

## ABSTRACTS

# The Fifteenth Meeting of the European Chemoreception Research Organization

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The following also include abstracts from satellite symposia on 'Trigeminal Chemoreception', 'Olfaction in *Drosophila*' and 'Sensitivity to PROP (6-*n*-Propylthiouracil)'.

### Magnetic resonance imaging of the olfactory system

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Magnetic resonance imaging (MRI) offers a wide spectrum of possibilities to acquire information on the olfactory system. It is a radiation free technique which can be utilized repeatedly—respecting certain exclusion criteria—without any adverse effects. The high soft-tissue contrast can be enhanced by contrast agents. Contrast in MRI is based on tissue-specific parameters, such as proton density. Additionally, microscopic motion (diffusion), macroscopic motion (flow) and the level of oxygen are used for functional MRI. As a rule of thumb, T2-weighted sequences are selected to visualize pathology or fluid-surrounded structures, while T1-weighted sequences are used to display anatomy or contrast material enhancement. In terms of 'olfactory questions', further improvement can be reached through adequate choice of the type of scanner (B0 between 0.2 and 3 T). Using a low field MRI scanner, repeated acquisitions of the nasal cavity have been done to evaluate the nasal cycle, which contributes to the perception of odors. The nasal cycle revealed a high intra- and interindividual variability. Other studies used MRI to visualize the cavity of the vomeronasal organ (VNO). As indicated by the variability of the shape and volume of the vomeronasal cavity function, the functionality of the human VNO is questionable. Using a high field MRI scanner, two- and three-dimensional data sets on the olfactory bulbs (OB), tracts (OT) and sulci (OS) of healthy subjects and patients suffering from inborn or early-acquired anosmia were measured. In healthy subjects OB volume was 120–140 mm<sup>3</sup>, while anosmic patients had an OB volume of 15–30 mm<sup>3</sup>. Accordingly, in healthy subjects the OS had an average depth of 8.9 mm while the OS in subjects with hypoplastic OBs and/or OTs exhibited a mean depth of 5.5 mm. This difference was present only in a selected coronal plane called the 'plane of the posterior tangent through the eyeballs' (PPTe). Additionally, it was possible to differentiate OB aplasia from hypoplasia by measuring the depth of the OS. Aplasia of the OB went together with an OS depth of ≤4 mm, hypoplastic OBs were accompanied by an OS depth of >4 mm. This points to the hypothesis that gyrus growth may be influenced by the development of peripheral structures. A wide variety of tumors, infections and degenerative

disorders affect the olfactory system, e.g. olfactory schwannoma, olfactory meningioma, superficial siderosis, frontal head trauma or anterior cerebral artery aneurysm. The majorities of these pathologies require tailored imaging. Usually, this needs a close interaction between radiology and the chemosensory sciences. In conclusion, MRI is the imaging method of choice to evaluate the olfactory system. If a patient suffering from anosmia presents in an ENT department, MRI is mandatory to complete the pre-therapeutic diagnostics. Growing radiological experience in 'olfactory imaging' will stimulate the chemosensory sciences.

### Differential function of RNCAM isoforms in precise target selection of olfactory sensory neurons

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Olfactory sensory neurons (OSNs) are individually specified to express one odorant receptor (OR) gene among ~1000 different and project with precision to topographically defined convergence sites, the glomeruli, in the olfactory bulb. While ORs partially determine the location of convergence sites, the mechanism ensuring that axons with different OR identities do not co-converge is unknown. RNCAM (OCAM) is assumed to regulate a broad zonal segregation of projections by virtue of being a homophilic cell adhesion molecule that is selectively expressed on axons terminating in a defined olfactory bulb region. We here identify NADPH diaphorase activity to be an independent marker for RNCAM-negative axons. Analyses of transgenic mice that ectopically express RNCAM in NADPH diaphorase-positive OSNs show that the postulated function of RNCAM in mediating zone-specific segregation of axons is unlikely. Instead, analyses of one OR-specific OSN subpopulation (P2) reveal that elevated RNCAM levels result in an increased number of P2 axons that incorrectly co-converge with axons of other OR identities. Both Gpi-anchored and transmembrane-bound RNCAM isoforms are localized on axons in the nerve layer, while the transmembrane-bound RNCAM is the predominant isoform on axon terminals within glomeruli. Overexpressing transmembrane-bound RNCAM results in co-convergence events close to the correct target glomeruli. In contrