing lateralization indicated that the ability of intensity discrimination increased during the experiment. When repeating the completely randomized version, where the subjects were deprived of any clue, laterality judgements again became completely random. The experiments demonstrated that it was possible to learn to lateralize pure olfactory stimuli when subjects were able to rely upon a predictable, intensity-based, cognitive clue.

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259. Effects of training and task on the perception of binary odour mixtures

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Little is understood about how individual odours combine in mixtures and, in particular, what determines whether an odour mixture is perceived synthetically as a distinct complex odour or analytically as a mixture of discrete odours. During training in descriptive analysis to assess the sensory qualities of foods and beverages, panelists are required to respond analytically to complex sensory stimuli. We investigated the impact of such strategies on the perception of binary odour mixtures. Separate groups of trained and untrained panelists undertook a non-analytical sorting task involving two odours at three concentrations, as well as their binary mixtures. These data were compared with the results of a descriptive analysis of these same stimuli by the trained panel. For the trained panel, multidimensional scaling (MDS) maps (sorting task) and principal component analysis maps (descriptive analysis) showed great similarity: the unmixed compounds were associated with the relevant main axes, and the binary mixtures occupied the intervening space. However, the MDS map for the untrained panelists showed little similarity to either map derived from the trained panel data, and there was little evidence for a systematic organization underlying the perception of the different samples.

Because the trained panelists had received prior training with the compounds that were used in the experiment, this may have increased their tendency towards analytical evaluation of the mixtures, even when a non-analytical method was employed. To determine if this could account for the results, the trained panel subsequently performed the non-analytical sorting task with two unfamiliar odours and their mixtures. The resulting MDS map showed a very similar organization to the map obtained with the original mixtures, suggesting that familiarity with the odours was not a factor in their analytical approach. We conclude that the differences between the trained and untrained panels in evaluating the odour mixtures are indicative of the difference between a synthetic perceptual framework and an analytical perceptual framework, and that the training of a descriptive panel predisposes the panelists towards treating the samples in an analytical manner, regardless of the nature of the task. In contrast, the untrained panelists' data imply that some synthetic processes are occurring.

260. The effect of odour, hue, saturation and form of an object on the perceived odour intensity

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Gilbert *et al.* (1996) found synaesthesias between colours and odours. This led to the hypothesis that there are combinations of hue and odour with special effects on perceived odour intensity (DuBose *et al.*, 1980). Zellner and Kautz (1990), however, found no hue-specific changes of perceived odour intensity. The increase of perceived odour intensity existed for every hue. These results led to the assumption that saturation, and possibly the form of an object together with the type of odour, can have an influence on perceived odour intensity.

In our experiment subjects had to rate odour intensity of bananas and strawberries (pieces of decor). There were 24 conditions. The pieces of decor were coloured yellow or red with one of two saturation values. Each object was treated with one of three different odours (strawberry, banana, neutral). Subjects had to rate odour intensity on a scale from 0 to 10 relative to the same amount of odorant presented on a piece of paper before of the first trial.

Computations excluded the neutral odour condition in order to reduce error variance. Contrary to the hypotheses most of the conditions decreased perceived odour intensity with respect to the comparison standard, where the odorants were concentrated on a very small area. The result was a main effect of form and hue. The effect of form is that the smell of banana forms is stronger than the smell on strawberry forms. This may be caused by a reduced recognizability of the strawberry forms as compared with the banana forms. The main effect of hue indicates that red objects smell stronger than yellow objects. The reason may be that red is associated with the ripeness of fruits. The interaction of form and odour was significant. The interaction of form and hue just missed significance. The effect size, however, is not small.

Taking into account the covariates sex, smoking, alcohol consumption, pill, menstruation cycle or caffeine consumption, statistical significance and effect sizes change rather strong. This means that these variables influence the perceived odour intensity and the data are rather unstable. we thus conclude that in further studies more subjects are needed and a more precise control of the odour stimuli is necessary.

261. Influencing the achievement in a test of intelligence through odours

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Baron (1990) found that pleasant odours could influence achievements. The theory is based on the assumption that the

odours induce a positive emotional state, which is the reason for the increase in achievement. Further, Ellis and Ashbrook (1988) described an resource allocation model of the effects of negative mood on cognitive achievement.

In the experiment it should be shown that the achievement in the CFT-3 test of Cattell and Weiß (1971) could be increased by a positive odour (mint), decreased by a negative one (skatole) and also decreased by an appetite-sharpening odour (strawberry). In addition to a part of the CFT-3 and a few emotion and motivation scales (happiness, relaxation, disgust, hunger, thirst,) a part of the d2 (Brickenkamp, 1972)—an attention test—was presented in a repeated measures design. Odour was placed on a cotton bud, which was stuck to the nose, so that the odour was 2 cm in front of the nose. The results show no effects of the odours on the achievement in the CFT-3. Also, the achievement in the d2 was not influenced significantly. There was a significant effect on relaxation, disgust and thirst.

In summary, we can say that the odours changed the emotional and motivational state. This had no measurable influence on the intelligence achievement. In further studies the d2 should be run in full to get greater differences, alongside another situative, more dependent intelligence test. Fluid intelligence (Cattell, 1971) does not seem to be influenced by odours, but perhaps crystallized intelligence is.

262. Investigation between cognition and agreeable concentration of taste substances in human

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It is well known that human taste is affected by the concentration of taste substances. There seem to be differences between individuals, related to the concentration at difference threshold and terminal threshold, in cognition of taste; for concentrations of taste substances higher than the terminal threshold, humans often complain of discomfort at that taste concentration. The author tried to investigate the relationship between cognition and agreeable concentration of taste substances.

Subjects were seven males and three females, average age 24.1 years (SD = 1.01), who had no illness and without smoking habits, feeling comfortably full after a meal. Before the investigation subjects were given an explanation, and they agreed with the aim of this investigation. The method of gustatory test utilized was the whole mouth method that was often used in clinics; the order of taste substances was first sucrose for sweetness, next was NaCl for saltness, tartaric acid for sourness and finally quinine for bitterness; and only when changing taste could subjects rinse their mouth. The substances were dropped above the center of subject's tongue, from low concentration of taste to high in five steps, and subjects were asked to identify the taste during 3 s of taste stimuli. At the same time, they were asked whether the taste concentration was agreeable or not in five steps (step 1, not agreeable; step 2, less agreeable; step 3, neither; step 4, more agreeable; step 5, agreeable).

The result was that all subjects with quinine scored 'not agreeable (step 5)' for each concentration greater than terminal threshold. With the other three taste substances, there were differences between individuals. With NaCl and tartaric acid, in higher concentration than terminal threshold, subjects complained more. With sucrose, there were two groups that complained about agreeable or not agreeable taste.

The author reached a conclusion that there was resemblance in taste cognition in agreeable taste concentration steps between saltness and sourness, there were differences between individuals for sweetness, and there were no differences between individuals for bitterness. The clinical gustatory test was sensuality inspection, an agreeable taste concentration showed that it needed the measurement of feeling together with taste stimuli in gustatory test, and it was proposed to develop a new clinical gustatory test or method especially for sweetness.

263. Effects of wine aroma on relaxation. II. Quantitative EEG data analysis

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The aroma of wine can induce relaxation in human brain activity. The aroma effects of white and red wines were evaluated on brain activity using quantitative electroencephalography (EEG) analysis from normal female subjects.

Twenty healthy right-handed female volunteers were recruited from the local community. The subjects were exposed to eight aroma samples; three red wines (Cabernet Sauvignon, Merlot, Concord), three white wines (Chardonnnay, Sauvignon Blanc, Muscat), 12% (w/w) ethanol solution and distilled water (DW). EEG activity was measured for 3.5 min after exposure of each aroma sample, with a 5 min interval. A frequency power spectrum was analysed on selected EEG waves for 30 s from each session. The relative power spectrum was calculated for six frequency bands (8–9, 9–10, 10–11, 11–12, 12–13, 13–14 Hz) from each sample relative to those from DW at 12 electrode sites. Repeated measurement ANOVA (Sample and Electrodes as within factors) was applied with the relative power on each frequency band for statistic analyses.

Significant main effect of sample was found in all frequency bands, except 11–12 Hz. Follow-up ANOVA suggested that Chardonnay and Concord significantly increased power for the 9–10 and 10–11 Hz bands compared with ethanol solution. In addition, ethanol solution did not change the power spectrum compared with DW in any frequency bands.

Wine aroma increased brain activity for the 9–11 Hz frequency band. These findings suggest that the aroma of wine has relaxatory effects.

264. How easy is it to speak the unspeakable? Using verbal information to describe wines and faces

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It has long be known that the world of smells is difficult to pin down in words. When ask to describe an odour, subjects generally generate hedonic or idiosyncratic descriptions. The difficulty in expressing smells in language has been attributed to physiological

and evolutionary causes. The olfactory brain structure, being an old structure, would have developed only weak connections with the more recent brain structures involved in higher cognitive processes such as language. Another plausible explanation is that this difficulty is not limited to smell but applies to the more general class of non-verbal perceptual stimuli, such as faces, music or painting. These stimuli have the particularity of being learned through repeated exposure and without explicit learning instruction. They are holistically perceived, and their verbal description is not essential for everyday life.

To test these alternative hypotheses, we realized a series of experiments in which we compared the ability of subjects to describe two types of complex perceptual stimuli: wine odours and facial images. The quality of the descriptions was assessed by asking other subjects to match them to the original wines and faces. To vary the difficulty of the task, the similarity of the stimuli within each set was manipulated across experiments, as well as the expertise of the subjects with the stimuli.

In all experimental conditions, non-wine-experts' matching performance was significantly greater than chance level for faces, but not for wines. This difference in performance decreases with expertise: wine experts' matching performance was above chance level for both types of stimuli but remained higher for faces than for wines. The vocabulary used to describe faces is more precise, various and extended than that used to describe wines. A plausible explanation for the difference between faces and wines is that subjects are more familiar with faces than they are with wine. To test this hypothesis, we are currently evaluating the effect of perceptual familiarity on wine descriptions.

265. The effects of learning strategy on perfume classification

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Thirty-four subjects (28 women, six men, aged 26.0 ± 7.3 years, mean ± SD) responded to an invitation to take part in a free perfumery course. They all received general information about perfume composition (top, heart and base notes) and then were divided into two main groups (A and B). Two different learning strategies were used: *prototype learning*, in which they worked with four perfumes from only one perfume class during two sessions and then with four perfumes of another class during two sessions, etc., and c*lass difference learning*, in which they received one perfume from each of four different classes in each of eight sessions. In the first part of the experiment four relatively simple perfume classes (floral, green, citrus tobacco, leather) and in the second part four relatively complex perfume classes (chypre, fougère, aldehyde, oriental) were studied. Group A started with prototype learning for the simple perfume classes and then was submitted to class difference learning for the complex perfume classes. For group B the order of the learning strategies was reversed. The effects of the learning strategies on odor classification were tested by asking the subjects to classify perfumes at three moments: after the first eight lessons, five known and five unknown (not presented during learning) simple perfumes; after the second eight lessons, five known and five unknown complex perfumes; and a week later, eight known and eight unknown perfumes from all eight classes. The results showed that for the relatively simple classes the results obtained with the two methods did not differ, but that when classification of the more complex perfumes was involved the class difference method was superior to prototype learning.

266. The mediating role of odours on cardiovascular responses in a computer-simulated driving task

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Previous research has shown that odour may exert an influence on the task-demands of a given situation, such as vigilance (Warm *et al.*, 1991, J. Soc. Cosmet. Chem., 42: 199–210), cognition [Knasko, 1997, Int. J. Aromather., 8(3): 28–33] and social interaction (Baron, 1997, Aromachol. Rev., 6: 3–11). In particular, evidence suggests that the 'arousing' nature of peppermint may play a mediating role, improving concentration and enhancing performance. The experiment reported here investigated the effect of olfactory stimuli on performance in a computer-simulated driving task.

Heart rate was monitored whilst subjects were randomly assigned to one of four groups and exposed to the intermittent odour of peppermint, lavender or 'malodour', or to a 'no-odour' control. Subjects were provided with brief instruction in a driving-related computer game (TOCA Touring Cars). After a period of orientation with the game and its controls, subjects were required to complete a three-lap 'time-trial'. Task performance was reflected in 'lap times' and these were correlated with cardiovascular responses. It was hypothesized that task performance would increase in the presence of a peppermint odour relative to a lavender odour. It was further hypothesized that performance would diminish in the presence of a malodour.

Findings are discussed in terms of the mediating influence of odours on task performance as reflected in error rate and cardiovascular responsivity. Implications of odorizing vehicular environments are discussed in the light of these findings.

267. Characterization of the perception of the malodours butyric acid and *N*-valeric acid in human subjects

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The characteristics of the psychological perception of the malodours butyric acid and *n*-valeric acid were studied by olfactometry. The odours were delivered to the nostrils via a Teflon canula in a continuous airstream with a total flow rate of 3 l/min. The duration of a random number of odour pulses was set by computer-controlled solenoid valves to last for 35, 50, 75, 100 and 200 ms with inter-stimulus intervals of 2.5, 5, 10 or 60 s. Subjects were required to indicate the number of pulses they could detect. The results showed that the number of odour pulses detected increased with increasing the pulse duration or inter-stimulus interval. 3D curve fitting with an exponential function revealed that the perception of odour (the percentage of odour pulses detected) was positively correlated to the concentration (pulse duration) and the inter-stimulus interval in both odours tested. However, more interesting phenomenon were revealed by analysing

the data in terms of gender. The perception of *n*-valeric acid was different between male and female subjects. The perception was correlated both to the concentration of the odour and the inter-stimulus interval in male subjects, while it was only correlated to the inter-stimulus interval in females. As for butyric acid, there was no significant gender difference in the correlation of perception with the concentration and inter-stimulus interval. Furthermore, the threshold of the perception of both odours was higher in male than in female subjects. The results suggest that there are different perceptual models for different odours and that, for certain malodours, women are more sensitive than men.

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268. Olfactory receptor potentials in humans

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The electro-olfactogram (EOG) is considered to be the summated generator potential of the olfactory receptor cells and therefore represents the peripheral olfactory events. The aim of the present investigation was to study EOGs in healthy human subjects. Fourteen volunteers participated in the study. Electrical activity of the olfactory epithelium was recorded with a Ag/AgCl electrode (0.8 mm dia) positioned in the olfactory cleft, in response to amyl acetate delivered by an olfactometer. EOGs were recorded in 11 out of 14 subjects. No responses were obtained when control air was used or when the electrode was positioned in the nasal respiratory mucosa. Three different patterns of responses were obtained from the olfactory epithelium. A negative electrical potential was recorded in 9/22 (40.9%) recordings. A second type of electro-negative on-off-EOG was found in 2/22 (9.09%) recordings. The third pattern was a positive potential recorded in 4/22 (18.18%) cases. These wave forms appeared after a latency of 40–154 ms (mean ± SE = 77 ± 10.36 ms). The variable polarity of these olfactory potentials needs further clarification. Similar findings were observed by Takagi and colleagues in frogs, who classified these EOGs into five fundamental forms (Takagi, 1989, Human Olfaction. University of Tokyo Press, Tokyo, Chapter V, pp. 147–187), and suggested that the positive potentials originated from the supporting cells whereas the simple negative potential represented the activated olfactory receptor cells. Further studies are being conducted to test these ideas.

269. Influences of estrateraenol and androstadienone on the behaviour in an initial encounter

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'Flirt signals' are a part of the human courtship behaviour, and have been defined, for example, by Moore (1985, Ethol. Sociobiol., 6: 237–247) and Tramitz (1992, Auf den ersten Blick. Econ.). The signals consist of several behavioural elements, such as turning of the head or the movements of the hands. This study investigates whether estrateraenol and androstadienone have an influence on the behaviour of men and women during their first encounter. One man and one women were invited to take part in a visual discrimination test (never met before). One was prepared in the experimental room while the other was prepared in another room and afterwards led into the experimental room. The subjects were initially unable to see each other because of a parting curtain. Both were sitting on a chair. In the treatment group estratetraenol was applied to the cheek of the man and androstadienone to the cheek of the women (explained by a cover story). The control received the solvent. The experimenters removed the parting curtain and left the room. With the removal the data collection started. The two subjects were videotaped for 10 min waiting for the visual discrimination test. After the test a questionnaire was administered. It included hidden questions about how they assessed the other and what they would like to do. The subjects were informed about the video and the aim of the study. Data from subjects were analysed only when they had given their permission; otherwise the data were deleted. The tapes were analysed concerning body positions, movements, flirtation signals and speech. A total of 120 men and 120 women took part in the study. Men in the treatment group would take the initiative to make contact more often ($P < 0.004$) and would feel hurt ($P < 0.000$) if they were rejected. Women in the treatment group would, for example, go to the movies with the men more often ($P < 0.005$). Women in the control group would show more body manipulations ($P < 0.007$), laugh out loud more ($P < 0.000$) and turn their head more often to the men ($P < 0.0.05$). However, the women and men in the treatment group expressed more coy smiles (flirt signal) ($P < 0.01$ and $P < 0.008$, respectively).

270. Do human infants have a distinctive body odour?

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It was the aim of the present study to investigate whether human neonates emit a distinctive odour which can be distinguished by adults from the odour of older children. A mixed group of both parents ($n = 45$; 23 women) and non-parents ($n = 51$; 26 women) was tested in a three-choice test in which a T-shirt worn for one night by an infant (<4 weeks, $n = 24$; 16 girls) should be distinguished from a similar shirt worn by an older child (1.5–4 years, $n = 11$; 6 girls) and from an unused control shirt.

Subjects showed a statistically significant (χ^2; $P < 0.001$) ability to distinguish the shirt that had been worn by a neonate from the older child's or the control shirt. Furthermore, when the data were divided according to parents and non-parents, both categories showed a significant ability to discriminate and identify the neonates' shirts (χ^2; $P < 0.001$ and $P < 0.05$, respectively). Despite a tendency for parents to perform better, this was not significant. Whether data of parents and non-parents were divided according to sex, male parents showed the highest scores, followed by non-parent males. All subjects together ranked the odour of neonates as significantly more intense than the odour of older children or of the controls shirts ($P < 0.001$). This sensory

evaluation was supported by GC- and GC-MS analysis. The GC samples of shirts (n = 3/group) worn by neonates contained a greater number of compounds and at higher concentrations than samples of older children's and control shirts. Of the 63 compounds identified, 16 were common to the samples from all three groups and two were common only to neonates and older children, whereas 13 and 19 were found only in the sample from neonates and older children, respectively.

These results support earlier studies showing that neonates and older children differ in skin lipid composition and glandular activity. Thus, the present results suggest that human infants may indeed have a distinctive body odour which adults can distinguish from that of older children.

271. Subjective pleasantness of an ambient odor varies according to the task

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The important role of associations between chemical senses and biologically (or emotionally) significant events has widely been emphasized (Rolls, 1999, The Brain and Emotion. Oxford University Press, Oxford), but has been demonstrated mainly on tastes (Logue *et al.*, 1981, Behav. Res. Ther., 19: 319–333). This study was aimed to clarify the process that subjective pleasantness of an odor would be varied by accompanied tasks. Twelve and 11 subjects were respectively assigned to a stressful task group (SG) or a non-stress task group (NSG). Subjects attended two sessions, 4–14 days apart. Trimethylundecylenic aldehyde (TUA) was selected as an appropriate odor from a previous odor conditioning study (Kirk-Smith *et al.*, 1983, Biol. Psychol., 17: 221–231). TUA was presented during tasks for both groups and in both sessions. In the first session, subjects in SG were asked to perform a series of 120 trials of a 7-digit memory and recall task with feedback of every performance. They were given a false instruction to maintain their motivation and to raise their stress level. On the other hand, subjects in NSG were asked to watch an animated video for children in the first session. During the second session, each subject of two groups performed the same photograph rating task at his/her own pace. All tasks took ~20 min. Dependent variables were pleasantness of the odor, mood and ratings of photographs. An experimenter presented TUA to a subject using a perfumer's paper smelling strip. The brief version of the Profile of Mood States (POMS) was filled in by subjects before (pre-task) and after tasks (post-task). An ANOVA of 2 (groups) × 2 (sessions) × 2 (pre/post-task) showed a parsimonious interaction of groups and pre/post-tasks. The pre-task pleasantness of the odor at the second session was affected by the content of the first session. A post-hoc analysis (Tukey's HSD) showed that the odor was perceived significantly more unpleasant for SG than for NSG at post-task of the second session ($P < 0.05$) and also for SG at post-task of the first session than for NSG at post-task of the second session ($P < 0.05$). An ANOVA of the sub-scales of POMS revealed several significant main effects and interactions. Moreover, the post-hoc analysis on two sub-scales, namely 'tension-anxiety' and 'confusion', revealed that those scores were rated higher by SG than by NSG at the post-task of the second session. In conclusion, this study revealed that subjective perceived pleasantness of an odor varied due to accompanied tasks. These change of odor pleasantness were concurrently with the change of mood which may indicate conditioning effects.

272. Serial position effects words and figures but not flavours

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Cognitive models of remembering have been based upon retrieval of words, and less commonly abstract visual figures. Recent studies have suggested that these models are either inaccurate in describing memory for taste and smell, or that the different senses requires such diverse methodological paradigms that direct comparisons become impossible. Interest had recently focused upon the serial-position effect—a characteristic of short-term memory whereby items early in a list (the primacy effect) and late in a list (the recency effect) are better retrieved than those in the middle of the list. Recent research (Anett and Lorimer, 1995, Percept. Motor Skills, 81: 787–794; White and Treisman, 1997, Br. J. Psychol., 88: 459–472) has obtained evidence that the primacy component of the serial position curve for odours may be mediated by the extent to which the items are subject to verbal labelling.

Two studies are described in which participants tasted a small set of flavours (12 or 9), and were exposed to a similar sized set of abstract visual items (Experiment 1) and a set of real and nonsense words (Experiment 2). For the flavours and figures, half the participants are required to generate names for the stimuli and half are not. Following the study task, participants are given a recognition memory task for randomly sampled items from the start, middle and end of the study list.

The results suggested the expected serial position effects for real but not nonsense words, and for labelled figures, but no primacy effects for any flavour list. Labelling did not influence overall recognition rate for flavours, and did not interact with serial position.

These findings are interpreted as supporting the view that verbal labelling is necessary, but may not be sufficient for producing serial position effects in non-verbal stimuli.

273. Examining hemispheric lateralization in odour perception and naming

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Considerable energy has been expended in studying the ways in which brain functions have been lateralized. In a study conducted by Herz *et al.* (1999, Chem. Senses, 24: 691–695), evidence was found for hemispheric lateralization of odour perception and naming. Subjects who smelled odours through their left nostrils named odours correctly more often than subjects who smelled odours through their right nostrils. Subjects inhaling through their right nostrils gave higher ratings for pleasantness. This experiment sought to examine and extrapolate the findings of Herz *et al.* (1999) by conducting a similar study with a between-subject design and by expanding rating scales to describe more hedonic features

of odours as well as ratings of familiarity and intensity. Naming was found to be significantly superior with subjects in the left nostril condition, confirming the findings of Herz *et al.* However, ratings for pleasantness were not higher with right nostril subjects. This experiment therefore provides supportive evidence for some degree of lateralization. However, more investigation is necessary.

274. Pheromone binding protein of *Sesamia nonagrioides*

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Chemical senses have since long been recognized as being of paramount importance. Terrestrial animals share some similar characteristics as an adaptive result to air. The presence of odorant binding proteins (OBP) in both terrestrial vertebrates and invertebrates and their absence in fish supports this adaptive hypothesis (Nagnan-Le Meillour *et al.*, 1996, Insect Biochem. Mol. Biol., 26: 59–67). In insects the OBPs are divided into the pheromone binding proteins (PBP) and the general odorant binding proteins (GOBP). In moths, PBPs are mainly localized to the lymph of the sensilla trichodea (organs responsible for pheromone perception) of the male antennae (Hansson, 1995 Experientia, 51: 1003–1027). On the other hand, GOBPs are found in both male and female antennae, and are mainly localized in sensilla that are believed to be responding to plant odors and other environmental chemicals.

The first PBP to be discovered in insects was that of *Antheraea polyphemus* (Vogt and Riddiford, 1981, Nature, 293: 161–163). Later other PBPs were found in other lepidopterans: *A. pernyi*, *Hyalophora cecropia*, *Bombyx mori*, *Manduca sexta*, *Lymantria dispar* and *Orgya pseudotsugata*. These PBPs exhibited similar tissue specificity and physico-chemical properties to the PBP of *A. polyphemus*, as, for example, a molecular weight near 15 kDa. PBPs should be able to: (i) solubilize the hydrophobic pheromone components in the sensillar lymph; (ii) transport the odor through the sensillar lymph and mediate its delivery to the specific receptor located in the dentritic membrane; and (ii) protect the pheromonal compound against the sensillar degrading enzyme (Nagnan-Le Meillour *et al.*, 1996).

Here we present evidence on the presence of PBP-like protein in the corn stalk borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae). Western analysis, using an antibody raised against the PBP1 of the male *L. dispar* (Vogt *et al.*, 1989, J. Neurosci., 9: 3332–3346), has revealed the presence of a near 15 kDa protein in the antennae homogenates of 2-day-old moths. This expression also seems to be tissue specific. Currently, binding experiments after tritiation of (*Z*)-11-hexadecenyl acetate, the major compound of the insect pheromone, are being carried out.

The corn stalk borer is major pest for maize crops. Extensive knowledge of the mode of pheromone perception for this pest apart from its basic significance may result in the development of novel approaches for control of this pest.

275. Elementary electrical events in moth olfactory cells: a three-state model of the receptor mechanism suffices

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In response to low pheromone concentration, pheromone-sensitive olfactory cells in *Bombyx* and *Antheraea* species generate spike activity which is accompanied by discrete 'bumps' of small amplitude and a few milliseconds duration. Bursts of such bumps are frequently observed. These electrical events are called elementary receptor potentials (ERP) (Kaissling and Thorson, 1980, in Sattelle *et al.*, eds, Receptors for Transmitters, Hormones and Pheromones in Insects. Elsevier, Amsterdam, pp. 261–282; Kaissling, 1994, in Kurihara *et al.*, eds, International Symposium on Olfaction and Taste XI. Springer, Tokyo, pp. 812–815). One bump reflects a transient depolarization of a receptor cell, which presumably results from the activation of one or several ion channels after a pheromone molecule is bound to a receptor molecule on the membrane of the receptor-cell dendrite. The durations of single bumps and their bursts look like random events, and can vary over a wide range, from a few to >100 ms. In the present study elementary receptor potentials (ERPs) and elementary receptor currents (ERCs, under transepithelial voltage clamp) were recorded from open tips of pheromone-sensitive sensilla in males of the silkmoth *Bombyx mori*.

Burst, bump and gap durations and the number of bumps in a burst were measured in the bombykal-sensitive receptor cells. It was found that the probability density functions (PDF) of bump duration and gap duration can be approximated by single exponentials, with time constants of ~6–10 ms for the bumps and 40–50 ms for the gaps. The PDF of burst duration is a combination of two exponentials, where a shorter and a longer time constant are in the range of 10–20 and 150–200 ms, their relative contributions being ~85 and 15%, respectively. The number of bumps per burst followed a geometrical distribution.

These data can be explained by means of the model of the olfactory receptor excitation (Kaissling, 1998, Chem. Senses, 23: 385–395) if a third ('excited') state of the receptor molecule is added. The bump and gap PDFs were used to calculate the expected parameters of the burst PDF which were found compatible with those observed experimentally. This agreement seems to support the suggested three-state receptor mechanism of bump generation in the insect olfactory cells.

We suggest that the excited state activates a step-like increase of concentration of the hypothetical messenger molecules. For computer simulation the characteristics of the pheromone-sensitive ion channels in *Antheraea polyphemus* (Zufall and Hatt, 1991, Proc. Natl Acad. Sci USA, 88: 8520–8524) were used. The bumps were sucessfully simulated by simultaneous activation of a small number (on the order of 10) of such channels. The parameters found experimentally were used to calculate the rate constants for transitions between the three states of the receptor molecule. For $r_{31} < r_{21}$ we find $r_{21} = 7.9$/s, $r_{23} = 16.8$/s and $_{32} = 98$/s.

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276. Excitation and inhibition by linalool in olfactory receptor cells of *Bombyx mori*

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In both sexes of the silkmoth *Bombyx mori* the antennae bear a large number of long trichoid sensilla, each holding a pair of olfactory receptor cells. In males each cell of the pair is excited by one of the sex pheromone components bombykol and bombykal, while in females one is best excited by 2,6-dimethyl-5-hepten-2-ol and the other best by benzoic acid. The cell most sensitive to 2,6-dimethyl-5-hepten-2-ol also responds to the plant volatile linalool (3,7-dimethyl-1,6-octadiene-3-ol). Linalool has been shown to inhibit responses of pheromone receptor cells in the male, though it can also excite some of these cells. In this study we compared the specificity of excitation by linalool in the female with the specificity of inhibition in the male. We tested linalool and 14 structurally related compounds with more or with fewer carbon atoms, with the double bonds in a different position or absent, and with the hydroxyl group replaced by a ketone or amine. We found that the specificity for excitation in the female differs from the specificity for inhibition in the male. The molecular sites of action of linalool must therefore differ in the two sexes. In the male a stimulus of linalool by itself did not hyperpolarize the cell but, when applied as a background odour, it did decrease the amplitude of the receptor potential in response to a pheromone stimulus. We hypothesize it acts on the pheromone receptor directly; the mode of inhibition is unclear. Interestingly, the receptor cells in the female had similar thresholds for the three most stimulating compounds tested (2,6-dimethyl-5-hepten-2-ol, linalool and sulcatol) but differed significantly in their response amplitudes at higher stimulus intensities. This will be discussed.

277. Plant odour detection by heliothine moths: signal receptor behaviour

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A major type of plant odour receptor neurons responding specifically to germacrene D has been identified in the American tobacco budworm moth *Heliothis virescens* (Røstelien *et al.*, 2000, Chem. Senses, 25: 141–148). The neuron occurred in 80% of all experiments of gas chromatography linked to electrophysiological recordings from single cells (GC-SCR). The same technique has been used to study receptor neurons in the related Eurasian species, *Helicoverpa armigera*, demonstrating the presence of the same receptor neuron type. In both species these neurons showed a secondary response to another sesquiterpene (unidentified) present in a concentrated non-host material of spruce sawdust. Responses to germacrene D were obtained when testing headspace volatiles from a large variety of plants, both hosts and non-hosts including sunflower, tomato, corn and spruce. Germacrene D was also found in commercial turpentine pulp (TMP) and in the sesquiterpene fraction (*Piper cubeba*) of the pepper plant *P. nigera*. These were used as sources for isolating germacrene D by several parallel separations through medium pressure liquid chromatography (MPLC) and finally isolated by preparative GC. The purity of the product was tested by GC-MS and NMR. This isolation procedure demonstrated the instability of germacrene D. The isolated product was used in behavioural tests of mated *H. virescens* and *H. armigera* females. The significance of germacrene D as a biological plant odorant, indicated by the large number of neurons evolved for detection of this compound, was evaluated in behavioural studies.

278. A comparison of the sexual dimorphism in the olfactory system of two phylogenetically distant honey bee species

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In *Apis mellifera*, drones and workers show a conspicuous dimorphism in the number of olfactory sensilla and in glomerular organization of the antennal lobes. Drones have more olfactory poreplate sensilla on their antennae than workers and four macroglomeruli (MG) in their antennal lobes (Esslen and Kaissling, 1976, Zoomorphology, 83: 227–251; Arnold *et al.*, 1985, Cell Tissue Res., 242: 593–605; Brockmann, 1999, Doctoral Thesis, University of Bremen; Galicia *et al.*, 1999, Cell Tissue Res., 295: 383–394; Brockmann *et al.*, in preparation). EAG recordings indicate that in drones the number of the receptor neurons sensitive to 9-ODA, the main component of the queens mandibular pheromone, is increased (Brockmann *et al.*, 1998, Naturwissenschaften, 85: 283–285). We compared the antennae and the antennal lobe organization in the smaller and distantly related *A. florea* with *A. mellifera*. The antennae of *A. florea* drones and workers (Gupta, 1992, Apidologie, 23: 47–56) are much smaller and bear less sensilla than those of *A. mellifera*, and the sexual dimorphism of the antennae is much less pronounced (see table). In the drone antennal lobes we identified two MGs, which occupy regions homologous to those occupied by the MG 1 and MG 2 in *A. mellifera*, but are remarkably smaller in volume.

	Flagellum length (mm)	No. of poreplates[a]	No. of glomeruli	MG-volume ($\times 10^3$ µm^3)
A. florea				
Worker	1.97	~60	150	
Drone	1.97	~140	90 + 2 MGs	MG1 44.3 MG2 77.3
A. mellifera				
Worker	2.4	~220	170	
Drone	3.9	~1300	100 + 4 MGs	MG1 593 MG2 1414.1

[a]On the dorsal surface of one flagellar segment.

The reduction of sensilla number and the decrease in glomerular volume indicate a reduced olfactory perception in *A. florea*

compared with *A. mellifera*. On the other hand, the elongation of the drone antenna in *A. mellifera* facilitates the detection of smaller amounts of pheromone and thus increases the pheromone detection range. The comparison of the olfactory system of both species promises to give insight into the question of what happens to olfactory systems and perception when the sensory input diminishes or increases.

279. Novel types of antennal lobe neurons characterized in the female tobacco budworm moth *Heliothis virescens*

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The olfactory system of the female heliothine moths includes receptor neurons responding to plant odours and antennal lobe neurons with dendritic arborizations in 62–65 ordinary glomeruli. A pheromone system, similar to that of males, is not present. However, the antennal lobe of the *Heliothis virescens* female includes two large glomeruli located at the entrance of the antennal nerve, corresponding to the position of the macroglomerular complex involved in the processing of pheromone information in males. In the present study intracellular recordings were carried out in combination with stainings and confocal microscopy recontructions. Out of several successfully stained interneurons, three showed a particular morphology by directly connecting the two antennal lobes. These bilateral neurons targeted olfactory glomeruli in homotopic areas via the axon following the antennal commissure. One of the neurons arborized also outside the antennal lobes, in the area of the ventro-lateral protocerebrum (the optical foci). Furthermore, the results showed the presence of local and projection interneurons of types similar to those previously reported in males. The local interneurons had extensive arborizations in nearly all glomeruli and cell body in the lateral cell cluster. Projection neurons of the uniglomerular type followed the inner antenno-cerebral tract to the calyces of the mushroom bodies and the lateral protocerebrum. The cell bodies of this neuron type appeared in all three cell clusters. One multiglomerular projection neuron projected via the outer antenno-cerebral tract to the lateral protocerebrum. In addition, a ventrally located branch targeted the ventro-lateral protocerebrum. The cell body of this neuron was located anteriorly in the medial part of the antennal lobe.

The responses to odour stimulation of the antennae were recorded as excitation (depolarization and firing of action potentials) as well as inhibition by all neuron types. Strong hyperpolarization was frequently recorded to stimulation with leaf vapour of some plant species. Response characteristics of the antennal lobe neurons to stimulation with single odorants and headspace volatiles are under further investigation.

280. Mapping responses in the antennal lobe of the moth heliothis virescens using calcium imaging

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Receptor neurons responding selectively to insect and plant produced compounds have been identified in the tobacco budworm moth *Heliothis virescens*. In the males, four sex-specific neuron types are classified, each projecting in one compartment of the macroglomerular complex (MGC) of the antennal lobe (AL). Neurons responding selectively to the pheromone components A (*cis*-11-hexadecenal) and B (*cis*-9-tetradecenal) projected into the cumulus and dorso-medial compartment, respectively, whereas the neurons responding to interspecific signals projected into the two ventral compartments. In the females, several plant odour receptor neuron types have been identified, each responding to one or two mono- and sesquiterpenes compounds. In this study, we used *in vivo* calcium imaging in order to measure the responses of olfactory glomeruli. We found that in the male moth stimulation with the pheromone component A and B leads to activation in the area of the cumulus and the dorso-medial compartments. Only one ventral compartment was visible in the recordings; this glomerulus was activated by an interspecific signal. No responses to the insect-produced compounds were found in the females. Stimulation with the plant odours did not elicit responses in the MGC area of males. Plant odours elicited activity in the 'ordinary' glomeruli. In both sexes, different glomeruli were activated by the various odorants and blends, showing an across-glomeruli code. In a cluster analysis of the evoked patterns, ocimen and *trans*-β-myrcene, which activate the same receptor neuron type, appeared as separate clusters but direct neighbours.

These data confirm and extend electrophysiological results from pheromone receptors and AL projection neurons, showing that pheromone information is coded in a combinatorial manner, with each glomerulus corresponding to one information channel. The results further suggest that the principle for coding plant odour information in the AL is also based on a functional organization of the ordinary glomeruli, where specific glomeruli are activated by a particular compound, similar to what has been shown in other species.

281. Calcium imaging of glomerular properties of output neurons in the honey bee antennal lobe

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Odours are represented by specific ensembles of activated glomeruli in the olfactory bulb of vertebrates or the antennal lobe (AL) of insects. Within the glomeruli the olfactory receptor neurons (ORN) are connected through local interneurons (LIN) to the olfactory projection neurons (PN), which represent the output of the AL. In previous studies, using calcium imaging in the honey bee *in vivo*, we studied the glomerular activity as a 'compound' signal of all cell types (ORNs, LINs, PNs with a probable emphasis on ORNs) in the AL (Galizia *et al.*, 1999, Nature Neurosci., 2: 473–478; Sachse *et al.*, 1999, Eur. J. Neurosci., 11: 3970–3982). We addressed here the question of how the olfactory code is modified by the throughput of the AL. We therefore measured the calcium responses of selectively stained PNs to five different odours. Furthermore, we mapped these responses to identified glomeruli.

As for the compound signal, we also found the glomerular pattern of PNs to be odour specific and conserved within the species *Apis mellifera*. Furthermore, there is a high correlation between the compound spatial activity and the spatial patterns measured when selectively staining PNs, though the latter appear sharpened. The temporal properties of the PN responses can be classified as excitatory or inhibitory responses (including

stimulus-off responses). These properties are odour specific and are also conserved between individuals: for example, glomerulus T1–24 reveals an inhibitory response for 1-hexanol and an excitation for 1-octanol and 1-nonanol, whereas glomerulus T1–38 is inhibited by 1-hexanol, is also inhibited by 1-octanol in half of the animals measured but excited in the other half, and is activated by 1-nonanol, and glomerulus T1–35 shows an excitatory response for 1-hexanol and an inhibition for 1-octanol and 1-nonanol. Note that these glomeruli show oppositional responses for chemically related odours. However, glomerulus T1–29 reveals an inhibitory response for all three odours, but an excitation for isoamylacetate.

In order to analyse the influence of GABAergic LINs of the PNs, we applied GABA and picrotoxin. GABA totally abolishes the calcium signal in the AL, whereas picrotoxin changes both temporal and spatial aspects of the odour responses. These pharmacological treatments provide us with information about the AL network.

282. Pheromone and odorant binding proteins of *Sesamia nonagriodes*

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The odorant-binding proteins (OBPs) studied so far in moths appear to fall into two different classes according to their expression in cells located in pheromone- or general-odorant-sensitive sensillae (Du and Prestwich, 1995, Biochemistry, 34: 8726–8732). Pheromone-binding proteins are expressed in the sensillum lymph of Lepidoptera and play an important role in the perception of pheromones (Krieger *et al.*, 1991, Biochim. Biophys. Acta, 1088: 277–284). Studies in different insect species are useful to search for the presence of related proteins, and to study their diversity and/or compound specificity among other species. In the context of molecular recognition of the hydrophobic compounds, we searched for PBP/OBP sequences in the antennae of *Sesamia nonagriodes*, a corn borer insect.

Molecular cloning was based on the use of degenerated primers designed for *Mamestra brassicae* GOBP/PBP (Maibeche-Coisne *et al.*, 1998, Insect Biochem. Mol. Biol., 28; 815–818) and *Lymantria dispar* PBP (Merritt *et al.*, 1998, J. Mol. Evol., 46: 272–276) sequences using DNA isolated from *S. nonagriodes* antennas. Sense primer sequences were deduced from amino acid of the N-terminal and antisense primer from published lepidopteran PBP/GOBP sequences. The PCR amplification fragments produced were directly sequenced on an ABI 310 Genetic Analyser, using Big-Dye Terminator cycle sequencing. Sequences with similarities to previously published PBP/GOBP were used to design new *S. nonagriodes* sequence-specific primers. These primers were then used as sense primers for RT-PCR with oligo $(dT)_{12–18}$ as antisense primers on total RNA isolated from frozen (–70°C) antennas. The produced fragments were subsequently cloned into the *EcoRV* site of the pGEM-T vector. Recombinant plasmids were subjected to sequencing analysis and sequencing results of one PCR fragment revealed 47% (79/167 bp) sequence identity with *M. brassicae* PBP2 (Maibeche-Coisne *et al.*, 1998) and 39% (65/167 bp) with *L. dispar* PBP1 (Merritt *et al.*, 1998).

The above results based on sequence similarities indicate that the cloned PCR fragment contain specific PBP sequences of *S. nonagriodes* and can be used as probe for screening purposes of antennal cDNA libraries to obtain full-length sequences.

283. The effects of amiloride on the labellar taste receptor cells of the fleshfly *Boettcherisca peregrina*

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Amiloride is known to inhibit the taste response of vertebrates to salt by blocking the amiloride-sensitive sodium channel (Lindemann, 1996, Physiol. Rev., 76: 719–766). In this study, we investigated electrophysiologically the effect of amiloride on the taste response of an invertebrate, the fleshfly *Boettcherisca peregrina*. When 0.5 mM amiloride was included in taste solutions, the response of the salt receptor cell (salt response) to sodium chloride (NaCl) was not depressed but those of the sugar receptor cell (sugar responses) to sucrose, glucose, fructose, L-valine (L-Val) and L-phenylalanine (L-Phe) were strongly depressed. These stimulants are typical ligands specific for each of the four receptor sites of the sugar receptor cell: sucrose and glucose react specifically with pyranose site; and fructose, L-Val and L-Phe react with the furanose, alkyl and aryl sites, respectively (Shimada, 1987, Chem. Senses, 12: 235–244). The concentration–response relationship for sucrose revealed that an inhibitory effect of amiloride was dependent on stimulus concentrations. After pretreatment of a chemosensory seta with 0.15 mM amiloride for 10 min, the salt response to NaCl was not affected compared with that before the treatment. On the other hand, the sugar responses to sucrose, fructose, L-Val and L-Phe were depressed just after amiloride treatment. While the sugar response to adenosine 5′-diphosphate (ADP) mixed with 0.5 mM amiloride was not depressed, the response to ADP alone was depressed after the amiloride treatment. After all, amiloride equally affects all the sugar responses regardless of receptor site types. The effects of amiloride on the taste response of the fly are therefore rather different from those of vertebrates.

284. Plant odour receptor neurons with narrowly tuning and non-overlapping response spectra in the tobacco budworm moth *Heliothis virescens*

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Females of the herbivorous heliothine moths seem to use plant volatiles as cues for locating the host. By the use of gas chromatography linked to electrophysiological recordings from single cells (GC-SCR), the objectives were to identify which of the hundreds of naturally produced plant chemicals can be detected by the sensory organs of the females. In these studies of the tobacco budwoorm moth *Heliothis virescens*, it was identified one major plant odour receptor neuron type 1, occurring in 80% of all recordings, which responded to a sesquiterpene identified as germacrene D (Røstelien *et al.*, 2000, Chem. Senses, 25). In addition, 12 types of less frequently occurring plant odour neurons have been classified accoring to the compound(s) they respond to, e.g. *E*-β-ocimene and β-myrcene (type 2), *E*,*E*-α-farnesene (type 3)

and homo-farnesene (type 4). Some of these neuron types showed weaker responses to one or two additional compounds. In cases where several components activated one neuron type, the chemical structures of the effective compounds were similar. Furthermore, no overlaps were found between response spectra of different neuron types. So far, the overall impression is that the plant odour receptor neurons in heliothine moths are as narrowly tuned as the pheromone receptor neurons, indicating that this kind of plant odour information is mediated to the brain by a labelled-line mechanism.

285. Divergence of pheromone blends and olfactory processing in heliothine moths

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Synaptic interactions between peripheral olfactory receptors and central interneurons occur within glomeruli, characteristic neuroanatomical structures of the primary olfactory neuropil across widely divergent taxa. In all moth species examined to date, males possess a sexually dimorphic glomerular region of the antennal lobe that is dedicated to processing olfactory information about the female sex pheromone. This area of the antennal lobe is known as the macroglomerular complex (MGC). The numbers and spatial arrangement of the glomeruli in the MGC vary extensively across species, but generally a large glomerulus [labeled the cumulus (Hansson *et al.*, 1991, J. Comp. Neurol., 312: 264–278; Vickers *et al.*, 1998, J. Comp. Neurol., 400: 35–56) owing to its complex appearance] dominates and is surrounded by smaller, satellite glomeruli. Data from several species suggest that the main component of a specific pheromone blend is processed within the cumulus. In *Heliothis virescens*, the MGC is comprised of four glomeruli which have a characteristic spatial arrangement recognizable across individuals. Recording and staining of projection interneurons that innervate each of these glomeruli have established that the two components of the female sex pheromone blend necessary for upwind flight are represented by activity in at least two of the glomeruli. Compounds that inhibit upwind flight activate PNs within a third glomerulus.

Phylogenetic studies of the genus *Heliothis* have revealed that *Heliothis subflexa* is most closely related to *H. virescens* (Cho *et al.*, 1995, Mol. Biol. Evol., 12: 650–656; Fang *et al.*, 1997, Syst. Biol., 46: 269–283). In wind tunnel tests we have determined that male *H. subflexa* utilize the same major pheromonal component (Z11–16:Ald) as *H. virescens* but a distinctive set of secondary pheromone components. The spatial arrangement of MGC glomeruli in these two species is, however, identical. We report here on the physiological and morphological characteristics of projection interneurons found to arborize in glomerular compartments of the *H. subflexa* male MGC. As in *H. virescens*, Z11–16:Ald activates PNs located in the cumulus but similarly positioned satellite glomeruli now process information about the distinctive secondary components of the *H. subflexa* pheromone blend. These results suggest that divergence in the blend emitted by females and accompanying changes in the specificities of olfactory receptor and central interneurons has occurred before any alteration in the spatial relationships between MGC glomeruli.

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286. Olfactory responses of Kenyon cells in the mushroom body of the cockroach *Periplaneta americana*

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The possible roles of the insect mushroom body in the control of complex motor repertories and in associative olfactory learning are suggested. Physiological and morphological evidence has indicated that intrinsic interneurons (Kenyon cells) of the mushroom body receive inputs from the projection neurons of antennal lobe. The mechanisms of olfactory processing of Kenyon cells, however, are still little understood. In this study we applied a whole-cell current-clamp recording method to the soma of Kenyon cell in the mushroom body of the cockroach *Periplaneta americana in vivo* and examined their physiological characteristics and activities while stimulating the antennae with six odours. The average resting membrane potential of Kenyon cells was 64.7 ± 6.9 mV (n = 28). All Kenyon cells (n = 121) showed action potentials during prolonged depolarizing current injection. Fifty-six of these Kenyon cells were tested with food and female feces odour, and alcoholic stimuli (1-octen-3-ol, 1-octanol, 1-hexanol and 1-heptanol). These odorants elicited regular subthreshold, depolarizing postsynaptic potentials (EPSPs) and sometimes action potentials upon suprathreshold EPSPs. Almost all Kenyon cells responded to some of the alcoholic odours, female feces and food odours; some responded to all six odorants. The response patterns of these cells are odour- as well as neuron-specific. Kenyon cells responded to 1-octen-3-ol strongly, and the amplitudes of EPSPs and the number the action potentials depended on the concentration. The results suggest that Kenyon cells of the mushroom body probably receive different combinations of converging input from the projection neurons of the antennal lobe to different odours.

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287. Sex-specific non-pheromonal taste receptors in *Drosophila*

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Sex differences in chemosensory capacities in insects have previously been directly linked to pheromone detection. In *Drosophila melanogaster*, there is a clear sexual dimorphism for the number

and central projections of tarsal taste sensilla (Possidente and Murphey, 1988, Devl Biol., 132: 448–457). Using a limited set of non-pheromonal stimuli (salts, sugar, water), we were able to discriminate three types of tarsal sensilla in females and only two types in males. The female-specific type, which responds to sugar in a specific manner, is absent in males, except when male gustatory neurons were genetically feminized. [Genetic feminization was performed under the control of PGal4-Voila (Balakivera *et al.*, 1998, J. Neurosci., 18: 4335–4343), an enhancer-trap strain expressing Gal4 in the peripheral gustatory nervous system of the fly.] The fact that tarsal gustatory hairs exhibit a sexual dimorphism that affects the perception of non-pheromonal compounds suggests that sexual identity is more complex than had previously been thought.

288. Ultrastructure of mitral/tufted cell synapses in the glomerular region of the salamander olfactory bulb

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Synaptic interactions in the glomerular layer (GL) and external plexiform layer (EPL) of the olfactory bulb shape the spatio-temporal patterns of mitral/tufted (M/T) output cell responses that encode odor features. Interest in the glomerular synaptic interactions has recently been renewed by electrophysiological recordings showing that in rat slice preparations, prolonged depolarization often follows the initial M/T cell response, apparently due to recurrent excitation in the GL (see Carlson *et al.*, 2000, J. Neurosci., 20: 2011–2021). With fluorescence imaging methods, a prolonged depolarization that attains the maximal amplitude in the juxtaglomerular EPL has also been observed in intact salamander preparations (Cinelli *et al.*, 1995, J. Neurophysiol., 73: 2053–2071). During preliminary studies to begin quantifying the glomerular synapses of the salamander (*Ambystoma* spp.) olfactory bulb, we have identified synaptic arrangements that could shape both the initial response patterning and prolonged depolarization of the M/T cells.

Dendritic profiles of M/T cells were readily identified by their parallel arrays of microtubules and predominantly spherical vesicles. Gray's type 1 (excitatory-type) and type 2 (inhibitory-type) synapses were also readily identified using established criteria. The dendritic profiles received type 1 synapses from olfactory nerve (ON) axon terminals and type 2 synapses from periglomerular (PG) cell dendrites and axons, and they formed type 1 synapses onto PG cell dendrites (Allen and Hamilton, 1998, Chem. Senses, 23: 554). These synaptic arrangements presumably shape the initial patterning of the M/T cell responses. In both the GL and juxtaglomerular EPL, however, the dendritic profiles also formed type 1 synapses with each other (Allen and Hamilton, 2000, Brain Res., 860: 170–173). These synapses could be a source of prolonged M/T cell depolarization, and they could serve to amplify or to synchronize neighboring M/T cell responses. Because the pre- and post-synaptic profiles could be derived from different M/T cell types (Kosaka *et al.*, 1998, Neurosci. Res., 30: 101–110), additional studies are required to determine if the net effect of activating the synapses is in fact excitatory.

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289. Mistargeting of primary olfactory axons—where do the axons go?

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The convergence of all primary olfactory axons expressing the same odorant receptor to topographically fixed glomeruli in the mature olfactory bulb has led to the suggestion that axon navigation occurs without error in this pathway. However, this is certainly not the case during embryonic and early postnatal development. Primary olfactory axons do not always target their appropriate topographical position, but instead over-shoot into inappropriate areas (Tenne-Brown and Key, 1999, J. Comp. Neurol., 410: 20–30). Primary olfactory axons are guided to their appropriate topographical positions by a combination of molecules which allows the axons to project to the olfactory bulb, defasciculate in the nerve fibre layer, refasciculate and project to their appropriate region before homing in on their target glomerulus. While many primary olfactory axons do find their correct target, others appear to become lost in the olfactory bulb. We postulate that primary olfactory axons that overshoot the glomerular layer are confined to the immediate vicinity of their target glomerulus as a result of a combination of both chemo-attractive and chemorepulsive guidance signals. To gain insight into the extent of mistargeting of primary olfactory axons in mice we studied the trajectory of axons that extended beyond the glomerular layer of the olfactory bulb. Two strains of wild-type mice, C57BL/6 and BALB/c, as well as two lines of transgenic mice, P2-IRES-tau-LacZ mice and heterozygous OMP-tau-lacZ (Mombaerts *et al.*, 1996, Cell, 87: 675–686), were examined. Primary olfactory axons were identified by immunohistochemistry using antibodies against OMP and by LacZ enzyme histochemical staining. We found that primary olfactory axons that projected beyond the glomerular layer were not restricted to a small region, but often projected vast distances throughout the external plexiform and granule cell layers. Over-projecting axons were detected at all ages in immature animals, although with increasing age the number of axons decreased. However, even in the adult mouse primary olfactory axons were detected in the external plexiform layer. These results indicate that for at least some primary olfactory axons the guidance signals are insufficient to enable the axons to target their appropriate glomerulus or the surrounding neuropil.

290. Pattern of vomeronasal nerve projections in mammalian accessory olfactory bulb

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Mammalian vomeronasal (VN) systems are essential for the pheromone reception and concerned with manifestation of various

reproductive and social behaviors by influencing the neuroendocrine system of the recipient animal. Pheromonal information is received by VN neurons in the VN organ, and then transmitted to the accessory olfactory bulb (AOB) via the VN nerves, which terminate on the dendrites of mitral/tufted cells in the glomerulus. Rodent AOBs have a segregated projection pattern of the VN neurons, which express two alpha-subtypes of the G protein, namely Gi2 and Go, to the rostral and caudal regions of the AOB, respectively. Although this segregated pattern was considered as a common feature for mammals, we showed that goat VN systems had a different pattern from those of rodents (Takigami *et al.*, 2000, Chem. Senses, 25: 387–393). In the present study, we extended our previous study to examine AOBs of several other mammalian species (horse, musk shrew, dog and marmoset) immunohistochemically by using antibodies to Gi2 and Go.

In the horse, musk shrew, dog and marmoset, the Gi2-immunoreactivities were found in the VN nerve and glomerulus layer throughout AOBs, whereas the Go-immunoreactivities were observed on the glomerulus, mitral/tufted cell and granule cell layers. Thus only Gi2-expressing VN nerves terminated in the AOBs and no Go-immunoreactivities were found in the VN nerve layers of the AOBs. This 'uniform pattern' of the VN projections was the same pattern that observed in the goats.

These results suggest that in most mammalian AOBs the VN nerve projections are not the segregated pattern as seen in rodents. In mammalian species there are at least two types of VN systems (segregated and uniform pattern), which may be related to morphological differences of the VN organs or to the ecological diversity among these species.

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291. A combination of two male urinary components induces region specific Fos-expression in rat accessory olfactory bulb

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The vomeronasal organ exists in many vertebrates for receiving pheromones related to sexual and social behavior (Keverne *et al.*, 1986, Chem. Senses, 11: 119–133; Halpern, 1987, Annu. Rev. Neurosci., 10: 325–362). In female rats, pheromones in urine excreted from males and females induce various changes in gonadal functions, such as reflex ovulation (Johns *et al.*, 1978, Nature, 272: 446–448) and a reduction in the oestrous cycle of female rats (Chateau *et al.*, 1976, Acta Endocrinol., 82: 426–435). These results suggest that urine contains plural pheromones. However, no study has been carried out to explore the molecular properties of pheromones contained in rat urine. In the present study, vomeronasal organs of female Wistar rats were exposed with sprayed urine preparations of male Wistar rats prior to sacrifice. Exposure to diluted urine preparations induced Fos expression, which is correlated with cellular activity, in the mitral/tufted cell layer of the accessory olfactory bulb (AOB) in a dose-dependent manner. Exposure to urine preparation treated with papain induced expression of Fos-immunoreactive cells in the rostral region of the AOB, but did not induce such expression in the caudal region (Tsujikawa and Kashiwayanagi, 1999, Biochem. Biophys. Res. Commun., 260: 222–224). Exposure to urine preparation treated with pronase induced urine-specific Fos-immunoreactivity neither in the rostral nor in the caudal region. These results suggest that at least two different peptides carrying pheromonal activities are contained in male Wistar rat urine. Exposure to the remaining substances after dialysis (>100 Da) induced a significant expression of Fos-immunoreactive (Fos-ir) cells, while exposure to the dialyzed urine preparation (<100 Da) did not. Exposure to ultrafiltrated urine preparation (<5000 Da) induced significant Fos expression, while exposure to the remaining substances after the ultrafiltration (>5000 Da) did not. Exposure to either the dialyzed urine preparation (<500 Da) or the remaining substances (>500 Da) did not induce expression of Fos-ir cells, whereas exposure to a mixture of these preparation did induce expression. These results suggest that a combination of a low molecular weight substance (<500 Da and >100 Da) and a high molecular weight substance (>500 Da) is necessary for the increases in Fos-immunoreactivity in the AOB.

292. Hyperpolarization-activated cation current (I_h) in hamster vomeronasal receptor cells

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In a previous study (Trotier and Døving, 1996, Prim. Sensory Neuron, 1: 245–261) we demonstrated that frog vomeronasal receptor neurons express a cation current I_h that is activated upon membrane hyperpolarization and plays an essential role in setting the resting membrane potential of these cells (Trotier and Døving, 1996, J. Physiol., 490: 611–621). In these cells the membrane potential (~–80 mV) is essentially set by the sum of the hyperpolarizing sodium pump current and the depolarizing current I_h.

We recently recorded hamster vomeronasal receptor cells. In the whole cell configuration the current I_h was observed in a number of cells. I_h confered a strong inward rectification to the current–voltage curve negative to –60 mV. The time constant of activation was voltage-dependent, the current kinetics being accelerated with hyperpolarization. Once activated the current did not inactivate. The current was blocked by 5 mM external Cs^+. The I_h current increases with increasing potassium concentration in the extracellular environment.

In current clamp conditions, hyperpolarizing current pulses activated I_h and reduced the amplitude of the hyperpolarization. At cessation of the hyperpolarization a depolarizing rebound elicited an action potential.

The role of I_h in hamster vomeronasal receptor cell is discussed.

293. Patch-clamp recordings of hamster vomeronasal receptor neurons

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We recorded hamster vomeronasal sensory neurons bearing a long dendrite and numerous microvilli. In many whole-cell recordings the membrane potential presented spontaneous jumps between the resting level near –60 mV and the firing threshold (near –45 mV).

These transitions were caused by inward current waves presenting a slow kinetics of activation adapted to the time constant of integration of the cell membrane. Many of these spontaneous short depolarizations triggered solitary action potentials or, in most cases, action potentials grouped in bursts. The current clamp configuration demonstrated a high input cell resistance and indicated that depolarizing current pulses of <4 pA shifted the resting potential to the firing threshold. This suggests that small transductive currents are efficient to trigger spiking.

To test whether this activity was endogenous or induced by the whole-cell configuration, we developed a method to estimate the resting intracellular potential and to follow its spontaneous fluctuations in the cell-attached patch clamp configuration, which does not interfere with the cytosol composition. Spontaneous depolarization waves were recorded as outward current waves flowing through the patched membrane. Some of these events triggered single action potential or short bursts of action potentials. The frequency of these events greatly varied among cells, some of them being almost silent during 1 h recordings whereas others elicited more or less frequent and short depolarizing waves, triggering bursts of action potentials, separated by silent periods. Although the instantaneous frequency of firing within the bursts was high, the mean frequency of firing of active cells over long periods was low. The analysis of the interburst interval suggested that the duration of the interburst intervals was a random variable.

Therefore both methods of recording detected the existence of an endogenous mechanism that opens depolarizing channels, depolarizes the membrane and triggers short bursts of action potentials. The existence of this endogeneous activity complicated the definition of significant responses elicited by natural ligands. The effect of extracellular lanthanium, a blocker of transient receptor potential channels (suspected to be involved in the transduction process in these neurons), as well as intracellular injections of GTPγ S, cAMP and IP_3 (an intracellular messenger involved in G protein coupled activation of vomeronasal neurons by native aphrodisin), was examined.

Suprathreshold depolarizing current pulses evoked a repetitive firing in some cells. Many other cells responded only by an initial short burst of action potentials. The relationship between the frequency of firing and the depolarizing current intensity was elusive. These observations suggest that the frequency of short bursts of action potential may be a significant signal.

These experiments help to understand how hamster vomeronasal receptor cells detect natural ligands and send the information to the mitral cells in the accessory olfactory bulb.

294. Co-expression of putative pheromone receptors in the sensory neurons of the vomeronasal organ

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Two large and divergent families of G protein coupled receptors (V1Rs and V2Rs) are expressed in subsets of cells in the vomeronasal organ. These receptors are likely to mediate pheromone responses, but it appears that many V2R genes may encode expressed pseudogenes rather than functional proteins. We have raised antibodies to the extracellular domains of representative V2Rs. V2R immunoreactivity was detected at the sensory surface of the vomeronasal organ in dendritic terminals, indicating that these V2R genes encode expressed receptors that are capable of directly interacting with pheromones and mediating physiological responses. Immunohistochemistry confirmed that three V2R receptors are expressed in small subsets of sensory neurons. However, very surprisingly we found that one V2R is broadly expressed in the Gαo-layer of the vomeronasal organ and is co-expressed in the same cells as other V2Rs. Therefore our results demonstrate that in the VNO, all Gαo-positive neurons express >1 V2R. This is in direct contrast to the main olfactory epithelium where sensory neurons express only a single receptor. The situation is probably equivalent to that observed in the goldfish olfactory neurons that express receptors related to the rodent V2Rs. Specifically it has been shown that a goldfish receptor, R5.24, which responds to basic aminoacid, is expressed in a large subset of olfactory neurons while a large number of related receptors are found in small subset of cells. It might be argued that this broadly expressed V2R (and also the goldfish receptor 5.24) is functionally distinct from the other V2Rs and that its roles is distinct. In part, this is supported by sequence analysis that places this receptor as an outlying member of the V2R gene family closer to fish olfactory receptors than to other V2Rs. This might suggest functional similarity between thisV2R and the fish receptor 5.24. An alternative hypothesis is that V2Rs, as for the GABA-b receptors, necessitate heterodimerization to elicit their function.

In conclusion, our data suggest that another mode of information processing may occur in the olfactory systems.

295. Molecular mechanisms of chemosensory transduction in the vomeronasal organ

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Chemosensory neurons in the vomeronasal organ (VNO) detect pheromones related to social and reproductive behavior in most terrestrial vertebrates. Current evidence indicates that the chemoelectrical transduction process in the VNO is mediated by G protein-coupled second messenger cascades. Our results indicate that stimulation of female rat vomeronasal organ microvillar preparations with male rat urine induce a rapid and transient IP_3 signal. In order to identify the G protein-subtypes which mediate PLC activation, G protein specific antibodies were employed to interfere with IP_3 formation induced upon urine stimulation; furthermore, in photoaffinity labelling experiments, the identity of receptor-activated G proteins was analyzed using [α-^{32}P]GTP azidoanilide followed by immunoprecipitation of the labelled G protein α-subunits. Both experimental approaches indicate that stimulation of female VNO membrane preparations with male urine induce activation of G_i- as well as G_o-subtypes. Employing different fractions of urine revealed that upon stimulation with lipophilic volatile odorants, only G_i proteins were activated, whereas G_o activation was elicited by the lipocalin α_{2u}-globulin, a major urinary protein (Krieger *et al.*, 1999, J. Biol. Chem., 274: 4655–4662).

Activation of PLC by pertussis toxin-sensitive G protein-subtypes, like G_i and G_o, appears to be mediated by βγ-subunits of

the trimeric G protein; therefore we speculated whether the simultaneously released α-subunit of G_i and G_o may affect other pathways. Since one classical function of G_i is the inhibition of adenylyl cyclase, attempts were made to characterize cAMP signaling in the VNO. These experiments revealed that male urinary components not only induce a rapid IP_3 signal in female VNO preparations, but in addition the concentration in cAMP decreases with a delayed and sustained time course. Since the urine-induced IP_3 formation and the decrease in cAMP did not occur simultaneously, our data suggest that the decrease in cAMP levels may not be mediated by $G\alpha_i$ but rather seems to be the consequence of the preceding activation of the phosphoinositol pathway (Rössler *et al.*, 200, Chem. Senses, 25: 313–322).

296. Perceptual learning of odor mixtures and their components

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Various studies have indicated that animals are capable either of responding to mixtures of odorants (lobsters: Livermore *et al.*, 1997) and tastants (rats: Rescorla *et al.*, 1985; Staubli *et al.*, 1987) as a unique configuration or, alternatively, they can respond to the individual elements of that mixture. However, it is not clear whether this change in responding reflects a change in perception of the stimulus, as is implied, or merely a change in the way that the elements of the mixture are classified by the animal. This is an important question as it has strong implications for theories of configural learning, perceptual learning and neural coding of olfactory mixtures. The reported studies explored this question. Odorant stimuli were composed of single chemicals, which are common constituents of food and wines, diluted in propylene glycol and presented at equivalent perceived intensity in sniffing bottles. Experiment 1 used 40 participants. The discrimination training phase consisted of 24 forced-choice triangular similarity judgements based on varying combinations of the stimuli. Group 1 was presented with the two mixtures ABC (+/– carvone, phenyl-ethyl alcohol, nerol) ABD (carvone, phenyl-ethyl alcohol, linalool) and geraniol. Group 2 received the mixture components AB, C and D. The next day participants were asked to make pairwise similarity judgements between the two mixtures and their components. In the third phase participants rated these same stimuli on a series of perceptual dimensions. Irrespective of the analysis (MDS, discriminant analysis, cluster analysis, *t*-test), the results supported the hypotheses and indicated that in G1 the mixture elements were perceived as being significantly more similar than they were in G2 but that discriminating elements (C andD) were significantly less similar in G1. Results are interpreted in light of models of perceptual learning (for a recent review see Goldstone, 1998). It is suggested that constant presentation of the mixtures (G1) creates strong inhibitory links between the discriminating components (C and D), emphasizing their discriminating features and decreasing their similarity. In contrast, common features (AB) are processed less deeply and so are less well discriminated from other components (C and D). Alternatives are considered and possible neural mechanisms underlying this process have been identified in the olfactory system. A second study, using two mixtures without common elements, was undertaken to further test this hypothesis.

297. Discriminability of fat content as a function of PROP sensitivity

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Variability in the degree to which individuals perceive the compound 6-*n*-propylthiouracil (PROP) to be bitter have also been found to be associated with differences in the perception of oral sensations other than tastes. Increasing sensitivity to PROP is associated with increased intensity of oral pungency (Prescott and Swain-Campbell, 2000, Chem. Senses, 25: 239–246), increased creaminess in foods (Tepper and Nurse, 1997, Physiol. Behav., 61: 949–954) and greater tactile sensations produced by oils and fats (Prutkin *et al.*, 1998, Proc. AChemS XX). A number of different sensory properties contribute to perceptions of creaminess in foods, including odour and mouthfeel characteristics such as viscosity and fat content. Tactile sensations produced by the relative density of fat globules contribute to creaminess perception independently of viscosity (Richardson and Booth, 1993, Acta Psychol., 84: 93–101). The present study investigated whether differential sensitivity to variations in fat content was the basis for differences in creaminess perception by different PROP taster groups.

Subjects were classified as non-tasters, medium tasters and supertasters based on their ratings of the intensity of a 0.032 M PROP solution using the labelled magnitude scale (Green *et al.*, 1993, Chem. Senses, 18: 683–702). Subjects undertook a signal detection discriminability task in which samples of milk containing 1.15, 1.9, 2.65 and 3.4% fat were compared with a reference sample containing 0.4% fat. All samples had approximately equivalent viscosity. Olfactory and visual differences between samples were eliminated by the use of nose clips and red light.

Overall, discriminability increased with increasing fat levels in the samples. Particularly at higher fat levels, supertasters showed increased dÕ values relative to medium- and non-tasters. These data suggest that differences in ratings of creaminess as a function of PROP taster group are mediated by fat content. Since sensitivity to PROP is positively correlated with the density of fungiform papillae, which is likely to be associated with greater number of lingual nerve fibres, such differences may be due to differences in the density of oral mechanoreceptors between the taster groups.

298. Comparison between two new sensory descriptive methods: Flash Profile and Comparative Free Commentary

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The aim of this study was to compare the efficiency between two fast sensory descriptive methods: Flash Profile (FP) and Comparative Free Commentary (CFC).

FP is derived from Free Choice Profiling (Williams and Langron,1984, J. Food Sci. Agric., 35: 558–568) and is based on individual description associated to product ranking. The whole product set is presented simultaneously (Sieffermann, 1995,

Doctoral Thesis, ENSIA, Massy). Thus, each expert panelist produces his own attributes according to the major differences he perceives among products. FP provides a quick and effective way of obtaining a product map (Dairou and Sieffermann, 2000, 60th IFT annual meeting, Dallas).

CFC is derived from open response questionnaires used in psychology and is based on the description of the differences in each related sample pairs in natural language by naive subjects. CFC provides a fast and sensitive way of mapping products and assessors.

The two methods were applied on five model synthetic aromatic solutions and validated on six champagne wines. Each experiment used a different panel (7–8 subjects) and was conducted in <3 h.

Data was statistically processed by Principal Components Analysis and Procrustean Generalized Analysis (FP) or Correspondence Analysis and Cluster Analysis (CFC).

The products were grouped and separated in a similar way.

In contrast, the attributes generated by the two methods were quite different. FP generated 24 attributes for the five model solutions and 29 for the six champagnes, whereas CFC extracted 85 terms from the five solution corpus and 103 from the six champagnes (from an original collection of 13 000 words). Only 19 attributes were common between the two methods for the five model solutions and for the six champagnes, thus rendering difficult any semantic interpretation of the otherwise strong consensual product map.

299. Gastrointestinal sintomatology and it's relationships between threshold taste of copper in drinking water

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The aim of the study was to determine taste threshold of copper in water and its relationship with appearance of nausea. A total of 75 'healthy' adult volunteers participated.

The triangular modified test was used. In these tests, five samples are presented to each panelist: four are identical and one contains the test solution. Individuals must identify the test sample and report its taste. Panelists received graded concentrations of copper, from 1.0 mg to 9 mg Cu/l (as $CuSO_4 \cdot 5H_2O$), increasing 1 mg at a time, until the threshold copper concentration was determined. Once the subject reached the threshold level he had the opportunity to confirm this value twice. If he succeeded, in the next trial he/she received a 'blank' solution (no copper added) and then a last confirmation of the threshold concentration was performed. Copper concentrations in the different solutions were checked by atomic absorption spectroscopy. In another set of experiments, the nausea threshold was determined in the same subjects. There were 44 subjects who felt nausea within the concentration range tested (1–10 mg Cu/l as copper sulfate) and 31 that did not feel nausea.

All of the panelists reported tasting at least one solution throughout the protocol. In 54.6% of them the threshold taste was between 1 and 2 mg Cu/l. The mean threshold value for the group was 2.3. The Spearman correlation test showed no correlation between taste threshold and nausea threshold ($r = 0.11$).

300. Gas chromatography–sniffing port detection of volatiles isolated from lovage (*Levisticum officinale* Koch.) at various phases of growing

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Volatiles from five different parts of lovage (leaves, stems, flowers, seeds and roots) were isolated by the dynamic headspace (DHS) method and characterized by gas chromatography–sniffing port analysis.

Differences in the composition of DHS constituents in various anatomical parts of the plants were not significant, whereas the amounts of a number of identified volatile compounds were different in the leaves, stems, flowers, seeds and roots. The effect of seasonal variations related to the phases of growing on the composition of headspace volatiles was determined. Except for roots, β-phellandrene was found to be the most abundant headspace component in all anatomical parts of lovage, constituting from 36.50 to 79.28% of the total GC peak area. The sniffing panel characterized effluents from the GC column and attributed odour descriptors to the detected constituents. α-Pinene and α-phellandrene + myrcene were the most frequently recognized compounds among 11 fractions constituting 12 identified and one unknown compound, which were detected by the members of the sniffing panel. None of the detected constituents was recognized as a lovage key aroma compound.

301. Chlorhexidine digluconate bitterness suppression using selected polyols and sodium salts

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Chlorhexidine digluconate is used as an anti-microbial agent in a wide range of oral health care products, such as toothpastes and mouthwashes. Its level of inclusion is limited by its intense bitter taste despite its low toxicity. It was against this background that three polyols (maltitol, xylitol and erythritol) and two sodium salts (sodium gluconate and sodium acetate) were assessed by a trained sensory panel for their ability to mask the bitter taste of chlorhexidine digluconate. Mixtures were prepared by adding polyols at 2 or 4% w/w and sodium salts at 1 or 2% w/w to a 0.1% w/w aqueous-ethanol (8% w/w) solution of chlorhexidine digluconate. Initially, bitterness intensity of mixtures was determined by an 18-member taste panel using a 'Labeled Magnitude Scale' (Green *et al.*, 1996, Chem. Sciences, 21: 323–334). Thereafter, bitterness intensity and persistence of selected mixtures were assessed by a 10-member subset-panel using the SMURF (sensory unit for measuring flux) potentiometric method in which time–intensity data were 'normalized' for each panellist to allow direct comparison of each potential inhibitor (Birch and Munton, 1981, Chem. Sciences, 6: 45–52). Magnitude

line scaling of mixtures showed that xylitol was ineffective while maltitol and erythritol were effective ($P < 0.05$) at suppressing the bitterness of chlorhexidine digluconate at both levels of addition. However, higher levels of inclusion did not produce greater bitterness suppression. In addition, sodium salts were only effective ($P < 0.05$) at suppressing the bitterness of chlorhexidine digluconate at the lower level of addition. Sodium gluconate (1% w/w), maltitol (4% w/w) and erythritol (2% w/w) were selected for further study using SMURF. Time–intensity flux measurements showed that the selected mixtures were effective ($P = 0.007$) at suppressing bitterness of cholorhexidine digluconate, with sodium gluconate > maltitol erythritol. However, the selected mixtures were ineffective at reducing the bitterness persistence of chlorhexidine digluconate. It is postulated that bitterness suppression by sodium gluconate involves transportation of bitter molecules further down the epithelium where fewer bitter receptors are present as a result of increased (ionic-mediated) hydration of chlorhexidine digluconate. Suppression of bitterness by selected polyols may occur by (i) entrapment of bitter molecules and prevention of their access to bitter receptors, or (ii) steric exclusion of bitter molecules from bitter receptors by polyols attached to adjacent sweet receptors. Bitterness suppression mechanisms will be the subject of further research.

302. Somatosensory influences on odor localization

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It is not known what factors contribute to the referral of food odor sensations to the oral cavity. Olfactory sensation during ingestion is generally mistakenly perceived as emanating from the oral cavity and consequently labeled as 'taste'. Todrank and Bartoshuk (1991, Physiol. Behav., 50: 1027–1031) demonstrated that taste sensations could be localized by touch. The purpose of this study was to investigate whether oral somatosensory input might also contribute to the localization of food odors. Additionally, potential cross-sensory enhancement between gustatory and olfactory stimuli was investigated.

One or two unflavored, orange-flavored and/or sweetened (0.44 M sucrose) gelatin cube(s) were presented to one or both sides of the anterior dorsal lingual surface, both before and after administration of an inferior alveolar nerve block (2% lidocaine HCl with epinephrine 1:1 000 000) during a routine dental procedure. With closed eyes, closed mouth and retracted tongue (not touching the palate), 30 patients estimated the intensity of orange perceived on the tongue and throat and in the nose, and recorded it [Labeled Magnitude Scale (Green *et al.*, 1993, Chem Senses, 18: 683–702)] immediately upon expectoration. Additional ratings of sweetness, cold and heaviness on the tongue were also required.

Orange was localized to the tongue 98% of the time when at least one cube was on a non-anesthetized tongue surface and orange was part of the stimulus complex, even when orange was presented only to the nose, unbeknownst to the patient. A mixed model least squares analysis revealed the significant effect of stimulus condition on orange intensity estimates ($P < 0.0001$). Eliminating the somatosensory cue by lingual anesthesia caused the perceived orange sensation to shift to the throat, an effect that was significant ($P < 0.05$). Contrasts of individual comparisons (Holmes adjusted *P*s) likewise indicated that doubling the somatosensory component (two cubes), of which only one cube was flavored orange, resulted in a significantly higher lingual orange intensity rating than when a single orange cube was rated ($P < 0.05$). Sweetness enhanced the magnitude of the lingual-localized orange perception ($P < 0.01$).

It is concluded that oral somatosensation is an important determinant of flavor referral and both touch to the lingual surface and airflow in the pharyngeal regions are sufficient to produce this effect.

303. Retronasal and orthonasal identification of odorants: similarities and differences

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Twenty unscreened judges' [nine females, ages 18–31 years (mean = 21)] veridical name identifications of vapor phase odorants delivered by either a retronasal or an orthonasal route by odorant presentation containers (Pierce and Halpern, 1996, Chem. Senses, 21: 529–543) were studied. Odorants were food-grade liquid extracts of plant materials: banana, cinnamon, coffee, lemon, oil (canola), orange, strawberry, wintergreen, diluted 1:2 with the principal diluent of that extract. Orthonasal sniffing was not permitted; modified retronasal breathing was not taught. Judges first learned, with corrections, veridical identifications of odorants presented orthonasally; they were next tested retronasally and then orthonasally, both without corrections. The judges then relearned veridical identifications of odorants presented retronasally, with corrections, and were tested orthonasally and finally retronasally, without corrections. A list containing veridical names of the odorants, and 19 others odorants, was available.

In the retronasal testing that followed orthonasal learning, judges made 36 ± 24% errors (mean ± SD), and in the immediately following orthonasal testing, 16 ± 10%. The difference was statistically significant ($t = 3.518$, $P = 0.001$). Confusion matrix analysis (Wright, 1987, Arch. Otolaryngol. Head Neck Surg., 113: 163–168) of orthonasal minus retronasal correct identifications found the difference for coffee significant ($t = 5.63$, $P = 0.0063$), Bonferroni corrected; the seven other differences in correct identifications were also positive, but $P > 0.05$. Differences in incorrect identifications were significant for coffee identified as oil ($t = -4.8$, $P = 0.0062$). For all other differences in incorrect identifications, $P > 0.05$. In the second half of the experiment, after retronasal learning, differences between orthonasal and retronasal errors were not significant ($P = 0.062$).

Retronasal and orthonasal correct identification of vapor-phase plant extracts were similar, but retronasal indentification was significantly inferior to orthonasal overall, and specifically for coffee odorant. On incorrect trials, coffee was significantly more likely to be misidentified as oil on retronasal than on orthonasal presentations.

304. Lateralization of vanillin odor perception elicited by a birhinal stimulation–differential olfactometer method

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The Differential Olfactometer presented here was first developed by Mac Leod in 1972 (CEA Patent no. 2, 210, 298). The method permits an evaluation of accurate and reliable quantification of individual human subject olfactory sensitivity. The Differential Olfactometer delivers simultaneously an unknown odorant in one nostril and a reference odorant in the other. Provided the two stimulations are synchronized with a time interval of <5 ms, the subject perceives only the more intense odor of the two stimuli, although both are supraliminar.

This phenomenon was first observed by Von Bekesy (1964, J. Appl. Physiol., 19: 369–373), and contested by Schneider (1967, Physiol. Behav., 2: 305–309). In 1969, Leveteau and Mac Leod (in C. Pfaffmann, ed., Olfaction and Taste III. Rockefeller University Press, pp. 212–215) found a reciprocal interbulbar inhibition accounting for Von Bekesy's observations. The peculiar Differential Olfactometer design stems from this finding.

By an Up and Down variation of the reference concentration we obtained an intensity matching between the unknown odor and the reference.

The question of a possible contribution of the trigeminal input to this lateralization of the olfactory perception (Cain, 1974, Ann. N.Y. Acad. Science, 237: 28–34) remains open to discussion. A partial answer to this question was provided in 1980 by Degobert and Mac Leod (Olfaction and Taste VII, pp. 469–470), who found that equalization of butanol was not different from an equalization of butanol with butanol + 5% ammoniac.

In the present experiment, vanillin was chosen as purely olfactory stimulus (Doty, 1978, Physiol. Behav., 20: 175–185) and we sought to determine a pure olfactory lateralization in the Differential Olfactometer. A 100 ms stimulus triggered by the sniffing of 20 subjects (21 ± 2 years old) was used.

This experiment is currently in progress.

305. The use of Flash Profile for a quick sensory characterization of a set of 16 strawberry yoghurts

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Understanding sensory perception often means being able to describe things with sensory profiling techniques. As an alternative to the traditional Quantitative Descriptive Analysis (QDA®) (Stone *et al.*, 1974, Food Technol., 28: 24–34) which is often criticized because it is expensive and time consuming, a very quick procedure designed to meet industrial needs is proposed. This method, named Flash Profile, combines the benefits of Free Choice Profiling (FCP) (Williams and Langron, 1984, J. Food Sci. Agric., 35: 558–568) and of ranking procedures in terms of both training and evaluation rapidity (Sieffermann, 1995, Doctoral Thesis, ENSIA, Massy; Rodrigue *et al.*, 2000, Food Qual. Pref., 11: 47–54). Flash Profile has been previously used to describe a set of jams from the French market and proved to be fairly satisfactory as compared with QDA®, which makes it a useful tool for a quick market analysis (Dairou and Sieffermann, 2000, 60th IFT annual meeting, Dallas). However, the tested products were easily discriminable. Therefore, the question arose whether the Flash Profile method is applicable to a set of closer products, which in turn, may be more relevant for product development.

For this study, a panel of seven assessors experienced in sensory analysis evaluated a set of 16 strawberry yoghurts, varying in sugar content and organic acids concentration according to a composite factorial design. As a control, five products maximally scattered within the boundaries of this experimental design were evaluated for preference by 100 consumers and yielded significant differences of degree of liking. Three sessions were needed to complete the experiment. A mono-dimensional data analysis shows that judges were poorly repeatable on most individual descriptors, confirming that the products were confusable. However, the performed Generalized Procrustes Analysis (GPA) (Gower, 1975, Psychometrika, 40: 33–51) combined with Principal Components Analysis (PCA) revealed that the products were clustered according to their sugar content, although the groups were overlapping. This result indicates an internal consistency in judges' representation of the product set. This view is supported by the expected opposition between descriptors related to sweetness (sugary, sweet) and those related to sourness (sour, tangy). Judges also generated aroma and texture descriptors positioned with some consistency on the first two-component plot. Our findings suggest that although Flash Profile is a fast and convenient descriptive method, the quality of the description is limited when judges are faced with a large set of products that are similar to each other. However, comparison with QDA® would be required to determine if any better description can be obtained with such a set of products.

306. Magnitude estimation: cost savings in sensory scaling using untrained panellists

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Sensory testing usually involves the testing of trained panellists within a sensory laboratory. The food science tradition asserts that untrained panellists cannot validly profile the sensory aspects of stimuli (Moskowitz, 1996, J. Sens. Studies, 11: 19–37). Although ratio scaling has considerable advantages over other forms of scaling, e.g. the measurement of absolute differences and the avoidance of end-effects, it is not much used because of the time and costs involved in training panellists how to assign ratios to reflect sensory perceptions.

In contrast to this assertion, Stevens and Poulton (1956, J. Exp. Psychol., 51: 71–78) showed that magnitude estimation (ME), a form of ratio scaling, could be used by untrained panellists to scale auditory stimuli. The aim of the present study was to determine whether panellists untrained in ME (n = 13) could scale the sensory intensity of food-related stimuli as accurately as panellists trained in ME (n = 14). The training involves judging geometric figures of varying sizes (Stevens, 1975, Psychophysics. John Wiley & Sons,

New York). All testing was carried out in a six-booth sensory evaluation laboratory and assessor responses were collected using a computerized sensory system (PSA System 3, version 2.05). Both panels assessed the saltiness of four randomly presented potato samples (0, 1, 2, 3,% salt) using non-modulus ME, i.e. they were allowed to assign any number they felt appropriate to the stimuli, such that the more intense the attribute of a stimulus, the higher the rating given to it, with each successive sample rated relative to the value that they assigned to the first.

Comparison of equalized sensory data (Lane *et al.*, 1961, J. Acoust. Soc. Am., 33: 160–167) revealed no significant difference in mean absolute errors in performance between trained and untrained panellists [$t(25) = 0.19$; $P < 0.05$].

Therefore, the responses of untrained people using ME were equivalent to those of trained panellists. In addition to its ratio scale properties and the ease of data collection, this opens up the possibility of testing in the field, with consequent ecological and cost benefits. This study suggests that 'untrained' ME may be an effective and economic method for scaling food-related stimuli with significant cost and practical advantages over current scaling methods.

307. PROP sensitivity of Japanese university students

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The sensitivity for the taste of 6-*n*-propylthiouracil (PROP) was studied with 395 Japanese university students (309 females and 86 males). The perception of bitterness of the PROP (3.2×10^{-5} M, 1.6×10^{-4} M) and saltiness of NaCl (0.3 M) was measured with a 100 mm visual analogue scale (0 for no taste, 100 for extremely strong). The results revealed that the perceived intensity of taste showed a unimodal distribution for the saltiness of NaCl but a bimodal distribution for the bitterness of the PROP. The subjects could be classed into three groups based on their PROP sensitivities (Sakai and Imada, 1999, Chem. Senses, 24: 226–227): nontaster (14.2%), taster (64.1%) and supertaster (21.8%). There is no difference in the classification between females and males ($\chi^2 = 1.79$, df = 2, ns). These results are in accordance with those shown by Bartoshuk and her colleagues (1994, Physiol. Behav., 56: 1165–1171).

To clarify the differences between the three groups, we investigate the detection threshold and the cognitive threshold for the PROP. Sixty-five participants were recruited from the participants of the first experiment. There are significant differences in the detection threshold and the cognitive threshold among the three groups [$F(2,62) = 22.69$, $P < 0.001$ and $F(2,62) = 44.87$, $P < 0.001$, respectively). *Post hoc* analysis revealed that nontasters have higher threshold for the PROP (both thresholds are $P < 0.001$). On the other hand, there are no differences between tasters and supertasters ($P > 0.10$).

In a third experiment, we investigate the relationships between the PROP sensitivity and the sensitivity for four basic tastes (sucrose, NaCl, citric acid and quinine hydrochloride). Ninety-two participants were recruited from the participants of the first experiment. There are no differences in the sensitivity for the basic tastes among the groups based on the PROP sensitivity (all $F < 2.95$, $P > 0.05$) nor sex (all $t < 0.95$, $P > 0.10$).

Finally, the relationships between the ratings of the bitter taste of PROP and the preferences for the stimulative foods were studied. The several researchers suggest that there are some relationships between the PROP sensitivity and the preferences for foods (Bartoshuk *et al.*, 1994). We asked some of the participants in experiment 1 to answer the questionnaire including the Japanese version of the Food Neophobia Scale and the food attitude survey for the several foods [Imada and Yoneyama, 1998, Stud. Human. Sci., 38, 493–507 (in Japanese)]. However, we could not clarify the relationships between PROP sensitivity and food attitudes. We are now investigating the preference and the intake behaviour to the foods by presenting the real foods (not by questionnaire) and its relationships with PROP sensitivities.

308. The influence of glass shape on wine aroma

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A common belief among wine enthusiasts is that to fully appreciate the beverage, one must use the appropriate glass. Wine glasses can vary not only in shape, but also in material (e.g. leaded crystal or glass). Differences in both the materials and the physical shape of glasses could potentially influence proportions of aromatic compounds trapped in the headspace of a filled wine of glass, and thus alter the olfactory perception of a wine.

To investigate this possibility, naive subjects were asked to assess the aroma of a California Cabernet Sauvignon presented in four different wine glasses. Two of the glasses were from a speciality line of crystal wine glasses. Of these two, one was designed for Chardonnay while the other was designed for Bordeaux/Cabernet Sauvignon. A typical, restaurant-style wine glass and a leaded crystal goblet of inappropriate shape were the remaining vessels used. The non-expert judges were asked to assess the wine aroma for total intensity, fruitiness, oakiness/woodiness, mustiness and vinegar intensity, as well as how much they liked the aroma.

During their assessment of the wine aroma, the subjects were blindfolded. The subjects never saw the glasses, nor did they touch the glasses. Subjects placed their heads in a chin rest and their noses were kept a set distance from the glasses. To create a standardized swirling motion, the wine glasses were placed upon a vortex machine. The presentations of the Cabernet Sauvignon were intermixed with an equal number of presentations of a California Chardonnay, also presented in the four different glass types. Unfortunately, the Chardonnay proved to be too delicate to withstand the length of the sessions, oxidizing over time, and its data were not reliable. However, its presence in the sessions introduced variety into the aromas being assessed.

Over most assessments, there was no significant difference between the glasses in the ratings of the Cabernet. The wine in the Bordeaux glass was rated as having a significantly lower total intensity than in the other three glasses, and it also received a slightly lower rating in oakiness that approached borderline statistical significance.

This work suggests that when smelling wines, the glass has limited impact upon the olfactory experience. However, differences in glass shape and materials could still potentially influence the overall perception of wine when it is drunk, and could certainly

influence the perceived pleasantness of the wine-drinking experience.

309. Characterization of umami taste in the water extract of fermented soybean paste

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Most enzymatic hydrolyzates of food proteins have a generally savory taste, and several savory tasting peptides of protein hydrolyzates have been isolated and highly acidic oligopeptides with high molar contents of glutamic acid residues have been reported as bouillon-like ('umami' in Japanese) (Arai *et al.*, 1972, Agr. Biol. Chem., 36: 1253–1256). Doenjang, fermented soybeans with salt and koji (cereal grains with *Aspergillus oryzae* and *Bacillus subtilis*), is widely used in Korea as a soup base or as a seasoning agent (Han *et al.*, 1993, Chem. Mikrobiol. Technol. Lebensm., 15; 150–160). As Doenjang contains high content of soybean (56.32%) and Koji serves as enzyme source, the production of savory tasting peptides during fermentation are expected. The objectives of this study were to investigate the savory tasting effects of Doenjang and the contribution of naturally hydrolyzed peptides during fermentation to its taste. The extract was obtained from commercial Doenjang by treatment with 3 vol of hot water (85–100°C) for its weight for 3–15 min and then filtered with a filter press followed by evaporation. The resulting concentrate (66 brix) itself had an umami taste and the addition of it to various dishes though in a small amount enhanced the whole flavor characteristics and gave a complex savory, rich and mild taste to them. It also had bitter-masking activity. The extract was fractionated to five fractions by ultrafiltration, fraction IV of mol. wt 500–1000 possessing the strongest umami taste of them, and was fractionated subsequently to aromatic, basic, acidic and neutral peptide fractions by column chromatography with activated charcoal, Amberlite IRC 50 and IRA 400, successively. Umami taste was found to be considerable in the acidic fraction, and a favorable after-taste effect was noted. Further isolation of umami peptides in the fraction is being carried out.

310. Receptor and synergistic mechanisms of umami taste as revealed by electrophysiological and neuropharmacological studies in the rat

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Umami substances such as monopotassium L-glutamate (MPG) and 5′-inosine monophosphate (IMP) elicit a unique taste called 'umami' in humans. This taste does not belong to any of the four primary taste qualities. Recently, Chaudhari *et al.* (1996, J. Neurosci., 16: 3817–3826; 2000, Nature Neurosci., 3: 113–119) found that the metabotropic glutamate receptor, mGluR4, was expressed in lingual tissues only with taste buds by using the reverse transcriptase (RT)-PCR method in rats. They also showed that monosodium glutamate and an agonist for mGluR4, L-amino-4-phosphonobutyrate (L-AP4), elicited a similar taste in the behavioural experiment using the conditioned taste aversion technique. In spite of these findings, it is known that rats trained to avoid umami substances cannot discriminate between the umami taste and sweet substances (Yamamoto *et al.*, 1991, Physiol. Behav., 49: 919–925). In the present study, therefore, we designed electrophysiological experiments to answer the following questions. (i) Does L-AP4 elicit synergistic effects when it is mixed with IMP? (ii) Does *s*-2-amino-2-methyl-4-phosphonobutanoic acid (MAP4), a metabotropic glutamate receptor antagonist, or gurmarin, an anti-sweet peptide, suppress the umami response? (iii) Does the binary mixture of L-AP4 and sweet substances elicit synergistic effects?

Results were as follows: (i) L-AP4 (5 mM) showed synergistic effects like MPG when mixed with 0.01M IMP. (ii) MAP4 (40 mM) did not suppress the responses to 5 mM L-AP4 or a mixture of 5 mM L-AP4 + 0.01 M IMP. Gurmarin (50 μM) suppressed the responses to both the mixtures of 0.1 M MPG + 0.01 M IMP and 5 mM L-AP4 + 0.01 M IMP. (iii) The responses to mixtures of 5 mM L-AP4 + sweet substances were synergistically enhanced, but they were not suppressed by 40 mM MAP4 or 50 μM gurmarin. These results suggest that umami receptors may not be simply understood by established glutamate receptors, such as mGluR4, in the nervous system, and that there are more than two types of mechanisms for eliciting the synergistic effect of umami taste.

311. Taste-related functional changes in the hypothalamus during intake of glucose, monosodium L-glutamate and NaCl in awake rats fed normal and non-protein diet

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The gustatory and anticipatory cephalic stimuli that are detected during a meal yield nutritional information and aid in the efficient digestion of food and maintenance of homeostasis. In general, foods having a umami, sweet or sufficiently salty taste are palatable, but those that are sour and bitter are usually aversive. Foods having a familiar or pleasant taste may be swallowed without caution. However, it is not clear whether the brain relates a taste of nutritional significance to dietary needs. It is possible that animals, including humans, can detect the amount of dietary protein ingested during a meal and use this information, via cephalic relays, to initiate digestion. They must, of course, ultimately have a means of determining whether the level of dietary protein intake is sufficient for bodily needs or not. It has been shown that foods with an umami taste character contain high quality animal protein that can be used for growth and maintenance.

The present study focused on showing that umami (monosodium glutamate, MSG) taste perception in the brain signals dietary protein intake. We developed a functional magnetic resonance imaging technique (fMRI, 4.7 T) to determine oxygenation and blood flow changes in awake rats. Overnight-fasted rats were fixed to a head platform with four pencil-like bars in the middle of the fMRI magnet. Rats ingested preferable taste

solutions (0.06 M MSG and NaCl, 0.6 M glucose) or distilled water. Brain blood flow decreased in the hypothalamus in all groups following solution intake. As 8% of neurons in the lateral hypothalamus (LH) differentially respond to umami taste stimulation, these findings suggest that umami taste perception could be integrated into the LH to identify protein intake, just as perception of saltiness serves to identify electrolytes and sweetness energy sources. In addition, the effects of taste solution intake on interstitial levels of norepinephrine (NE) were measured in the LH. Rats, housed in standard operant boxes, were fed either normal or non-protein diet for 3 days. Animals had limited fluid access, other than a daily bar-mediated drinking session (75 min). Microdialysates, collected from the LH during the 75 min of drinking, were analysed using HPLC. No significant responses of the LH NE to the drinking of distilled water, MSG, NaCl (0.06 M) and glucose solution were found in normally fed rats. However, a specific decline in LH NE release was detected during MSG solution-drinking in rats fed non-protein diet. Thus, LH NE may serve as a neurochemical substrate for association between umami preference and protein intake.

312. Inosinic acid and glutamate in fish and soy sauces

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Fermented beverages and foods such as sake and bread contain inosinic acid (IMP) and glutamate as taste compounds, produced through fermentation process by yeast (Fujisawa and Yoshino, 1994, in: Olfaction and Taste XI, p. 385; Fujisawa and Yoshino, 1998, in: Food Flavor: Formation, Analysis and Packaging Influences, pp. 227–231). Inosinic acid is formed from adenine nucleotides by AMP deaminase, which acts as a control system of glycolysis in yeast (Yoshino and Murakami, 1982, J. Biol. Chem., 257: 2822–2828). Stimulation of fermentation is thus closely related to the formation of IMP in yeast. IMP and glutamate also accumulate as a result of degradation of ATP and protein during storage of meat and fish. Soy and fish sauces are produced from soy beans and fish through fermentation, and used as a flavor and taste enhancer in East and Southeast Asia. However, composition of the taste compounds in these sauces has remained obscure.

In this study we determined contents of IMP and glutamate in various soy and fish sauces enzymatically. Fish sauces, including Japanese, Vietnamese, and Siamese sauces, contained higher levels of IMP and also glutamate, though to a lesser extent. On the other hand, soy sauce showed a negligible amount of IMP, but a considerable level of glutamate in comparison with those of fish sauce. IMP in fish sauce may originate from degradation of adenine nucleotides by higher activity of muscle AMP deaminase. The lack of IMP in soy sauce can be explained by negligible activity of AMP deaminase and the lower level of ATP concentration in soy beans.

313. Learning deficits in rats with low brain docosahoexaemoic acid

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Dietary deficiencies during development can result in cognitive deficits. In developing an animal model for such studies, we assessed the acquisition of an olfactory learning set of the F2 generation of rats raised on a docosahexaenoic acid (DHA)-deficient or DHA-supplemented diet. The Bailey Mental Scale test and other studies suggest that babies raised on DHA-free formula may have lower cognitive function scores and learning set tasks have been used to assess the ability of rats to adopt a response strategy in solving a series of similar problems.

Fifteen males per dietary group were placed on partial water deprivation and, at 10 weeks old, were trained by operant conditioning, in a multi-channel olfactometer, on a series of 'GO/NO-GO', discrimination tasks. In each 60-trial task novel odors were used; responding to the presence of one odor (S+) was reinforced with water, while responding to the other odor (S–) was punished by an extended inter-trial interval.

Analysis of fatty acids revealed an 85% decrease in brain DHA in n-3-deficient rats. Over the first 15 problems, both dietary groups performed equally well. In problems 16–20, the n-3-adequate rats improved further and most achieved near-errorless performance, while the n-3-deficient animals did not improve or improved more slowly. As measured by olfactory learning-set performance, our results point to a mild cognitive deficit in n-3-deficient rats.

314. 'Electronic nose' detects major histocompatibility complex-dependent odor components

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Mice prefer to mate with individuals expressing different MHC genes from their own. Volatile components presenting MHC-dependent odor types are present in urine and can be smelled by mice, as shown by extensive behavioral studies in the last 15 years (Yamazaki *et al.*, 1994, Proc. Natl Acad. Sci. USA, 91: 3735–3738). Similar odor types influencing human behavior are suspected (Wedekind *et al.*, 1995, Proc. R. Soc. Lond. B. Biol. Sci., 260: 245–249). Although a recent report indicates that MHC expression influences the ratio of volatile compounds such as phenol acetic acid (Singer *et al.*, 1997, Proc. Natl Acad. Sci. USA, 94: 2210–2214), there has been no way to date to assess odor types other than by studying the behavior of mice or rats. We report the use of a gas sensor array (referred to as 'electronic nose') to detect MHC-dependent odor types. The 'electronic nose' used in our investigations consists of an array of chemophysical detectors, in our case quartz crystal microbalances (QMB) and semiconducting metal-oxide sensors (MOX) that change their parameters

(frequency and conductivity, respectively) upon binding of very small numbers of individual molecules present in the gas phase of odorous fluids (Mitrovics *et al.*, 1998, Acc. Chem. Res., 31: 307–315). The pattern of changes is characteristic of a particular smell. The electronic nose is able to distinguish the urine odor types of MHC congenic mouse strains, MHC class I mutant mice and HLA-A2 transgenic mice. In addition, MHC-dependent odor types can be detected in serum of mice. The device also clearly differentiates between individual odor types of human sera from HLA homozygous individuals; however, HLA expression seems to have only a secondary influence. To be able to interpret our results comparatively, independent measurements have been performed by gas chromatography/mass spectrometry (GC/MS), which serves as a well-established analytical method. GC/MS results are in good correlation with e-nose data. Thus odor-type research can now be carried out with a more objective and fast through-put system independent of behavioral studies.

315. Factors involved in the establishment of odor and taste aversion learning

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When ingestion of flavoured (odor and taste) solution is followed by malaise, animals learn the association and acquire aversions to the odor and taste. The purpose of the present study is to examine the conditions which influence the establishment of odor and taste aversions in rats. Isoamyl acetate (banana-like smell) and saccharin were used as the conditioned stimuli (CS). An i.p. injection of 0.15 M lithium chloride was used as the unconditioned stimulus (US). In the present study, we focused on the CS–US interval and the method of presentation of odor stimulus. The CS–US interval was either 0 or 30 min. Odor stimulus was dissolved in the liquid or put in front of the nose. In the former case, rats ingested flavoured water or flavoured saccharin. In the latter case, rats smelled the odor stimulus during ingestion of water or saccharin. In experiment 1, 0.001% isoamyl acetate and 0.0025M saccharin were used for odor and taste stimuli, respectively. Rats were divided into five groups: group 1 rats ingested flavoured water (odor, water and ingestion group; OWI), group 2 ingested flavoured saccharin (odor, taste and ingestion group; OTI), group 3 ingested taste solution (taste group, T), group 4 smelled the odor during ingestion of water (odor, water and smell group; OWS) and group 5 smelled the odor during ingestion saccharin (odor, taste and smell group; OTS) as the CS. OTI and OTS were experimental groups, and OWI, T and OWS were control groups. The CS–US interval was 0 min in all groups. Rats in all groups acquired aversions to the odor or taste. These results suggest that the method of presentation of odor has no effect on establishment of odor and taste aversions when the CS–US interval is 0 min. In experiment 2, the CS–US interval was changed from 0 min to 30 min. The other conditions were the same as those in experiment 1. OTS, OWI and OWS did not acquire aversion to odor, whereas OTI did acquire weak aversions. These results indicate that aversions to odor are acquired when the CS is the flavoured taste solution even if the CS–US interval is long (30 min). Although OTI acquired odor aversions, OTS did not acquire them. We changed the concentration of taste from 0.0025 M to 0.005 M in experiment 3. The other conditions were the same as those in experiment 2. OTI and OTS rats acquired aversions to odor and taste. These results suggest that the concentration of taste influences the establishment of aversions to odor in odor and taste aversion learning.

316. Preference for corn oil in normal and anosmic mice in the conditioned place preference test

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Oily and fatty foods are consumed for their good taste and flavor, and we have consume them br preference. In mice, a vegetable oil such as corn oil alone was also preferably taken in a two-bottle choice test (Takeda M. *et al.*, 2000, Life Sci., in press). The conditioned place preference (CPP) test has been used to evaluate the rewarding (reinforcing) effects of addictive drugs and food reward (Schechter and Calcagnetti, 1998, Neurosci. Biobehav. Rev., 22: 827–846). In the present study, we evaluated the palatability of corn oil, focusing on its rewarding effects by the CPP test in normal and anosmic mice, since previous reports pointed out the contribution of olfactory stimulation (flavor) of the oil to its preference (Ramirez, 1993, Am. J. Physiol., 265: R1404–R1409).

We used the test chamber consisted of two boxes (dark and light) of equal size and a small connecting zone. It took 12 days to complete the CPP test cycle: days 1–3, measurement of baseline preference to the light and dark boxes; days 4–11, conditioning in each box; and day 12, measurement of changes in preference by conditioning. On days 1–3 and 12, the time spent in each box by each mouse (adult male ddY) was measured for 30 min. On days 4–11, each mouse was confined to one box and given corn oil or water from the bottle which was inserted through the ceiling in the light or dark boxes, respectively, for 30 min. Conditioning of corn oil and the light box or water and the dark box were alternated four times. The time spent in the light box on day 3 was used as the baseline and compared with that on day 12. Olfactory blockade was induced by infusion of $ZnSO_4$ solution into the nasal cavity in mice (Brouette-Lahlou *et al.*, 1999, Physiol. Behav., 66: 427–436).

Voluntary intake of 100% corn oil in the light box in the CPP test showed place preference but its peroral administration 60 min before the conditioning did not show either place preference or aversion. In the anosmic mice, the corn oil intake also induced place preference in the corn oil-related box as well as in olfactory normal controls. These results suggest that corn oil has rewarding effects in mice derived from the other stimuli rather than olfaction in the oral cavity. These effects may be responsible for the high palatability of corn oil.

317. Function of antennular grooming behaviour in Caribbean spiny lobsters

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Antennular grooming behaviour (AGB), observed in crustaceans, consists of two repetitive components: antennule wiping followed by auto grooming of the third maxillipeds. In the Caribbean spiny lobster, *Panulirus argus*, AGB is induced by one chemical found in

prey extracts: L-glutamate (Glu) (Barbato and Daniel, 1997, Biol. Bull., 193: 107–115). We have observed similar response specificity in other decapod species as well, including *Panulirus guttatus* and *Homarus americanus*. The behaviour appears to be mediated through the aesthetascs, the olfactory sensilla on the antennules, according to ablation studies. In investigating the function of AGB we considered two questions: does AGB maintain olfactory function and why is AGB elicited by only one chemical? Our hypothesis was that Glu is the most sensitive indicator of biofouling because its charge characteristics in normal sea water make it very adherent to the antennule cuticle.

To answer the first question, experiments were performed at the Hofstra University Marine Laboratory in Jamaica, where we maintained lobsters in aquaria with flow-through seawater and restricted their ability to groom their antennules. Olfactory responses were monitored over a 2–3 week period by observing the magnitude of AGB towards Glu. Grooming was restricted by either excising the grooming setae on the third maxillipeds ($n = 12$) or removal of the third maxillipeds ($n = 3$). Antennules from lobsters in these two treatment groups and lobsters with maxillipeds unmodified were examined using SEM to determine the amount of fouling and damage. To answer the second question, excised sections of antennule cuticle were incubated with radiolabelled (^{3}H) chemicals, washed, and then measured for amount of bound isotope via scintillation counting. Isotopes consisted of chemicals found in prey extracts and varied in charge number at pH of sea water [Glu (3), aspartate (3) taurine (2), AMP (1), hypoxanthine (0)]. It was hypothesized that isotopes with higher charge number would adhere more to cuticle.

There was no change in responsiveness to Glu in lobsters with grooming setae removed even after 16 days. However, responses of lobsters with maxillipeds removed for 14 days were ~70% less responsive than lobsters with maxillipeds removed for <24 h. Antennules from lobsters with excised maxillipeds were the most fouled and damaged; however, fouling was observed in the other treatment as well. These results support our hypothesis that AGB maintains olfactory function. There were no differences in binding of the five chemicals. We propose as an alternative that Glu may be excitotoxic at high concentrations and AGB may serve to facilitate its removal. Preliminary evidence indicates that repeated application of high concentrations of Glu results in long-term reduction in AGB magnitude.

318. Behavioural study of truffle hunting animals: a modelized approach for key-odor compounds identification

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Black truffles (*Tuber melanosporum* Vitt.) are fructifications of a mycelium which grows in symbiosis with certain trees, especially oaks. The presence of truffle mycelium may be observed at the surface of the ground by an area without any vegetation. Truffles develop inside this 'burnt patch' at a depth of 0–25 cm. As truffles have an exclusive underground biological cycle, their localization remains a delicate and uncertain operation for which the acute sense of smell of certain animals was required, traditionally pigs or trained dogs. A preliminary study stated that the steroidic pheromone, 5α-androstenol, previously identified in truffles (Claus *et al.*, 1981, Experentia, 37: 1178–1179), had low importance in comparison with major aroma volatiles compounds (Talou *et al.*, 1987, J. Agric. Food Chem., 48: 774–777) in the detection process of truffles by both dogs and pigs (Talou *et al.*, 1990, Mycol. Res., 94: 277–278). The present paper reports a modelized behaviour study carried out among selected trained truffles dogs by using different mixtures of truffle volatiles for identifying key-odor compounds for truffle localization.

Oily based mixtures of one, two or three pure chemicals identified as the nine major black truffle volatiles and Nature-Identical Truffle flavouring (Talou *et al.*, 1990, European Patent No. 257 666) at a fixed total chemicals concentration of 300 µl/l were tested in different burn areas of experimental truffle fields. The test consisted of burying 20 samples (a 25g mature fresh truffle, 2 ml of flavouring and 2 ml of nine odorous mixtures in duplicate) at 3 cm depth within a 30 m^2 area. Samples were placed 1 m apart and the trained dog was free to move inside the area. The test conditions were similar to a truffle dog contest: (i) duration: 6 min; (ii) a gift was given to the dog when it indicated a place with its paw. The behaviour of the dog is also categorized: (i) no interest; (ii) smelling the plot; (ii) indicating the site with a paw; iii) digging the soil to extract the product. Tests were carried out among three trained dogs every week during a truffle production season (December–March). Complementary tests were performed with odorous volatiles which are not present in truffles (e.g. benzaldehyde, 1-octen-3-ol, cumminaldehyde, anethol, skatole, various sulfurous compounds) in order to validate this modelized approach.

Genuine truffles and flavouring samples were located with 100 and 95% success rates, respectively. Dimethyl sulfide appeared to be the key-odor compound for truffle localization by dogs (100%) and 2-methyl butanal was another major contributary one. None of the other odorous volatiles generated the typical dog's behaviour in truffle hunting.

319. Olfactory detection of truffle volatiles by *Suillia* flies

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Black truffles are the fruiting bodies of the hypogeous fungus *Tuber melanosporum* Vitt., which grows in symbiosis with the roots of certain trees. During its limited harvesting season, mainly through the winter, one finds them in several regions of southern Europe, especially Spain, Italy and France. Their harvesting is problematic because, even when ripe, truffles remain underground. While pigs and trained dogs are commonly used for truffle hunting, a particular fly species (genus *Suillia*) could also be used for truffle localization. Indeed, these flies, which lay eggs above truffles to provide food for their larvae, often hover above the place where a truffle is hidden (Coutin, 1983, Insectes, 75: 6–8). During sunny winter days, by lying on the ground and watching these tiny insects, truffles could be efficiently localized. In order to identify the

key-odor compounds responsible for this attraction, an electroantennography study was carried out on wild *Suillia* species flies by testing 35 chemicals identified as truffle aroma volatiles (Talou, 1992, Doctoral Thesis, INPT).

Larvae of *Suillia pallida*, *Suillia humilis* and *Suillia gigantea* were collected in overmature truffles and placed in a vivarium in order to obtain the corresponding insects several days after. After feeding for 3 weeks with pollen and water/honey mixtures, sexually mature flies were collected for systematic identification and further olfactory tests with an electro-antennographic device (Thiery *et al.*, 1990, J. Chem. Ecol., 16: 701–711) equipped with a dedicated processing software (Marion-Poll and Tobin, 1991, J. Neurosci. Methodol., 37: 1–6). Twenty microliters of each of the 35 chemicals and 1-hexanol (standard) diluted in paraffin oil (dilution 1% v/v) were deposited on filter paper placed in a glass pipet. Two milliliters of dry air was flushed through the pipet during 1.6 s and the corresponding odorized airstream was sent into contact with the fly antenna equipped with two electrodes. The corresponding electroantennograms (EAGs) were recorded from the removed heads of 10 *Suillia pallida* flies (seven females and three males). Olfactory stimulation by the standard was delivered after five samples and the inter-stimulation interval was fixed at 30 s. The responses measured were the EAG amplitude versus standard ones and the RECO time (time of return to half-amplitude value).

Eighteen compounds, including methyl sulfide, generated EAG responses of >50% of the standard ones, though some of them had a high SD (>20%). Among these, diethyl sulfide, bis-methyl-(thio)methane, 1-octanol, 1-octen-3-ol, 2-octanone and benzaldehyde were identified as minor compounds in mature truffles, but were reported as key-flavor compounds of several flowers which could be collected during the truffle production season.

320. Does olfactory imprinting exist in nematodes?

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Environmental sensory signals present during critical developmental periods can influence and partially fix patterns of sensitivities at the adult stage. Imprinting of the early olfactive environment is well documented in a number of animal species. Nematodes have complex olfactory systems, and rely heavily on their chemical sense for survival. In *C. elegans*, it involves 11 pairs of morphologically different amphid neurons, and each pair is thought to express a limited number of chemoreceptors out of several hundred members. As demonstrated for the AWA and AWB neurons, which respectively control attraction or repulsion to volatile odorants, the identity of the neuron in which a given receptor is expressed direct worms chemotactic behaviour toward the ligand of this receptor. Genetic analysis on the chemoreceptor transducing pathways demonstrated activity dependence of sensory neurons' morphofunctionality during development (Peckol *et al.*, 1999, Development, 126: 1891–1902), and a growing number of evidences support the idea of a relatively high degree of plasticity in the nematode chemosensory system. For example, in adults, reversible adaptative desensitization after prolonged exposure to some odorants as well as odorant discrimination vary with environmental signals and previous experience (Colbert and Bargmann, 1997, Learn. Mem., 4: 179–191). It seems, however, that adult nematodes cannot be sensitized to odorant stimuli even through associative learning protocols. We want to adress the question of a possible influence of environmental olfactory stimuli on chemoreceptor genes expression in functionally distinct sensory neurons. Chemoreceptors are thought to be expressed very early during development, as transcripts are present even before hatching (Troemel *et al.*, 1999, Cell, 99: 387–398). By exposing worms to odorants at different stages of their development, and measuring the resulting modifications of chemotactic responses in the adults, we hope to be able to define a critical period during which chemosensitivity could be experimentally manipulated. This potential odorant 'learning' process by early imprinting could then be further analysed pharmacologically and genetically.

321. The importance of CREB synthesis and phosphorylation in the olfactory bulb for olfactory learning in young rats

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Cyclic AMP response element-binding protein (CREB) is involved in hippocampal long-term potentiation. CREB mutant mice exhibit impaired memory. Transgenic flies dominant-negative for CREB protein show impairment in olfactory learning (Silva *et al.*, 1998, Annu. Rev. Neurosci., 21: 127–148). Not only CREB synthesis but also CREB phosphorylation hves been considered to be crucial processes in long-term structural or functional changes (Bito *et al.*, 1996, Cell, 87: 1203–1214). Therefore we have undertaken behavioral and biochemical experiments to clarify the function of CREB in olfactory learning in young rats.

Pups of Long–Evans hooded rats were bilaterally implanted with cannulae made of 6-mm-long 23G stainless steel tubings in the olfactory bulb (OB) on postnatal day (PND) 10.

After training with simultaneous olfactory stimulation and foot shock on PND 11, young rats showed aversion to the odor at PND 12 in an odor preference test on an open arena (Okutani *et al.*, 1999, Neuroscience, 93: 1297–1300). Animals were infused with 4 nmol/2 ml of CREB antisense, scrambled or sense oligodeoxynucleotide (ODN) solution into the OB 6 h prior to training through the cannulae (Guzowski *et al.*, 1997, Proc. Natl Acad. Sci. USA, 94: 2693–2698).

Nuclear extracts were obtained from the OB homogenates of animals after training. Western blot analysis was carried out on a nitrocellulose membrane following electrophoresis by SDS–PAGE (Hu *et al.*, 1999, Neuroscience, 89: 437–452). The membrane was incubated with primary antibodies against CREB and phosphorylated CREB (pCREB).

Animals infused with CREB antisense ODN prior to training did not show aversion to the odor. Western blot analysis revealed higher pCREB immunolabeling expression in the OB of odor + shock training animals than that of odor only or no exposure animals. These results suggest that CREB synthesis and CREB

phosphorylation in the OB play critical roles in olfactory learning in young rats and that the OB is a critical site for synaptic plasticity responsible for the learning.

322. Factors influencing the ingestion of linoleic acid by the rat

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It has been shown that it is quite likely that rats taste fatty acids that represent potential chemical cues contained in dietary fat (Gilbertson, 1998, Curr. Opin. Neurobiol., 8, 447–452). Corn oil is a commonly used fat in rodent studies (Smith *et al.*, 2000, Physiol. Behav., in press). The fatty acids that can be cleaved from corn oil are linoleic (~60%), oleic (~25%), palmetic (~12%) and stearic (~2%). Linoleic acid is an essential fatty acid that must be obtained from food. If the rat 'tastes' corn oil, linoleic acid would be a large component of this gustatory experience. The studies that will be reported here are behavioral experiments, looking at factors influencing the ingestion of linoleic acid. The general technique involved is a conditioned taste aversion (CTA) design, where the rat is given a particular concentration of linoleic acid (typically ~20 μM) for a few minutes followed by an injection of LiCl. The 'sickness' induced by the LiCl injection conditions an aversion to the linoleic acid, indicating that the rat can discriminate the linoleic from its ethanol vehicle. Control rats are given an injection of NaCl and they develop no flavor aversion to the linoleic acid.

A variety of experiments were conducted to show that linoleic acid acts in the same manner as many other taste stimuli.

1. Extensive experience with this fatty acid before the conditioning inhibits the development of the aversion.
2. Bilateral sectioning of the chorda tympani nerve blocks the development of the CTA tolinoleic acid. This latter finding is not the result of disruption of salivary glands, since removal of the submaxillary and sublingual glands has no effect on the development of the CTA to linoleic acid.
3. The concentration threshold for linoleic acid was measured to be ~10 μM when using the taste aversion technique.
4. A conditioned aversion to linoleic acid generalizes to both oleic and palmetic acids, but not to arachidonic acid nor terfenidine.
5. Rats can distinguish 22 μM linoleic acid mixed in 0.25 M sucrose from the sucrose solution alone.
6. Rats conditioned with corn oil as the CS show some generalization to linoleic acid.
7. Rats conditioned with linoleic acid as the CS show some generalization to corn oil.
8. The microstructure of the development of the aversion to linoleic acid is shown with a resolution of measurement equal to 6 s.

The implications of these studies for the ingestion of corn oil and other fats are to be discussed.

323. Seasonal and environmental modulation of proliferation rate underlying adult neurogenesis in the olfactory brain of decapod crustaceans

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Recent studies showed that in the olfactory brain of the shore crab, *Carcinus maenas*, special proliferation zones continue to generate new neurons during the juvenile and adult life of the animal. Labeling adult crabs *in vivo* with bromodeoxyuridine (BrdU), a thymidine analog inserted into DANN during S-phase of the cell cycle, revealed a group of proliferating cells in the right and the left lateral soma clusters (LC) containing the cell bodies of olfactory projection neurons (OPN). Furthermore, a group of BrdU-positive cells was present in the soma clusters (HBC) attached to the hemiellipsoid bodies, the secondary neuropils in the eyestalks to which the OPNs project (Schmidt, 1997, Brain Res., 762: 131–142). Morphology and immunocytochemical tests with antibodies to neuropeptides proved the labeled cells to differentiate into neurons, and unilateral ablation experiments indicated that the rate of neurogenesis is regulated by olfactory afferents (Hansen and Schmidt, 1999, Chem. Senses, 24: 531). The aim of the present study was to elucidate whether the lifelong proliferation in the olfactory brain of *C. maenas* is also affected by the time of year and environmental conditions like the keeping in tanks. For this reason, late juvenile and adult shore crabs were caught at the same place in the North Sea every 6 weeks. Four days after catching the crabs, a batch of 15 animals was injected with BrdU and fixed 14 h later. A second batch of 15 animals was kept in aerated tanks filled with water from the North Sea at a constant temperature of 10°C, and fed once a week with pieces of beef heart. After a time interval of 3 months, these crabs were also injected with BrdU and fixed 14 h later. Brains and eyestalks of all fixed animals were processed for BrdU-immunocytochemistry, and labeled nuclei were counted in a confocal microscope (LEICA). Our results show that the time of year exerts a very prominent influence on the number of proliferating cells and on the intensity of labeling, with a low during wintertime in November/December. In winter, some of the animals had no BrdU-positive nuclei at all, while others had ~5–10 labeled nuclei. In summer, numbers of proliferating cells went up to ~80 nuclei per cluster. Moreover, keeping the animals in tanks for an extended period of time—though at a constant temperature throughout the year—slightly affects the rate of proliferation in the brain of the shore crab.

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324. Maternal stimuli contributing to olfactory learning in the newborn rabbit

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In rabbits, a maternal pheromone is essential for the release of nipple-search behaviour and sucking. Nevertheless, if the mother's

ventrum is scented, pups learn the novel odour in a single, 3 min nursing episode. When subsequently placed on a rabbit fur scented with the experimental odour, conditioned but not naive pups respond with the stereotyped nipple-search behaviour characteristic of the pheromonal response.

To examine the contribution of maternal stimuli to this learning, we formed five experimental groups (n = 12/group) of 3-day-old pups: (i) naturally nursed in the nest box (NAT); (ii) searching in the arena on does injected with oxytocin so that pups could obtain milk (AM); (iii) searching in the arena without obtaining milk (NM); (iv) only sucking (SUK, pups hand-held to nipples); and (v) only searching (SER, pups prevented from attaching to nipples by taping these over). Three groups of control pups exposed to unscented does were formed (n = 12/group): one for NAT, one for AM and one for all other arena groups (without milk).

In all groups, the pups' nipple-search behaviour was tested by placing them for 3 min in an arena on an unscented rabbit fur, and then for 3 min on a scented fur. Total search times on each fur were compared, as well as relative times spent searching inside the central stimulus area versus outside.

The search time spent on scented fur versus unscented fur was significantly different across groups, with NAT > NM > SUK > AM > SER > cNat > cAM > cNM (Kruskal–Wallis, $P < 0.0001$). *Post hoc* analysis showed the differences to be significant between experimental and control groups. When relative search times in the perfumed centre of the arena versus the unperfumed surround were calculated, differences between the SER pups and the other experimental groups became significant. The findings suggest that learning is dependent on both the pheromone and the peri-oral tactile stimuli provided by the mother's nipples.

325. MHC-mediated fetal odortypes play a functional role in regulating social interaction

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Mice can recognize one another by individually characteristic phenotypic body odors (odortypes) that reflect their genetic constitution at the highly polymorphic major histocompatibility complex (MHC) of genes on chromosome 17. We have shown that the urine odor of the pregnant mouse is a combination of her own odors and that of her fetuses, and individuals can discriminate between identical females carrying fetuses differing in MHC type. Theoretically, it should be possible for a mouse to determine the MHC type of the sire based on the odortype of the pregnant female. This study was designed as a first step in investigating the functional roles that fetal odortypes might play in mouse behavior. Untrained male and pregnant female mice were given two preference tests where the choices were between two genetically identical pregnant females who carried 15- to 18-day-old fetuses that differed only in MHC type. The results indicated that among untrained male and pregnant female, fetal MHC type influenced choice behavior presumably via fetal odortypes expressed in maternal secretions/excretions. They also demonstrated that previous housing and/or mating experience modulated choice. These data also provide the first evidence that fetal odortypes are salient cues under normal conditions and suggest that they play a role in modulating social interactions in this and perhaps other species.

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326. Specific anosmia to androstenone and aggressive behavior in inbred mice

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CBA/J and NZB/B1NJ mice and offspring from specific matings were examined in two behavioral tests: olfactory sensitivity to the bovine pheromone androstenone (AND) and for the level of aggressive behavior exhibited toward other male mice. Thresholds to AND were estimated in a quinine aversion Y-maze test and buried cookie test. Differences in sensitivity to AND between CBA and NZB mice were estimated to be at least 2000-fold, with CBA mice being much more sensitive than NZB mice. Analysis of the results of AND sensitivity tests of the segregating F2 generation indicated probable involvement of X and Y chromosomes in the control of sensitivity to AND. Our model fits if we assume the existence of a gene(s) that suppresses sensitivity to AND on the X chromosome of NZB mice and the existence of gene(s) that promotes sensitivity to AND on the Y chromosome of CBA males. The level of aggressiveness was quantified using a standard test, with castrated male intruders serving as target mice. Although highly sensitive to AND, 93% of CBA mice did not attack the intruder, though they showed clear investigatory reactions. Of the AND insensitive NZB males, 88.2% exhibited high levels of aggression. We also investigated sensitivity to AND and aggressiveness in the F1 hybrids from matings between CBA females and NZB males and vice versa (n = 13 and n = 12 respectively), and in the F2 generation (n = 127). Only 23% of CBA (female) × NZB (male) F1s were aggressive; however, 92% of the males from the reciprocal, NZB × CBA, cross revealed high levels of aggression. In the F2 generation, the level of sensitivity to AND is correlated with the level of male aggressiveness (r = 0.78).

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327. Olfactory discrimination of amino acids and their mixtures by fish that inhabit different ecological niches

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How fish use chemical and visual stimuli in food-searching behavior is presented. Depending on the ecological niche occupied, visual, olfactory and taste systems are differentially developed (either genetic differences or differential expression) in different fish species. Depending upon the ecological group of fish, food search is accomplished primarily by: (i) olfaction and taste (e.g. brown bullhead catfish, *Ameiurus nebulosus*), and in some species

(e.g. channel catfish, *Ictalurus punctatus*) alternatively by vision; (ii) vision or olfaction (e.g. rainbow trout, *Oncorhyncus mykiss*); and (iii) vision (e.g. European huchen, *Hucho hucho*, and walleye, *Stizostedion vitreum*). For fish that rely on both olfaction and taste to locate food, well-developed olfactory and taste systems participate in the control of feeding excitation. The olfactory system of catfish and carp (*Cyprinus carpio*) enable discrimination of odors of low molecular weight. With the exception of a few amino acids (e.g. L-valine/L-isoleucine and L-alanine/glycine/L-serine), catfish can be trained to discriminate all amino acids. Catfish initially detect binary and ternary mixtures of amino acids as the more stimulatory component [determined experimentally by the amplitude of electrophysiological response (EOG)] in the mixture. However, with additional discrimination training, the less stimulatory components sufficiently modify mixture perception in catfish such that a mixture is then discriminated from its more stimulatory component. Current evidence indicates that multi-mixtures of >10 components are detected by brown bullhead catfish as novel sensations, as there is no indication for the detection of the components within the multimixture. Rainbow trout predominantly hunt by vision; however, food search can be released by olfactory (e.g. amino acid) stimuli alone. European huchen and walleye, which are primarily visual hunters, do not respond to chemical stimuli alone and do not accept non-living foods; however, when non-living food is available at an early phase of their ontogeny, fry and fingerlings of both species start to feed on non-living foods and start to respond behaviorally to olfactory stimuli. For example, fingerlings of walleye have been conditioned to discriminate L-proline from other amino acids.

328. Roles of vision and olfaction in homing of hime (land-locked sockeye) salmon

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Salmonids are well known for their ability to return to their natal streams for spawning. It is generally accepted that the juvenile salmon at the smolt stage learn site-specific odors of their home stream. As adults, they use this imprinted odor memory for homing (Hasler and Wisby, 1951, Am. Natur., 85: 223–238). Many behavioral experiments have supported this 'olfactory imprinting hypothesis' (Satou *et al.*, 1996, Bull. Natl Res. Inst. Aquacult., Suppl. 2: 49–57). However, there still remains a possibility that other senses, such as vision, play some roles since the odors of rivers should not extend to the open sea. Thus, we conducted an experiment to examine the role of the senses involved in salmonid homing using hime salmon (land-locked sockeye salmon, *Oncorhynchus nerka*), which has accurate homing ability.

Adult salmon caught by shore seine at the mouth of Shobu-shimizu river and Senju-shimizu river flowing into Lake Chuzenji, Nikko, Japan (wild salmon) and mature fish reared in Shobu-shimizu water from eggs (hatchery-reared salmon) were used in this study. Fish were tagged and released at the center of the lake or the mouth of a non-home stream. Visual impairment was done on one group of wild salmon. Fish migrating into streams were recaptured by fish traps and their tags checked.

Wild salmon showed a homing rate of 65–88.3% (nuo. of fish returned to home stream/no. of fish released × 100), whereas hatchery-reared salmon and visually impaired wild salmon showed 21.9–52.4 and 50%, respectively. However, home stream selectivity (returned to home/migrated into rivers × 100) was almost 100% in all groups. Homing duration for returning to natal stream from the center of the lake was much shorter in intact wild salmon than in hatchery-reared salmon and visually impaired wild salmon. It seems that hatchery-reared fish and visually impaired wild fish swam randomly and happened to reach the mouth of their natal stream by chance. These results suggest that hime salmon use visual cues from open water to the mouth of their natal stream, then discriminate the odors of their natal stream by olfaction.

329. Behavioral response to a novel bitter amino acid, pulcherrimine, in C57BL/6 and BALB/C mice

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The gonad of the green sea urchin (*Hemicentrotus pulcherrimus*) is a popular seafood in Japan. However, mature ovaries often taste bitter and are not accepted as food. In our previous study, a novel sulfur-containing bitter amino acid, named pulcherrimine, was isolated from mature ovaries of *H. pulcherrimus*. This study presents behavioral experiments of pulcherrimine using a conditioned taste aversion (CTA) paradigm in C57BL/6 and BALB/c mice.

Subjects were adult male and female mice (20–30 g body wt) of C57BL/6 and BALB/c strains. The conditioning and test stimuli were pulcherrimine, four sulfur-containing bitter substances [6-*n*-propylthiouracil (PROP), phenylthiourea (PTC), $MgSO_4$, quinine sulfate (QH_2SO_4)], five non-sulfur-containing bitter substances [sucrose octaacetate (SOA), denatonium benzoate (DEN), quinine hydrochloride (Qui), strychnine (Str), brucine (Bru)], seven bitter amino acids [L-valine (Val), L-leucine (Leu), L-isoleucine (Ile), L-phenylalanine (Phe), L-arginine (Arg), L-lysine hydrochloride (LysHCl)] and a sulfur-containing amino acid [L-methionine (Met)]. The behavioral experiments were carried out by a single bottle test counting the number of licks per 10 s for each test solutions.

Behavioral aversion thresholds to pulcherrimine were 1.0 mM in C57BL/6 mice and 0.1 mM in BALB/c mice. An aversion conditioned to pulcherrimine significantly generalized to PROP, PTC, $MgSO_4$, QH_2SO_4, SOA and DEN in C57BL/6 mice, and generalized to PTC, QH_2SO_4 and SOA in BALB/c mice. The number of licks for Met, Val, Leu, Ile, Phe, Arg and LysHCl were not significantly suppressed after CTA to pulcherrimine in both strains of mice.

Taste sensitivity to pulcherrimine is higher in BALB/c than

in C57BL/6 mice. Generalization patterns across various bitter and other taste stimuli in the two strains of mice suggest that pulcherrimine may taste similar to other sulfur-containing bitter compounds but different from other bitter amino acids and L-methionine.

330. Whole-cell response characteristics of ciliated and microvillous olfactory receptor neurons to amino acids, prostaglandins and urine in the rainbow trout

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The urine of mature rainbow trout contains pheromones that control their spawning and homing behaviors. Urine collected from ovulated females serves as a releaser for immature rainbow trout when treated with methyltestosterone (Yambe, unpublished data) and as a primer for mature males (Scott *et al.*, 1994, J. Fish Biol., 44: 131–147). However, what chemical components in the urine serve actually as pheromones and how the pheromones are detected by different types of olfactory receptor neurons (ORNs) in their olfactory organ are not yet clear. In this study, we examined the response difference in ciliated and microvillous ORNs of the rainbow trout, *Oncorhynchus mykiss*, to an amino acid mixture (containing 1 mM each of glutamate, arginine, alanine and norvaline), prostaglandins (0.05–0.1 mM $PGF_{2\alpha}$ and $15KPGF_{2\alpha}$) that were identified as pheromones in the goldfish (Sorensen *et al.*, 1988, Biol. Reprod., 39: 1039–1050), and urine samples (diluted 20-fold) collected from immature and ovulated female rainbow trout using whole-cell patch-clamp techniques. Ciliated and microvillous ORNs were isolated from immature rainbow trout (70–100 g body wt; 17–25 cm fish length) by a Ca^{2+}-free Ringer's solution treatment. Stimulants loaded into a three-barrelled micropipette were applied focally to the olfactory knob of an isolated ORN by a pressure ejection system. Current responses of ORNs were recorded from 50 ORNs (36 ciliated and 14 microvillous ORNs) at a holding potential of –60 mV and were all phasic inward currents. Sixteen ciliated ORNs responded specifically to the amino acid mixture and 12 responded to both the amino acid mixture and urine samples, with eight responding specifically to urine samples. Microvillous ORNs responded only to the amino acid mixture. None of the ciliated and microvillous ORNs responded to prostaglandins. The response profiles of ciliated and microvillous ORNs did not change significantly by methyltestosterone treatment of immature fish. The results suggest that ciliated ORNs may be generalists that respond to various odorants, including pheromones, whereas microvillous ORNs may be specialists which are specific to amino acid odorants. Amino acid analysis by HPLC showed that the urine sample from immature rainbow trout contained mainly urea, taurine and ammonia (>100 mM), together with 35 amino acids (1–40 mM). The number of amino acids and their concentration of the urine sample from ovulated females were a little higher than those of the sample from immature fish. Bile acid analysis by HPLC also showed that both urine samples contained a small amount of glycoursodeoxycholic acid (20–100 nM) and taurocholic acid (600–800 nM).

331. Comparison of transduction mechanisms underlying NaCl- and KCl-induced responses in non-dissociated mouse taste cells

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Amiloride-sensitive Na^+ channels are thought to be a main contributor to the transduction of the salt-induced response because a diuretic, amiloride, greatly suppresses the salt-induced response recorded from the chorda tympani nerve in the mammalian gustatory system (Sato *et al.*, 1994, Zool. Sci., 11: 767–780; Lindemann, 1996, Physiol. Rev., 76: 719–766). The chorda tympani nerve responses induced by K-salts are, in general, known to be insensitive to amiloride (Sato *et al.*, 1994; Lindemann, 1996), but the responses induced by lower concentrations of K-salts are only partially suppressed by amiloride (Lundy *et al.*, 1997, Am. J. Physiol., 273: R1923–R1931).

The transduction mechanism of salt-induced responses of mouse taste cells was investigated using the patch-clamp and locally stimulating techniques under the quasi-natural condition. The local stimulation of the apical membrane with 0.5 M NaCl induced inward current responses of –14 ± 2 pA at a holding potential of –80 mV. Some NaCl-induced responses were suppressed by the diuretic amiloride, with a K_i of 0.2 μM, but other responses were not affected by amiloride. The reversal potential (E_r) of the amiloride-sensitive current was 103 ± 5 mV. The E_r of the amiloride-insensitive current of NaCl-induced responses greatly varied from negative to positive potentials. A Cl^- channel blocker, NPPB (25–100 μM) reversibly potentiated NaCl-induced responses with a positive shift of the zero-current potential (V_0), while responses induced by NaCl were suppressed by a nonselective cation channel blocker, 1 mM Cd^{2+}, with a negative shift of V_0. An apically applied 0.5 M KCl induced an inwardly rectifying current (I_{ir}) of –42 ± 6 pA at a holding potential of –80 mV. The E_r was more positive than the equilibrium potential of K^+ (E_K). The I_{ir} induced by 0.5 M KCl was not affected by amiloride. On the other hand, KCl-induced responses were suppressed not only by external Cs^+ and Ba^{2+}, which are blockers of inwardly rectifying K^+ channels, but also a Cl^- channel blocker, 500 μM niflumic acid. The E_r of KCl-induced response was independent of the apical ionic concentration, but rather was close to the equilibrium potentials of Cl^- (E_{Cl}) at the basolateral membrane. The KCl-induced I_{ir} displayed a fast rundown under the condition of the conventional whole-cell clamp method, but no rundown was observed using the perforated patch method. NaCl-induced responses also did not show any rundown. A similar I_{ir} was induced by internal 300 μM GTPγS, and an adenylate cyclase blocker, 250 μM SQ22536, partially suppressed the KCl-induced I_{ir}, suggesting that the transduction mechanism for KCl response requires some intracellular factors involving G_s-coupled adenylate cyclase. It is concluded that the transduction mechanism of NaCl-induced responses is completely different from that of KCl-induced responses in mouse taste cells.

332. Electrophysiological recordings of the responses to quinine and denatonium in isolated mouse taste cells

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Multiple transduction pathways are proposed for the perception of bitter taste. In mouse taste cells, it is suggested that bitter substances block ionic channels or activate G proteins via taste receptors. G proteins will activate the effectors and change the levels of the second messengers, cyclic nucleotide or IP_3. To date, we have recorded the electrophysiological responses to bitter substances, and shown that several responses were involved in isolated mouse taste cells (Seto *et al.*, 1999, Cell. Mol. Biol., 45: 317–325). In the present work, we investigated the responses to quinine and denatonium in isolated mouse taste cells. Also, the mechanisms of transduction pathways were examined by using some modulators of signal transduction cascades.

Taste cells were isolated from the tongue of an 8-week-old female mouse (C57BL/6J) by enzyme treatment. The electrophysiological responses were recorded by the whole-cell patch-clamp technique under the voltage-clamp mode (holding potential –80 mV). Current–voltage relationships were observed by using ramp voltage commands. Both 10 mM quinine and 1 mM denatonium were dissolved in Ringer solution (pseudo-extracellular solution) and applied to a taste cell by pressure ejection from a capillary glass. The filling solution of the electrode pipette (pipette solution) was Cs^+ pipette solution, which substituted Cs^+ for K^+ in the pseudo-intracelleular solution. To modulate a part of signal transduction cascades, IBMX, GDP-βS, etc., were added the pipette solution.

Quinine induced an inward current response with the normal pipette solution. While 1 mM IBMX was applied to the pipette solution, quinine still induced an inward current response, and the size of response was larger than that observed with the normal pipette solution. This is consistent with the result that was obtained when cGMP was applied to the pipette solution (Shigeta *et al.*, 1998, Jpn. J. Taste Smell Res., 5: 479–482). Quinine also induced an inward current response in the presence of 1 mM GDP-βS in the pipette solution. This suggests the existence of a cascade which does not involve G protein. Denatonium induced an outward current response with the normal pipette solution. One millimolar IBMX in the pipette did not affect the outward current response. This is consistent with the result that was obtained when cGMP was applied to the pipette solution (Shigeta *et al.*, 1997, Jpn. J. Taste Smell Res., 4: 441–444).

333. Responses of pharyngeal taste nerve fibers to fatty acids in rats

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Fat in food is an important constituent that affects the food's palatability. Although it is generally accepted that the texture of fat is the most prominent feature of the palatability, the mechanisms by which fat is detected in the mouth have not been clarified. Recent behavioral studies suggest the existence of chemosensory mechanisms in the oral cavity for the detection of fat. However, there are few studies that have examined directly whether fat influences the activities of the taste nerves. This study was therefore designed to investigate the responsiveness of the glossopharyngeal nerve to fat or fatty acids. In particular, we studied the response properties of pharyngeal branch of the glossopharyngeal nerve because, in recent years, there has been increasing attention given to taste reception in the pharynx. Wistar rats were anesthetized with urethane and placed in the supine position. The pharynx and larynx were surgically opened to expose the posterior pharyngeal wall, posterior pillars and soft palate. Fat or fatty acids were applied to these regions of the pharynx. Oleic acid and linoleic acid were used as the fatty acid stimuli because these long-chain fatty acids are preferred by rats (Tsuruta *et al.*, 1999, Physiol. Behav., 66: 285–288). The nerve activities were recorded from the whole bundle or pauci-fiber bundles of the pharyngeal branch of the glossopharyngeal nerve. Oleic acid elicited vigorous discharges in the pharyngeal nerve. The response activities immediately increased after the application and continued for >10 s. Linoleic acid also elicited an excitatory response, but the magnitude of the response was smaller than that for oleic acid. Triolein, which was used as a pure fat, had no effect on the nerve activity. Vegetable oil (safflower oil) also had no effect. Paraffin oil and mineral oil, non-fat substances with a fat-like texture, did not evoke any response. These results indicate that only fatty acids had potent excitatory effects on the pharyngeal branch of the glossopharyngeal nerve. The present findings provide the first evidence for the existence of taste nerve fibers which respond to long-chain fatty acids.

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334. Electrophysiological recordings of mouse taste cell responses to ibotenic acid as an umami substance

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Monosodium glutamate (MSG) elicits a unique taste in humans called 'umami'. Recent studies suggest that several mechanisms are involved in the transduction of umami taste. Ibotenic acid (IBO) is a non-selective agonist of NMDA and metabotropic glutamate receptors. In humans, IBO elicits a significantly stronger umami taste than MSG. We have previously shown that MSG (10 mM) induced three different responses in mouse taste cells under whole-cell voltage-clamp: a transient inward current, a sustained inward current and an outward current. We report here responses of mouse taste cells to IBO (1 mM).

Taste cells were isolated from 8- to 9-week-old female mice of the C57BL/6J strain. The mice were killed by cervical dislocation and their tongues removed. Ringer solution containing collagenase, elastase, DNase and amiloride was injected under the epithelium of the tongue. After incubation at 31°C in divalent-free Ringer solution for 15 min, the epithelium was peeled off and taste cells from the vallate and foliate papillae individually collected in a capillary. Cells were plated on a Concanavalin A-coated glass-bottomed dish and allowed to settle. Whole-cell currents were

recorded from individual taste cells under whole-cell conditions at a holding potential of –80mV. Stimulus solutions were applied by pressure ejection from glass pipettes.

IBO induced two different responses in taste cells under whole-cell patch-clamp recording: a transient inward current and a sustained inward current. The amplitude of the transient inward current was 160–360 pA, while that of the sustained inward current was 10–40 pA. The amplitude of the transient inward current elicited by IBO was considerably larger than the transient currents evoked by MSG (20–160 pA). In Na^+,K^+-free Ringer, only transient inward currents were observed in response to IBO, suggesting that these currents could be carried by Ca^{2+}.

335. Inositol-1,4,5-trisphosphate and cyclic ADP-ribose induce responses in turtle olfactory sensory neurons

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Using the whole-cell mode of the patch-clamp technique, we recorded inward currents in response to inositol-1,4,5-trisphosphate (IP_3) in turtle olfactory sensory neurons (Kashiwayanagi *et al.*, 2000, Eur. J. Neurosci., 12: 606–612). Dialysis of IP_3 into the neurons induced inward currents with an increase in membrane conductance in a dose-dependent manner under the voltage-clamp conditions (holding potential: –70 mV). The application of Ca^{2+}-free Ringer solution to neurons previously dialyzed with IP_3 induced inward currents that were reversibly inhibited by application of Na^+,Ca^{2+}-free Ringer solution, normal Ringer solution or 10 μM ruthenium red. The present study demonstrated that IP_3-mediated transduction pathways exist in turtle olfactory receptor neurons.

Next, we investigate a possible role of cyclic ADP-ribose (cADPR) as a novel second messenger in the olfactory transduction pathway by using a combination of electrophysiological and calcium imaging methods. The cells responded to dialysis with cADPR with an inward current, an increase in membrane conductance and an increase of the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$. Dialysis of cADPR induced inward currents in a dose-dependent manner. Flooding of cells with 100 μM cADPR from the pipette also induced an inward current without changes in $[Ca^{2+}]_i$ in Ca^{2+}-free Ringer solution. In Na^+-free Ringer solution, cADPR induced only small inward currents with increases in $[Ca^{2+}]_i$. Inward currents and an increase in $[Ca^{2+}]_i$ induced by cADPR were completely inhibited by removal of both Na^+ and Ca^{2+} from the outer solution. The experiments suggest that cADPR activates a cation channel at the plasma membrane, allowing inflow of Na^+ and Ca^{2+} ions. The magnitude of inward current responses to cAMP-increasing odorants were reduced by desensitization of cADPR responses achieved by dialysis of high concentration of cADPR or dialysis of 8-Br-cADPR, an antagonist, while that to IP_3-increasing odorants were not changed. These results suggest that a large component of responses to cAMP-increasing odorants may be generated via cADPR while those to IP_3-increasing odorants may not be generated via cADPR in turtle olfactory cells.

336. Electrical properties of reciprocal synapses in the mouse accessory olfactory bulb

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Female mice develop a long-lasting olfactory recognition memory of a partner male at the first relay in the vomeronasal system. The goal of our research is to understand the mechanism of synaptic transmission in the accessory olfactory bulb (AOB), which is involved in olfactory memory. Recent research, including molecular biological, pharmacological, electron-microscopic and electrophysiological studies, revealed that reciprocal synaptic events in the AOB are critical to memory formation for male pheromones. Relatively little is known, however, about the mechanism of synaptic transmission between dendrites in the AOB. To investigate the properties of the synaptic transmission, evoked synaptic currents were measured from mitral /tufted cells in slice preparations with the patch-clamp technique in nystatin-perforated whole-cell configuration.

AOB slices were prepared from 10- to 35-day-old BALB/c mice. Gravity was used to deliver a constant stream of solutions from an array of microcapillary tubes. All experiments were performed at room temperature.

To evoke dendrodendritic inhibition, a depolarizing voltage step from –70 to 0 mV (5–50 ms) was applied to a mitral/tufted cell. Under control conditions, the voltage step evoked a fast inward current which was primarily carried by Na-channels and was followed by inhibitory postsynaptic currents (IPSCs). The IPSCs were greatly enhanced by washout of extracellular Mg^{2+}. Following addition of tetrodotoxin (TTX, 1 μM), the fast inward current was blocked, but a slow inward current followed by a slowly decaying barrage of IPSCs still remained. The IPSCs were blocked by addition of a $GABA_A$ receptor antagonist, bicuculline (10 μM). These results demonstrate that IPSC in mitral/tufted cells can be elicited through purely dendritic interactions and is mediated by $GABA_A$ receptors. The present results obtained so far suggest that the reciprocal synapses in the mouse AOB have similar characteristics to those reported in the rat main olfactory bulb in respect of the sensitivities to TTX, bicuculine and extracellular Mg^{2+}.

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337. Effects of NMDA receptor agonists and antagonists on oscillatory signal propagation revealed by optical and electrophysiological recordings

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A characteristic oscillatory signal propagation elicited by a single electrical stimulation to vomeronasal nerve layer (VNL) was found in a guinea-pig accessory olfactory bulb (AOB) slice (Sugai *et al.*, 1997, Neuroscience, 79: 871–885). To analyse the mechanism for

the oscillation, it is important to elucidate both inhibitory and excitatory synaptic interactions in the bulbar neural circuit. Our recent study showed that two types of GABAergic inhibitory actions, $GABA_A$ and/or $GABA_B$ action, had different effects on the signal propagation in the different bulbar layers (Sugai *et al.*, 1999, Eur. J. Neurosci., 11: 2773–2782).

In this study, the role of NMDA receptors in oscillatory signal propagation was investigated by using optical and electrophysiological recordings. In response to VNL stimuli, characteristic optical signals appeared in each layer of the AOB; in the VNL, there was a transient presynaptic response; in the glomerular layer (GLL), pre- and postsynaptic responses; and in the external plexiform, mitral cell layers (EPL/MCL) and granule cell layer (GCL), a damped oscillatory response. Bath application of a non-NMDA receptor antagonist CNQX suppressed most of the postsynaptic response in the GLL and the EPL/MCL/GCL, but not a small late component. The CNQX-resistant component was slightly reduced or unaffected by the subsequent application of an NMDA receptor antagonist 2-amino-5-phosphonovalerate (APV). These results suggest that the neurotransmitter released from the vomeronasal nerve terminals in the GLL is glutamate and that the synaptic receptors would be predominately a non-NMDA type. Perfusion with APV or MK-801 alone increased the frequency of oscillation in the EPL/MCL, where mitral cells make reciprocal dendrodendritic synapses with granule cells. The removal of Mg^{2+} in the medium abolished the oscillation; the subsequent application of APV or MK-801 restored the oscillation, suggesting that inhibition of the oscillation may be mediated with NMDA receptors. Further, paired-pulse shocks delivered at an interval of 300 ms markedly depressed the oscillation in response to test shock. After application of APV or MK-801 the oscillation recovered moderately. These results suggest that, in the dendrodendritic synapses in the EPL/MCL, NMDA receptors on the granule cells may enhance GABA release, which could strongly inhibit the activities of mitral cells, resulting in a cessation or in a long lasting depression of the oscillation.

338. Vaginal stimulation modulates the dendrodendritic interaction between mitral/tufted and granule cells in the mouse accessory olfactory bulb

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Female mice, when mated, form an olfactory memory of the pheromones of the mating male. The neural changes underlying this memory occur at the accessory olfactory bulb (AOB) (Kaba *et al.*, 1989, Neuroscience, 32: 657–662), depend upon vaginal stimulation at mating, and involve changes at the reciprocal synapses between mitral/tufted and granule cells. Vaginal stimulation at mating promotes noradrenaline release in the AOB (Rosser and Keverne, 1985, Neuroscience, 15: 1141–1147) and removal of noradrenergic innervation prevents the formation of the olfactory memory (Keverne, 1983, Trends Neurosci., 6: 381–384). However, the action of vaginal stimulation on the reciprocal interaction remains to be elucidated. Therefore we have examined the effects of vaginal stimulation on paired-pulse depression at the reciprocal synapses and the single-unit activity of mitral/tufted cells.

Adult BALB/c female mice showing estrous vaginal smears were used. Following urethane anesthesia, the animals were placed in a stereotaxic instrument in the prone position and had two holes drilled in the skull to allow access to the AOB and amygdala. A co-axial bipolar stimulating electrode was guided into the amygdala. A glass micropipette was lowered into the AOB external plexiform layer to record potentials evoked by amygdala stimulation. The strength of paired-pulse depression of evoked field potentials was measured by applying paired stimuli of equal intensity at an intra-pair interval of 40 ms. Vaginocervical stimulation was carried out using a glass probe or a latex baloon catheter.

Vaginal stimulation suppressed the paired-pulse depression which is believed to be due to granule cell-mediated inhibition of the mitral/tufted cells. Consistent with the result, vaginal stimulation enhanced the single-unit activity of mitral/tufted cells. These results suggest that vaginal stimulation suppresses granule cell-mediated inhibition of mitral/tufted cells, thereby enhancing the activity of mitral/tufted cells.

339. Oscillations and coherent activities in the rat olfactory bulb

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Oscillatory and synchronized neuronal activities have been observed in many sensory systems. Recent data in the invertebrate (Laurent and Davidowitz, 1994, Science, 265: 1872–1875; Wehr and Laurent, 1996, Nature, 384: 162–166) seem to indicate that (i) odours appear to be represented by dynamical assemblies of transiently synchronized neurons; and (ii) the relative position of their spikes to the local field potential could be essential for coding.

Based on these works, we intended to evidence such processes in mammal. Extracellular unitary activities were recorded simultaneously with local field potentials (LFPs) in the olfactory bulb (OB) of anaesthetized rats, in response to odorous stimuli. Three sets of recordings were performed, depending on the distance between the two recording sites. In a first set, the two pipettes were placed in two different zones of the OB; in a second set, the two electrodes were lowered in the same bulbar zone but separated by at least 1 mm; in a third set, the tips of the two pipettes were glued together with an inter-tip distance of <50 μm. Recording sites were labelled with a dye and identified histologically.

Preliminary results show that LFPs are very different according to the OB layer. Even when electrodes are separated by <50 μm, LFPs are not completely similar but coherence is greater during γ activity. Concerning unitary activities, coincident spikes are very scarce between distant cells. They are more frequent in the close group, where we observed either coincident individual spikes or coincident bursts of spikes with small temporal and frequential shifts. Coherence and time-frequency analyses are in progress in order to determine whether synchronized spikes could appear more frequently during a particular oscillatory rate.

340. Low-frequency oscillations in the rat olfactory bulb *in vitro*

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Highly synchronized, low-frequency neuronal oscillations (LFOs) are found in neurons of the rat olfactory bulb (OB) upon disinhibition with $GABA_A$ antagonists (bicuculline 10 mM or picrotoxin) and/or removal of Mg^{2+} from external saline. LFOs demonstrated a highly organized temporal structure, with regular bursts occurring at ~0.05 Hz. In this study, LFOs were analyzed using whole-cell patch-clamp recordings in slices of rat OB.

LFOs were present in both relay (mitral and tufted cells) and intrinsic (granule and periglomerular cells) neurons throughout the OB, and consisted of large (~20 mV) depolarizations occurring every 18 s and lasting ~3 s. LFOs triggered high-frequency repetitive firing in relay neurons, whereas only single action potentials were observed occasionally in intrinsic neurons.

Paired-cell recordings from mitral cells showed that LFOs are in phase within the mitral cell layer. Under voltage-clamp conditions, LFO-underlying currents reversed at 0 mV. The pace of the oscillations was reset by olfactory nerve stimulation but not depolarizing currents injected directly into an oscillating cell. LFOs were reversibly abolished by Mg^{2+} and tetrodotoxin (0.3 μM), removal of $GABA_A$ receptor antagonists, substitution of Ca^{2+} with Ba^{2+}, blockage of Ca^{2+} channels with Cd^{2+} (100 μM), and by the NMDA receptor antagonist D-AP5 (50 μM) but not the non-NMDA receptor-channel antagonist NBQX (10 μM).

Sectioning a slice along the external plexiform layer, which separated the glomerular region from the inner part of the OB, irreversibly interrupted LFOs in neurons of the deeper bulbar layers. The results provided evidence that the bursting activity initiates in the glomerular region and propagates synaptically towards the interior of the bulb, thus suggesting an organizing role for the olfactory glomeruli.

The dynamics, pharmacology and origin of LFOs differ considerably from other oscillations in the OB described previously. LFOs are more akin to rhythmic oscillations that govern the creation of privileged pathways for propagating excitation in morphologically haphazard central neuronal networks. This robust phenomenon will be a useful test for computational models of the bulbar neuronal network.

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341. Neuromodulatory actions of taurine in slices of rat olfactory bulb

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In the mammalian brain, taurine is a ubiquitous free amino acid that is involved in several fundamental processes, including neuronal inhibition and neurotransmitter release. Among the brain regions studied, the main olfactory bulb (OB) has the highest levels (up to 10 mM) of taurine. In the OB, taurine is localized in various neurons and primary olfactory afferents, and appears to be the most abundant neuroactive amino acid. Although taurine is a conspicuous constituent of the OB, its physiological role in olfaction is unknown. We studied the effects of taurine on the membrane potential and EPSCs in identified bulbar neurons using whole-cell patch-clamp recordings in slices of rat OB.

Bath-applied taurine (1–10 mM) reversibly inhibited mitral and tufted cells in a concentration-dependent manner, and had no effects on a resting potential of periglomerular and granule cells. In mitral/tufted cells, a 75% reduction of the input resistance and a shift of the membrane potential towards E_{Cl} were observed. These effects were blocked by $GABA_A$, but not $GABA_B$, receptor antagonists and sustained under the blockage of synaptic transmission in a Ca^{2+}-free/Mg^{2+}-high solution. The data indicated that taurine increases chloride conductances via activating $GABA_A$ receptors and that this action is direct, not mediated by stimulating neuronal GABA release. Differential sensitivity of principal and intrinsic neurons to taurine may be due to the known difference in the molecular structure of $GABA_A$ receptors from these cells.

Voltage-clamp recordings from external tufted cells showed that taurine blocks spontaneous glutamatergic EPSCs, implying a significant decrease in the amount of the transmitter released from olfactory axons. Taurine also markedly depressed EPSC evoked in external tufted cells by olfactory nerve stimulation. This effect, observed at E_{Cl} and in the presence of bicuculline, could not be ascribed to activation of $GABA_A$ receptors and shunting EPSC by opening chloride conductances. The results suggest that taurine may moderate the excitability of some principal neurons in the bulb at both post- and presynaptic levels. Mechanisms of taurine actions on synaptic transmission in the OB remain to be elucidated.

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342. Voltage-gated currents of mouse taste bud cells in soft palates

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Taste buds occur not only in tongues but also in epithelia lining oral cavities in mammals. They are differently innervated and different in function, though their structural characteristics are similar to each other. For example, taste buds in soft palates are innervated by greater superficial petrosal nerves and are shown to sense nutrients in neonatal animals (Harada *et al.*, 2000, Physiol Behav., 68: 333–339). Taste bud cells (TBCs) in mammalian tongues have been investigated intensively. They functionally expressed various voltage-gated channels and appeared to use them in taste transduction, as reviewed by Lindemann (1996, Physiol. Rev., 76: 718–766). In contrast, little is known about the excitability of TBCs in the soft palate. In this study, we investigated their voltage-gated channels under in-situ patch-clamp conditions.

We applied an in-situ patch-clamp technique developed for TBCs in mouse tongues (Furue and Yoshii, 1997, Brain Res., 776: 133–139) to those in mouse soft palates.

TBCs fired action potentials and elicited various voltage-gated currents, including TTX-sensitive Na currents, TEA-sensitive

outwardly rectifying K currents, Cl currents and inwardly rectifying K currents. Almost all TBCs elicited Na currents and outwardly rectifying K currents. The magnitudes of voltage-gated currents (± SD, $n = 15$) were Na currents, 629 ± 424 pA at –15 mV; outwardly rectifying K currents, 515 ± 407 pA at +45 mV; and inwardly rectifying K currents, 77 ± 57 pA at –115 mV, where the holding potential of TBCs was –85 mV. The application of channel blockers or impermeant ions on either the receptor or basolateral membranes of TBCs showed that functional channels were expressed on the basolateral membranes, and that their receptor membranes expressed few channels. Under our in-situ patch-clamp conditions, the ionic environments on basolateral membranes are always physiological because epithelia and tight junctions prevent free diffusions of taste substances on receptor membranes. Therefore, these voltage-gated channels will co-operatively elicit action potentials in response to taste stimuli. Our previous study showed that TBCs in mouse fungiform papillae expressed various voltage-gated channels on their basolateral membranes (Furue and Yoshii, 1998, J. Neurosci. Methods, 84: 109–114), which agreed with the present results. These agreements suggest that action potentials are essential in the taste transduction mechanism of TBCs irrespective of differences in their location, function and innervation. It appears that action potentials effectively open HVA-Ca channels to release neurotransmitters. Alternatively, the capacitive currents generated by action potentials may stimulate taste nerves attached to TBCs.

343. Mapping odorant-responsive neurons in an intact coronal slice of newborn mouse olfactory epithelium by calcium imaging techniques

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Despite increasing molecular evidence of combinatory mechanisms for odorant–receptor interactions, we have little knowledge about the distribution of olfactory receptor neurons (ORNs) that respond to specific odorant molecules in the olfactory epithelium. In order to obtain spatial information on odorant discrimination and recognition at an epithelial level, we have developed a calcium imaging system to allow for the detection of odorant responses *in situ* in an intact epithelial slice preparation from a newborn mouse. First, we confirmed the presence of distinct spatial zones that express odorant receptors in newborn mice by *in situ* hybridization using probes from several odorant receptor genes that had previously been cloned. Next, we adapted calcium-imaging techniques using a calcium-sensitive dye, to monitor *in situ* responses of ORNs in intact coronal tissue slices from mouse olfactory epithelia. Stimulation with KCl solution, forskolin or IBMX elicited cytoplasmic calcium elevations in ORNs in the slice preparations. This method allowed us to visualize odorant-responsive ORNs in the coronal section, which were directly compared with the distribution and number of cells that express specific odorant receptor genes by *in situ* hybridization. Increasing odorant concentrations resulted in increases in the number of odorant-responsive ORNs, supporting the evidence that a single odorant molecule is recognized by multiple receptors that have differing dose–response properties. Furthermore, we found that the relative numbers of ORNs, which responded to specific odorant molecules, correlated well with the relative numbers of cells that showed responses in single cell preparations. We also investigated the spatial distribution of neurons that respond to a particular odorant of interest, such as odorants that show psychological effects. We are currently constructing a map of odorant responses in the olfactory epithelium in order to address the relationship between zonal expression of olfactory receptors and spatial patterns of odorant recognition, and to decipher the molecular architecture of odorant detection in the epithelium and signal inputs into the olfactory bulb.

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344. Effects of 3-methylindole on electrophysiological correlates of nasal chemosensitivity in rats

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Olfactory receptor cells and nociceptors of trigeminal nerve endings are the major elements responsible for nasal sensitivity to volatile chemicals. Intranasal stimulation with volatiles can activate both chemoreceptor systems, eliciting similar mucosal potentials. Data from human studies imply that vanillin mainly, if not exclusively, causes olfactory activation, whereas carbon dioxide (CO_2) is a trigeminal stimulant. It has been shown that exposure of rats to the toxic compound 3-methylindole (3-MI) results in a substantial loss of olfactory receptor cells and behavioral olfactory deficits. However, the influence of 3-MI on the electro-olfactogram (EOG) has not been studied, and it is unknown whether 3-MI affects the trigeminal system. We examined the effects of 3-MI (300 mg/kg, i.p.) on the EOG evoked by vanillin (35% v/v) and the trigeminal mucosal response to CO_2 (65% v/v) in rats.

Chemical stimuli (20 presentations each; duration 1 s, interstimulus interval 90 s) were delivered into the nasal cavity via an olfactometer in a constantly flowing (2 l/min) air stream. Potentials were recorded (electrode resistance ~20 kΩ255) from the nasal septum at 4, 8 and 16 days after 3-MI administration, averaged, and the mean amplitude for each post-3-MI time interval was determined. Responses obtained from non–3-MI-injected rats provided basal amplitude values which were similar for both stimulants. Relatively to the basal values, the EOG decreased to 6, 7 and 43%, and the trigeminal response decreased to 25, 38 and 51% at 4, 8 and 16 days post-3-MI, respectively. A marked depression of the EOG was expected because 300 mg/kg 3-MI is known to produce an almost total loss of olfactory receptor cells in rats. The significant increase in the EOG amplitude by day 16 may be due to a partial repair of receptor cells after toxic injury. The reduction of the response to CO_2 most likely resulted from 3-MI-induced damage to the tri- geminal system. Actions of 3-MI on the nasal mucosa presumably are restricted to olfactory regions. Therefore, it is possible that a residual response to CO_2 was generated by trigeminal elements located in the respiratory epithelium.

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345. GABAergic modulation in the frog olfactory bulb: electrophysiological study with baclofen and saclofen

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In the olfactory bulb $GABA_B$ receptors are selectively located in the glomerular layer. A current hypothesis is that GABAergic inhibition mediated through these receptors would be, at least partly, presynaptic and would exert by decreasing the release of the olfactory receptor neurons excitatory neurotransmitter. Here, for the first time, we assessed, in the frog, the *in vivo* action of baclofen, the $GABA_B$ agonist and saclofen, the $GABA_B$ antagonist upon both the spontaneous activity and the odour intensity coding properties of the mitral cells.

Baclofen (10^{-4} M) drastically affected mitral cell spontaneous firing rate: it suppressed spontaneous activity in 97% of the studied cells. By contrast, saclofen (10^{-3} M) had no effect on mitral cell firing rate. After baclofen application, all recorded cells still responded to odours, and the lack of spontaneous activity made their response bursts all the more salient.

Saclofen was tested in order to characterize the receptor target of baclofen. Saclofen did not produce any visible effect by itself on the spontaneous activity or on mitral cell responses. By contrast, the application of saclofen 10–15 min prior to baclofen was shown to prevent baclofen's action on spontaneous activity. Saclofen's action could be antagonized in a surmountable manner by successive applications of baclofen. Conversely, when saclofen was applied again after baclofen, a partial recuperation of the spontaneous discharge was observed.

The innovative aspects of this study show that $GABA_B$-mimicked inhibition suppressed mitral cells spontaneous activity, while odour responses were maintained. This suggests that olfactory receptor neurons partly drive spontaneous cell activity. Moreover, the effect of $GABA_B$-mediated inhibition was seen to be close to that we described previously for dopamine D_2 receptor-mediated inhibition (Duchamp-Viret *et al.*, 1997, Neuroscience, 79: 203–216). We propose that these two mechanisms would offer the possibility to reduce or suppress mitral cell spontaneous activity so as to make their response to odour especially salient.

346. Single unit extracellular recordings from the olfactory epithelium and patch-clamp recordings from isolated vertebrate receptor neurons reveal their ability to elicit excitatory and inhibitory odor responses

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Olfactory receptor neurons (ORNs) isolated from rat or toad chemosensory epithelia, obtained after a purely mechanical dissociation procedure, can be excited or inhibited by odors (Morales *et al.*, 1994, Proc. R. Soc. Lond. B., 257: 235). The excitatory or inhibitory response elicited by an ORN to a particular odor stimulus was not strictly related to the odor class. A small fraction of the tested neurons gave both types of responses. Excitatory responses, consisting of increases in action potential firing, were due to a depolarizing inward current (an Na^+ and Ca^{2+} influx through a non-selective cationic channel plus a Cl^- efflux through a Cl^--selective channel), leading to a depolarizing receptor potential. In contrast, inhibitory responses, consisting of decreases in the firing rate, were caused by a hyperpolarizing K^+ current (Morales *et al.*, 1994). In addition to these two response types, mediated by signal transduction cascades, odors affect firing through suppression, a non-specific reduction in the voltage-gated ionic currents (Sanhueza and Bacigalupo, 1999, Am. J. Physiol., 46: C1086). Similar excitatory and inhibitory transduction currents, as well as suppression effect, were observed both in toad (*Caudiverbera caudiverbera*) and rat ORNs. We tested whether excitatory and inhibitory responses were also generated by the ORNs present in the olfactory epithelium, and we verified in the toad that indeed they behave as the isolated ORNs, confirming previous observations in the rat (Duchamp-Viret *et al.*, 2000, J. Neurosci., 20: 2383) and in fish (Kang and Caprio, 1995, J. Neurophysiol., 73: 172), as well as in some invertebrates (McClintock and Ache, 1989, Chem. Senses, 14: 637). This complex neuronal behavior upon odor exposure indicates that an integrating sensory processing takes place at the first stage of the olfactory pathway, namely the ORN, followed by further integration stages at the olfactory bulb and higher levels of this sensory system.

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347. Modulation of the activity of olfactory receptor neurons by biogenic amines in *Mamestra brassicae*: an electrophysiological and molecular study

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Octopamine (OA) and serotonin (5-HT) are involved in the modulation of numerous functions in the insect central nervous system. Their effects on the peripheral system are less documented. Recently, *in situ* hybridization has revealed that OA receptors are expressed within the olfactory sensilla of *Heliothis virescens* (Von Nickisch-Rosenegk *et al.*, 1996, Insect Biochem. Mol. Biol., 26: 817–827). This prompted us to investigate the effects of OA and 5-HT on the electrical activity of specialist olfactory receptor neurons (ORNs) in the noctuid moth *Mamestra brassicae*.

Injection of OA at the antenna base enhanced the spontaneous firing of ORNs by a factor of four after 20 min. This enhancement was dose-dependent and reversible. The firing response of ORNs to Z11–16:Ac, the main component of the female pheromone, was enhanced in the same proportion as the spontaneous firing, independently from the dose of Z11–16:Ac. The slow depolarization of the sensillar potential in response to Z11–16:Ac was not altered.

Injection of clonidin, an OA-agonist in insects, also enhanced the spontaneous firing activity of ORNs. In turn, injection of 5-HT decreased the spontaneous firing in a dose-dependent manner.

Holomogous sequences to OA receptors were searched for by RT-PCR on *Mamestra brassicae* antennal cDNA. A cDNA encoding a protein presenting 98% homology with the *H. virescens* OA receptor gene has been cloned, confirming the expression of OA receptor within the olfactory tissues of *M. brassicae*.

In conclusion, OA and 5-HT modulate the activity of moth ORNs, either directly or by modifying the environment in the sensillum. Further experiments will be necessary to localize more precisely their site of action.

348. Discrimination between *R*-(–)- and *S*-(+)-carvones in mouse olfactory receptor neurons

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Each type of olfactory receptor neuron (ORN) generally responds to more than one odorant but can discriminate slight difference in stereochemical structure of odorants in a dose-dependent manner. Actually, human olfactory sense can discriminate between stereo-isomers of some odorants in stimulus strength or odor quality. The receptor (Krautwurst *et al.*, 1998, Cell, 95: 917–926) or glomerulus (Rubin and Katz, 1999, Neuron, 23: 499–511) responsive to both of the isomeric carvones has been reported, but little is known about alternative responses at the receptor level. We examined the discriminability to optical isomers of carvones in mouse ORN to understand the outline of the odor encoding.

We examined odorant tuning specificities of ORNs isolated from trypsin-treated adult male mouse epithelium by the tissue-printing method in Ca-imaging using fura-2 (Hirono *et al.*, 1992, J. Neurosci. Methods, 42: 185–194; Sato *et al.*, 1994, J. Neurophysiol., 72: 2980–2989). Each set of isolated ORNs was prepared from one of four zones of the olfactory epithelium in order to examine the difference of odorant responsiveness of ORNs among the zones for *R*-(–)-carvone (RC), *S*-(+)- carvone (SC), *n*-fatty-acids (nFA) and *n*-aliphatic-alcohols (nAA).

About 10% of tested ORNs responded to either of carvones. About 20% of 110 carvone-responsive ORNs (CR-ORNs) responded to RC with a lower concentration threshold than SC, that is, higher sensitivity to RC (RC-preferred ORNs), while ~10% of CR-ORNs showed lower threshold to SC (SC-preferred ORNs). The other CR-ORNs were sensitive similarly to both of isomers (non-discriminative CR-ORNs), although a part of them showed higher response magnitudes to either carvone than the other. CR-ORNs could be obtained from all epithelial zones, and the populations of them in each zone seemed to be less different than a factor of two. One-sixth of CR-ORNs also responded only to nAA, and a different one-sixth were responsive to both nAA and nFA. The maximal sensitivities in nAA or nFA were observed at 6–9 carbons chain length, especially at 9 carbons. The different tuning specificities of CR-ORNs to nFA or nAA suggested that there were more than three types of CR-receptors. The group of non-discriminative CR-ORNs which was a major population may contribute to a common part between RC-odor (spearmint-like) and SC-odor (caraway-like) and each group of RC- or SC-preferred ORNs may identify unique parts of the respective odors. In this manner, the receptor combinatorial code (Malnic *et al.*, 1999, Cell, 96, 713–723) enable us to discriminate optical isomers each other.

349. Chloride concentration in rat olfactory receptor cells measured with a fluorescent probe

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Inward-directed Ca^{2+}-dependent chloride currents are thought to prolong and boost the transient receptor currents generated in olfactory cilia in response to odorants (Kurahashi and Yau, 1993, Nature, 363: 71–74; Lowe and Gold, 1993, Nature, 366: 283–286). Chloride inward current, of course, requires a sufficiently high intracellular Cl^- concentration ($[Cl^-]_i$). In previous measurements, using a fluorescent Cl^- probe, $[Cl^-]_i$ of newt olfactory cells was estimated to only 40 mM (Nakamura *et al.*, 1996, Neurosci. Lett., 237: 5–8). This low value led us to re-examine the distribution of Cl^- by an improved procedure, using the fluorescent Cl^- probe MQAE and *in situ* calibration.

Isolated rat olfactory cells were loaded with fluorescent Cl^- probe MQAE. After recording the original fluorescent image of a cell containing MQAE under 380 nm excitation light, cells were permeabilized with ionophores and the *in situ* calibration was performed using bathing solutions of different $[Cl^-]$. The calibration was carefully corrected for the leakage of MQAE from the cell.

When isolated olfactory neurons of the rat were bathed in Tyrode's solution (150 mM Cl^-) at room temperature, the Cl^- concentration was 81.5 ± 42.6 mM in the tip of the dendrite (olfactory knob) and 81.8 ± 33.8 mM in the soma (n = 11). If the ciliary volume equilibrates with the knob chloride in the resting state, a Cl^- concentration near 80 mM would be present within the cilia also. The corresponding Cl^--equilibrium potentials (E_{Cl}) were –15.4 and –15.3 mV, respectively. Therefore, at resting potentials in the range of –90 to –50 mV, Cl^- currents are predicted to be inward and able to contribute to the depolarization induced by odorants. Yet, if the cell were to depolarize beyond –15 mV, Cl^- currents would be outward and facilitate re-polarization during excitation. This must be expected to occur while action potentials are fired. The Cl^- inward current is likely to cause a depletion of chloride within the narrow intraciliary volume. Model considerations predict, that this depletion is rapid enough—even with a starting value of 80 mM—to contribute to the time course of the receptor current. Future measurements of ciliary chloride dynamics will be required to resolve this point.

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350. Changes in ion concentrations in olfactory cilia during odorant detection: predictions from a numerical model

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Sensory cilia serve for odorant detection mediated by activated olfactory receptor proteins. On activation of the receptor, the ciliary concentration of the second messenger cAMP rises and cng-channels open. Ca^{2+} and Na^+ ions then flow into the filamentous intraciliary space (Nakamura and Gold, 1987, Nature, 325: 442–444). Subsequently, Ca^{2+}-activated chloride channels open

and inward chloride current now contributes substantially to the receptor current (Kleene and Gesteland, 1991, J. Neurosci., 11: 3624–3629; Schild and Restrepo, 1998, Physiol. Rev., 78: 429–466). Due to these currents, changes in concentrations of Ca^{2+} and Cl^- ions will occur within the small ciliary volume. These changes are counteracted by ionic equilibration between cilium and olfactory cell. The rate of equilibration is limited by the axial dimension (>100 μm in the frog) of the cilium. Therefore, which changes of ion concentration can be realistically expected within a cilium during odorant transduction?

To probe this question, a computer model of olfactory cilia was developed. It solves the partial differential equations of simultaneous transport and diffusion of several ionic species by numerical simulation based on morphological and biophysical detail. Length (typically 30 μm in the rat), mean diameter (0.1 μm) and number of ciliary segments (2–100) are choosable by the user. For each ciliary segment, the balance between membrane currents and axial electrodiffusion determines the concentration change of an ion. K^+, Na^+, Ca^{2+} and Cl^- ions can thus be monitored simultaneously. Axial diffusion between segments is calculated with the GHK formalism. The program adjusts diffusion potentials between segments such that electroneutrality is preserved (Kirchhoff's law).

At zero time, cAMP rises in a sigmoid time course, opening cng-channels. The rise can be restricted to one or a few segments, or be uniform throughout the cilium. The resulting local increase in Ca^{2+} activates Cl^- channels, boosting the receptor current. In consequence a local drop in Cl^- concentration occurs. Its magnitude, which varies along the cilium, is highly dependent on axial diffusion. Based on cytosolic diffusion coefficients and a realistic geometry, the model shows a significant drop in local Cl^- concentration during transduction. The drop is basically due to the difference in Cl^- selectivity of membrane channels and cytosol. The model predicts that the Cl^- depletion will co-determine the time course of the receptor potential or current.

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351. Characterization of somatosensory neural cells of petrosal ganglion in reference to food stimuli reception and response

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Taste stimuli, such as sweet and bitter, and somatosensory stimuli, such as pungency and hotness, all of which we received while eating, are then transmitted to the brain via cranial nerves that project to the pharyngo-oral region. Both gustatory and somatosensory neurons in glossopharyngeal nerves projected to the posterior tongue and pharynx are derived from the inferior glossopharyngeal ganglion, the petrosal ganglion (PG). However, it is still unclear in many respects about the gustatory and/or somatosensory cells in PG. To elucidate the properties of those cells that may control taste and somatosensory responses, we analyzed the expression patterns of neural marker molecules occurring in PG. Considering that somatosensory cells in PG share some common characteristics with trigeminal (TG) or dorsal root ganglia (DRG) cells, we first investigated somatosensory marker molecules in PG. Since vanilloid receptor subtype 1 (VR1) in DRG can be activated by capsaicin and nociceptive thermal stimuli and is also expressed in TG, this molecule probably functions to receive the sense of the hotness in the oral cavity. Also, the pungency and hotness to the posterior tongue and pharynx can be received by somatosensory neurons of glossopharyngeal nerve. However, it remains to be clarified whether VR1 contributes to the hotness reception in PG cells.

In the present work, we demonstrated by RT-PCR that VR1 is actually expressed in PG. Our *in situ* hybridization study revealed that VR1 mRNA is expressed in 50–55% of the PG cells investigated, and that all the VR1 mRNA-expressing cells are of small- to medium size. In about half of VR1 mRNA-expressing cells, substance P known as a marker molecule for somatosensory cells is expressed. When we analyzed the relation of VR1 to three neurotrophin receptors, TrkA, B and C, in terms of expression patterns, some of the VR1 mRNA-expressing cells are found to co-express either or both trkA and C mRNA. All the data above strongly suggest that VR1 in PG cells could be involved in the reception of food-derived pungency and hotness in the posterior tongue and pharynx.

352. Odorant-induced activities on piriform cortex of guinea-pig isolated whole brain with olfactory epithelium

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The odor discrimination initiates at the odorant receptor in the receptor neuron and completes in the central nervous system through sequential and parallel processing. The combinatorial receptor codes for odor has been supported by the evidence that a single type of odorant activated a unique set of receptors and each olfactory receptor neuron (ORN) expressed a single type of odorant receptor (Malnic *et al.*, 1999, Cell, 96: 713–723). The functionally convergent projection from the olfactory epithelium to the olfactory bulb (Mombaerts *et al.*, 1996, Cell, 87: 675–686) results in the odorant tuning specificity of the ORN (Sato *et al.*, 1994, J. Neurophisiol., 72: 2980–2989) looking quite similar to that of mitral/tufted cells (Mori *et al.*, 1992, J. Neurophysiol., 67: 786–789) and glomeruli (Rubin and Katz, 1999, Neuron, 23: 499–511) in the olfactory bulb. However, little is known about the functional role of the piriform cortex in the neuronal processing for odor discrimination, although the piriform cortex is the largest olfactory cortical area. In order to study the spatio-temporal properties of odor-induced activity of the piriform cortex, we developed an *in vitro* preparation of isolated whole brain with olfactory epithelium.

Whole brains were isolated according to previous papers (de Curtis *et al.*, 1991, Hippocampus, 1: 341–354; Muhlethaler *et al.*, 1993, Eur. J. Neurosci., 5: 915–926) and by adding some modifications for keeping the olfactory epithelium with the brain. Briefly, young adult Hartly guinea pigs were anesthetized with Nembutal and perfused for a few minutes through the aorta with cold oxygenated artificial plasma solution. After a complete craniotomy, the whole brain was removed with a part of the nose

cavity and started the arterial perfusion in the recording chamber at 14°C as the ventral side was up. The local field potentials (LFPs) were recorded in the piriform cortex at 27°C following the application of odorant solution to the olfactory epithelium via a tube inserted into the nose cavity.

The odor stimulation induced the slow negative LFPs recorded on the surface layer of the piriform cortex of the isolated whole brain. We observed that the oscillatory LFPs superimposed in the early phase of slow LFPs, as expected by the observed oscillations in the olfactory bulb (reviewed in Ketchum and Haberly, 1991, Olfaction as a Model System for Computational Neuroscience. The MIT Press, pp. 69–100; for recent papers see Kashiwadani *et al.*, 1999, J. Neurophysiol., 82: 1786–1792; Lam *et al.*, 2000, J. Neurosci., 15: 749–762) and the piriform cortex (Ketchum and Haberly, 1991). This result suggested that our *in vitro* preparations kept the original networks and activity as well as the intact tissue. The odor-induced LFPs in the piriform cortex were temporally and spatially more distinctive between different odors than those by the same ones in each preparation.

353. Novel subdivisions of the rat accessory olfactory bulb revealed by the combined method of lectin histochemistry, electrophysiological and optical recordings

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Two lectins, WFA and PNA, bound histochemically to the anterior and posterior regions of the vomeronasal nerve and glomerular layers (VNL and GLL), respectively, suggesting that an anatomical boundary exists in these two layers. Another lectin, RCA_{120}, bound strongly to the anterior region of the VNL and GLL, whereas it bound weakly and/or moderately to the rostral two-thirds of the posterior GLL but not to the caudal one-third of the posterior one. This suggests that the posterior region of the VNL and GLL is divided into further subregions, indicating that the rat AOB receives at least three different inputs. An electrophysiological mapping study also demonstrated that the rat AOB has at least three outputs.

Real time optical imaging showed that shocks to the anterior VNL produced neural activity which spread only within the anterior region of the external plexiform layer (EPL) and mitral cell layer (MCL), whereas shocks to the rostral two-thirds and the caudal one-third of the posterior VNL evoked, respectively, neural activity which spread only within the rostral two-thirds and the caudal one-third of the posterior EPL and MCL. Furthermore, the most anterior and posterior extents of the optical response evoked by shocks to the rostral two-thirds of the posterior VNL immediately adjoined, respectively, the maximum spatial extents of the optical responses evoked by shocks to the anterior VNL and to the caudal one-third of the posterior VNL. Moreover, the extreme distance of signal propagation in the granule cell layer (GCL) corresponded to that in the overlying EPL and MCL, indicating that the GCL also has similar boundaries. Thus, these optical imaging studies not only demonstrated individual precise boundaries in each layer of the AOB, which were positioned directly beneath the individual boundaries defined by lectin (RCA_{120}) histochemistry, but also confirmed the observations from the electrophysiological mapping study.

The presence of the functional segregation in each layer leads us to conclude that the rat AOB is distinctly divided into the anterior and posterior subdivisions, the latter of which admitted further subdivision, suggesting that there are at least three different input–output pathways in the rat vomeronasal system.

354. Penetration of amphipathic tastants through liposomal and taste cell membranes

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Some amphipathic tastants (i.e. those that possess hydrophilic and hydrophobic domains) may permeate liposomes and taste-cell plasma membranes (Peri *et al.*, 2000, Am. J. Physiol. Cell Physiol., 278: C17–C25), and activate G proteins directly *in vitro* (Naim *et al.*, 1994, Biochem. J., 297: 451–454). We hypothesize that amphipathic sweet and bitter tastants, due to their permeation into taste cells, stimulate downstream transduction pathways by means of receptor-independent mechanisms, in addition to their action on G protein-coupled receptors.

Our objectives were to study the permeation kinetics of the bitter tastants quinine and the cyclo(Leu–Trp) peptide, and of the non-sugar sweetener D-tryptophan into rat taste-bud cells. We also applied a commercially developed bitter taste-masking product (BMI-60) made up of phospholipids (Katsuragi *et al.*, 1997, Pharmaceut. Res., 14: 720–724) to investigate its effect on tastant permeation.

Circumvallate (CV) taste-bud sheets were isolated from rat tongue (Striem *et al.*, 1991, Cell. Physiol. Biochem., 1: 46–54), incubated with tastants for 30 s or 5 min, washed and permeabilized, and their intracellular content determined by HPLC using a fluorescent detector (Peri *et al.*, 2000). Intracellular tastant concentrations were estimated by approximate cell volume and the number of taste cells. Interaction of tastants with PC/CH liposomes and BMI-60 was tested using quenching of tastant fluorescence by KI (Lange *et al.*, 1994, Eur. J. Biochem., 226: 963–970).

Tastant accumulation inside taste cells was linear up to 30 s. The kinetics of quinine accumulation versus concentration appeared to favor accelerated diffusion or cooperative action (no clear saturation) whereas saturation kinetics occurred for D-tryptophan at concentrations of up to 60 mM. Thus transport proteins may be involved in the case of D-tryptophan. At a concentration of 90 mM, there was an accelerated accumulation that did not seem to be limited by transport number. Quinine and cyclo(Leu–Trp) translocated easily through liposomal membranes, whereas D-tryptophan did not. The former interacted with the bitter-taste masking agent BMI-60, which forms micelles/liposomes and thus may compete with the liposomes for tastants. BMI-60 at a concentration of 1% (w/v) significantly inhibited the accumulation of quinine and cyclo(Leu–Trp) in taste cells.

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355. Investigation of vibrational frequencies of small odorant molecules and their contribution in the partition function

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In a previous work (Ben Lamine and Bouazra, 1997, Chem. senses, 22: 67–75) we applied a statistical physics model on olfaction mechanism. By this model we could interpret some results which have been empirically established in literature. To calculate partition function Z_g in this model we neglected all internal degrees of freedom of odorant molecules. In this work we will introduce vibrational contribution in the partition function.

Starting from the fact that the vision and audition mechanisms induce an energy transfer (photon or a pressure acoustic energy), we postulate that an olfaction mechanism induces an energy transfer between the stimulus molecule and the receptor molecule. Considering all the possible energies that two molecules could exchange, we have deduced by an eliminatory process that vibrational energy is the only possible energy we could not eliminate. Thus we confirm that molecular vibrations originate the olfactory phenomenon, which has been advanced *ad hoc* in the literature before (Martin and Laffort, 1991, Odeurs et désodorisation, TecandDoc Lavoisier, p. 137). All the known theories (chromatographic steriochemical) are only preliminary stages to the final vibrational energy transfer.

Avoiding large molecules, as has been done before, we will be here interested principally in little molecules (mono-, di-, tri-, tetra-atomic . . .). This is in order to be able to localize their limited number of vibrational frequencies. This led us to study the vibrational frequencies of odorant and odorless molecules and to separate them into the complete vibrational band between 0 and 4160 cm^{-1}. This in turn enabled us to delimit an 'olfactible' band between 0 and 1350 cm^{-1} that contains all bands determined *ad hoc* in the literature (Turin, 1996, Chem. senses, 21: 773–791), such as Hamilton Wright's band between 100 and 500 cm^{-1} or Huey Wright's one between 100 and 1000 cm^{-1}. This reinforces spectroscopic character of odors. Turin's band (Turin, 1996), like Dyson's (Martin and Laffort, 1991), stretches out all over the vibrational band up to 4160 cm^{-1}. Nevertheless, in addition to the introduction of an unusual supplementary phenomenon (tunnelling), the contradictions in this band are numerous (Martin and Laffort, 1991).

We are led to take into account the vibrational contribution into the partition function. Some consequences have been deduced; in particular, we have studied how vibration contributes to the analytical expression of the olfactory threshold.

356. Time–frequency analysis on coherent activities in the rat olfactory bulb

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The functional meaning of oscillatory neuronal activities in the olfactory system has not yet been established, but some works suggest that a coherent activity in a neural assembly could be revealed. The examination of the relative positions of temporal sequence of spikes to the local field potential (LFP) is one such study. Some results in molluscs (Delaney *et al.*, 1994, Proc. Natl Acad. Sci. USA, 91: 669–673) and insects (Wehr and Laurent, 1996, Nature, 384; 162–165) are really just a first step, highlighting the unitary activity phase-locked to the oscillation and the importance of the precise temporal sequence of spikes. However, the situation is not so clear in the olfactory systems of mammals because of number of neurons, and also because the representation of odour seems to be more redundant (Slotnick *et al.*, 1997, Brain Res., 762: 89–96).

With the aim of clarifying some aspects of this problem in mammals, we deal with extracellular unitary activity recorded simultaneously with LFP in the olfactory bulb (OB) of anaesthetized rats, in response to odorous stimuli. Here we examine only one point on the OB with one or two pipettes stuck together with an inter-tip distance 50 μm. Recording sites were labelled with a dye and identified histologically. Moreover, together with these classical methods, we investigate electrophysiological sources using the latest techniques in signal analysis.

Our treatment of electrophysiological signals allowed us to separate spikes and LFP, and this work produced the first aspect of our results: (i) we found at the same location cells with activity on a large size scale (ranging from a few Hz to a few tens of Hz). Without consideration for its mean spontaneous frequency, the cell could respond or not to the odorous stimulus. For a positive case, a transient regime precedes a permanent one. The inter-spike variance is less than in spontaneous activity. (ii) Time–frequency analysis of the LFP showed a marked increase in the oscillatory rate with duration of stimulus and also a relative decrease in the local frequency inside wave stationed in gamma-band. The second aspect of our study dealt with reconciling two points (i) and (ii). It seems clear that there is no general rule of phase-locked spike/LFP: some bursts come with no wave, others do come with one. This depends on the part of the layer in OB. These results argues in favour of a sparse but redundant code.

357. Stimulus encoding at the early stages of an artificial olfactory system

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This work demonstrates how a simple front-end model to an artificial olfactory system can track changes in the chemical stimuli as measured by a population of fluorescent chemical sensors. It is clear from the results that a rate code representation of odour intensity, as is used at the receptor stage of the biological olfactory pathway, can be efficiently decoded at the first layer of the olfactory bulb by integrating spike-trains obtained from many sensory neurons. However, to do this accurately a large population of probabilistic neurons is required so that individual spikes can be aggregated and slow charging-time constants at the glomeruli are required to remove the high frequency components. In this paper we compare how the efficiency of information transmission

compares between a rate-coded and graded signal transmission of the stimulus at the receptor stage.

An important question related to how well glomeruli are able to track changes in the stimulus is whether using a rate-coded representation of the stimulus at the receptor level degrades the signal quality at each glomerulus. To investigate this issue, we compare two model of the early stages of the olfactory pathway, one in which probabilistic spike trains representing the receptor input are integrated at the glomerular cell and another in which the graded receptor input was transmitted directly to the glomerulus for integration. The signal-to-noise ratios (SNRs) obtained from both models were compared. The results demonstrate that direct transmission of graded receptor input gives rises to high SNR and is robust to variations in the charging time-constant of the glomerulus. For very short time-constants the rate-coded equivalent does very poorly, achieving an SNR at the glomerulus that is worse than that obtained from a single receptor. However, for longer integration periods the SNR achieved under the rate-coded regime approaches that of the graded signal transmission, showing that a similar efficiency of information transmission can be achieved under favourable conditions. The cost for adequately recovering a reasonable SNR at the glomerular is, though, a far longer integration period, which slows the dynamics of the system as a whole. A rate-coded spike modulated transmission has clear advantages in terms of its robust properties in the face of external noise sources, and so is preferable for transmission over long distances. This demonstrates the efficiency of spike-based communication at the early stages of an olfactory system, with the constraint that there is an apparent trade-off between integration time and signal quality.

This study demonstrates one aspect of the input stage to an artificial olfactory system that needs to be optimized so that the integrity of the signals generated at the receptor levels are maintained during the early stages of processing. Such issues are fundamental to the design of efficient artificial nose systems as well as postulating possible mechanisms that may be present in biology.

358. Some experiences with the application of e-noses to pharmaceutical samples

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The potential application of e-noses to food and beverage products have been explored in some detail. The literature contains examples of their use in taint detection and control, quality assurance and product development.

It is probably not widely appreciated that there are parallels to these applications in the pharmaceutical industry. The detection, and where possible quantitation, of volatile molecules provides a valuable insight into: chemical stability; degradation processes; the effect of environmental factors on products; quality control of raw materials; process control; effluent control; and changes in product flavour.

This information can be obtained non-invasively without destroying the sample. Examples based upon the examination of both ethical and OTC pharmaceutical products with conducting polymer and headspace mass spec. based e-noses will be presented to illustrate their actual and potential utility in the pharmaceutical sphere.

359. Sex-specific non-pheromonal taste receptors in *Drosophila*

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Sex differences in chemosensory capacities in insects have previously been directly linked to pheromone detection. In *Drosophila melanogaster*, there is a clear sexual dimorphism for the number and central projections of tarsal taste sensilla (Possidente and Murphey, 1988, Devl Biol., 132: 448–457). Using a limited set of non-pheromonal stimuli (salts, sugar, water), we were able to discriminate three types of tarsal sensilla in females and only two types in males. The female-specific type, which responds to sugar in a specific manner, is absent in males, except when male gustatory neurons were genetically feminized [genetic feminization was performed under the control of PGal4-Voila (Balakivera *et al.*, 1998, J. Neurosci., 18: 4335–4343), an enhancer-trap strain expressing Gal4 in the peripheral gustatory nervous system of the fly]. The fact that tarsal gustatory hairs exhibit a sexual dimorphism that affects the perception of non-pheromonal compounds suggests that sexual identity is more complex than had previously been thought.

360. Various kinds of inhibition in silkmoth pheromone receptor neurons

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An earlier study (Pophof *et al.*, 2000, J. Comp. Physiol. A, 186: 315–323) showed that the esterase inhibitor decyl-thio-trifluoro propanone (DTFP) inhibited electrophysiological responses of pheromone receptor neurons in the silkmoth *Bombyx mori*. From studies with tritium-labelled DTFP, it was suggested that this compound, probably in complex with PBP, competitively inhibits pheromone receptor molecules. A subpopulation of bombykol receptor neurons was shown to be inhibited by (±)-linalool (Kaissling *et al.*, 1989, J. Comp. Physiol A, 165: 147–154).

Here, the effects of DTFP and linalool on the extracellularly recorded elementary receptor potentials (bumps) elicited in silkmoth pheromone receptor neurons by weak bombykol and bombykal stimulation (Minor *et al.*, 2001, Chem. Senses, 26: 792) were investigated. Recordings were performed at 4°C to increase the bump amplitude. The occurrence of bumps in both the bombykol and bombykal receptor neurons of *B. mori* males was strongly suppressed by application of DTFP and recovered within

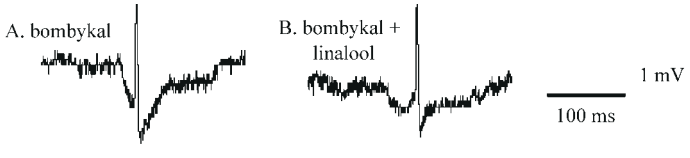

Figure 1. Bump with a nerve impulse elicited during continuous low bombykal stimualtion **(A)**, and addition of linalool **(B)**.

minutes in clean air. The amplitude and duration of bumps was not affected by DTFP, which supports the above idea that DTFP acts on pheromone receptor molecules.

In contrast to DTFP, application of linalool reduced the amplitude and duration of bumps (Figure 1) in the majority of both types of pheromone receptor neurons in *B. mori* males. The effect disappeared immediately after the end of linalool application. In a few receptor neurons, linalool either slightly increased the frequency of bumps, but not their amplitude, or remained without any effect. Similarly to DTFP, linalool inhibited only pheromone receptor neurons, but not other kinds of moth olfactory receptor neurons. Therefore, an unspecific effect as described for general anaesthetics (Stange and Kaissling, 1995, Chem. Senses, 20: 421–432) can be excluded. The gradual inhibitory effect of linalool indicates that it may modulate the properties of the receptor without inhibiting it completely, or act later on the transduction cascade, or directly on the ion channels mediating the responses to pheromone.

361. Olfactory event-related potentials: comparison of breathing technique in young and elderly

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Since the first recordings of cortical activity in response to olfactory stimuli were reported, constant improvements in recording methods and olfactometer design, especially by Kobal and colleagues, have rendered the olfactory event-related potential (OERP) a valuable and objective tool in the assessment of olfactory function in healthy individuals and a variety of clinical populations. Much of the previous literature on OERPs has employed the velopharyngeal closure (VC) method, in which subjects are trained in using the levator veli palatini muscle to elevate the soft palate in order to isolate the trachea from the nasal cavity. This procedure prevents intranasal respiratory airflow, and has limited the use of OERPs in populations unable to perform this artificial breathing technique.

The present study investigated the effects of normal breathing (NB) in comparison to VC on OERP component distribution in two populations.

Participants were 12 healthy young adults (mean age: 24 years) and 12 healthy elderly adults (mean age: 71 years). OERPs were recorded from three midline scalp electrodes (Fz, Cz, Pz) for 15 trials in each breathing condition, with an interstimulus interval of 3.5 min, using amyl acetate as the stimulus. A thermistor placed inside one nostril monitored nasal respiration and performance of VC. Subjects were exposed to both VC and NB in a counterbalanced block design and asked to report perceived stimulus intensity on the Labeled Magnitude Scale. In the NB condition subjects were instructed to breathe normally through mouth and nose, while stimulus presentation occurred during inspiration. In the VC condition subjects breathed only through the mouth.

Measurements included interpeak amplitudes for N1–P2 and N1–P3, and latencies for N1, P2 and P3. In both breathing conditions, elderly subjects showed significantly lower N1–P2 and N1–P3 amplitudes and longer latencies for N1, P2 and P3 than younger subjects. VC generated significantly larger N1–P2 amplitudes across all electrode sites compared with NB. The type of breathing technique used had no effect on N1 and P2 latencies. However, NB produced a trend towards shorter P3 latencies.

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362. Inhibition of sweetener time–intensity functions with 2-(4-methoxyphenoxy)propanoic acid: a kinetics analysis

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The purpose of this study was to determine whether a wide range of different sweeteners, representing multiple categories of compounds, would be inhibited by 2-(4-methoxyphenoxy)propanoic acid or a sodium salt (lactisole), and to what degree. The sweetness intensity of 10 sweeteners—sucrose, aspartame, acesulfame-K, Na-cyclamate, Na-saccharin, neohisperidin dihydrochalcone (NHDC), stevioside, glycyrrhizic acid monoammonium salt, thaumatin and Monellin—were rated on the Labeled Magnitude Scale (LMS) for 180 or 120 s.

All sweeteners were first approximated for equal sweetness intensity using a rough sweetness matching procedure. Next, sweetness intensity readings were taken automatically by computer every 100 ms over the first 10 s, then once every second over the next 50 s, and then every 5 s thereafter (solutions were expectorated at 5 s). Each sweetener was tested in the presence of 0, 50, 100 and 200 p.p.m. lactisole. 'Blank' sessions were included in which no sweetener was used, only different levels of lactisole, to test for the taste of the inhibitor. The compounds were tested in two experiments; thaumatin and NHDC appeared in both. Within an experiment, subjects rated every compound and the blank at the four levels of lactisole a total of three times. In any given test session a subject would only rate a single solution due to adaptation effects and the lingering aftertaste of lactisole.

The various sweeteners (without lactisole) had greatly differing time–intensity profiles, with the time to peak sweetness ranging from 1 to 30 s. They also differed greatly in their sweetness potency, ranging over six orders of magnitude in concentration when equally sweet. All 10 sweeteners were inhibited to varying degrees by lactisole. Some sweeteners were inhibited throughout the entire rating period in a dose dependent manner. Others were only inhibited during the first several seconds and were not inhibited at later times, occasionally showing later sweetness enhancement. Unsweetened lactisole solutions gradually grew in sweetness intensity beginning after ~20–30 s; the greater the lactisole concentration the greater the late-onset sweetness. Water rinses after lactisole were reported as intensely sweet.

Since all sweeteners were, at least initially, suppressed, lactisole appears to suppress the sweetness of compounds that vary widely in structure and sweetness potency. The time to peak sweetness intensity was loosely related to the size and weight of the compound. We calculated the binding affinity (K_d) for each compound by applying Beidler's taste equation, based on the concentration employed, the sweetness level stimulated and an estimated 'possible' maximum sweetness intensity between 'very strong' and 'strongest imaginable' on the LMS. Those compounds that were suppressed throughout the rating period had K_ds greater than lactisole and those that were only suppressed initially had K_ds less than lactisole. We therefore hypothesize that all sweeteners stimulate a single sweet receptor type (without excluding the

possibility of related subtypes), and lactisole competitively inhibits them at this site as a function of the ratio of the K_d of the sweetener to the K_d of lactisole.

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363. Psychophysical testing of olfactory function after pituitary surgery using the sublabial, trans-septal trans-sphenoidal approach

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Olfactory dysfunction following pituitary surgery is a rarely described complication and depends on the chosen approach. Operations performed sublabially, trans-septally and trans-sphenoidally seem to have a lower risk of postoperative olfactory dysfunctions than those using the pterional aproach.

We examined 24 patients (12 female and 12 male; mean age: 45.2 years, range 23–78 years) post-operation (2–44 months) psychophysically using the 'Sniffin' Sticks' test battery. Threshold, discrimination and identification (TDI) was tested and the TDI score calculated. All patients underwent ENT examination prior to testing including intranasal endoscopy. Moreover, all had to rate their subjective disturbance on a visual analogue scale (Vas). Three out of 24 patients were operated on twice and additionally one had postoperative radiotherapy. Six other patients had postoperative radiotherapy alone. In six patients endoscopy revealed a septal perforation.

One patient was bilateral anosmic, three unilateral anosmic, eight bilateral hyposmic, two unilateral hyposmic and 10 normosmic. The subjective disturbance was rated rather low in all patients. One of six patients with septal perforation was hyposmic, the other five being normosmic. Five of seven patients with radiotherapy were hyposmic, one was unilateral anosmic and one was normosmic.

The overall incidence of 58% of olfactory disorder is surprisingly high and, further, four patients had uni- or bilateral anosmia. Possible explanations may include damage to the olfactory epithelium on the septum caused by the preparation or postoperative radiotherapy. The unilateral disorder generally does not cause complaints by the patient due to normal odor perception on the contraleteral side. Nevertheless, a routinely performed preoperative smell test should be discussed.

364. Clinical testing of olfactory function with a new testing concept: random 24

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Testing olfactory function in the clinical routine should supply a maximum of diagnostic information whilst using financial and human resources sparingly. Routine examination of the olfactory sense is especially required in patients prior to surgery of the internal and external nose (e.g. septal correction, rhinoplasties), as well as surgery of the paranasal sinuses.

With a newly designed test procedure using 24 'Sniffn' Sticks', we tested 100 rhinological patients of the ORL Department. The test consists of two substances (phenylethanol and citronelal) in 10 dilution steps. The smelling substances (rose-like and lemon-like) are presented in pens, which are constructed like marker pens. Ten dilutions of each substance are arranged together randomly, together with four blanks. The patient has to decide after presentation of the pen whether he smells rose, lemon or nothing. The test was compared with the already established 'TDI'-test, which contains testing the threshold, discrimination and identification capabilities of the sense of smell.

The time required for testing was >10 min. Correlation to the TDI-score was high (0.74). The sensitivity of the test, the adequate identification of a hyposmic or anosmic patient, was as high as 95.2%. Only 2/42 people (hyposmic patients) were inadequately identified as normosmic. The normosmic people were correctly identified in 73%. The nine 'false positives' were all located in the border between normosmia and hyposmia.

Random 24 is an attractive test for clinical use. It is easy to use and of simple conception. The additional advantage is found in the simple terms to describe the substances as 'lemon', 'rose' or 'water', and in its multicultural knowledge. The high sensitivity and specificity make the testing especially attractive for clinical use.

365. A hypothetical short-term memory for integrating olfactory information: temporal distance from preceding stimuli influences olfactory event-related potential distribution

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The question analyzed was whether inter-stimulus intervals (ISIs) of different (and, for the experimental subjects, unpredictable) duration, within a series of equal olfactory stimuli, could influence the distribution of chemosensitive event-related potentials (CERP). The underlying hypothesis was that at longer ISIs some of the detected features of the preceding stimuli might not be fully preserved until the onset of the following stimuli. That could be because longer ISIs did not fit the temporal span of a hypothetical short-term perceptual memory mechanism, supposedly related at the psychological level to the phenomenon of 'actual present', enabling the integration of incoming information, and ongoing brain processes. A computer-controlled constant flow olfactometer was used for olfactory stimulation. The duration of inflow olfactory pulses was set at 200 ms. Phenyl ethyl alcohol (PEA) was used as the odorant. Odor onsets were triggered by inspiration. Five classes of ISIs were selected, their average duration being 3.6, 5.4, 9.4, 17.1 and 33.7 s. Odor stimuli were presented with a random sequence of ISIs of different duration. The subjects wore an electrode cap with 30 EEG electrodes. Maps of the spatial-temporal distribution of electrical potentials before, during and after odor presentation were analyzed. A late, positive polarity, 'P300'-like CERP component could be typically found within the temporal window of 1000–1400 ms after stimulus onset. With the shorter ISIs (3.6, 5.4 and 9.4 s) the amplitudes of positive potentials were equal over the anterior, central and posterior regions of the skull, approximately, whereas stimulations with the

longer ISIs (17.1 and 33.7 s) were characterized by lower amplitudes of positive potentials in the anterior region and higher amplitude potentials in the posterior region. These results point to the involvement of different brain structures in processing of olfactory stimuli, coming repetitively in series, and arriving after longer or shorter ISIs. These differences might be because after longer ISIs the incoming stimulus has to be processed as a new one, whereas after shorter intervals traces of previous stimuli are to some degree still preserved, and could, in some way, be integrated with the incoming information. It is possible to hypothesize that the duration of this hypothetical elementary integration period for olfactory stimuli might be somewhere between 9.7 and 17.1 s (which is longer than the 3 s period typical for some other sensory modalities and brain processes).

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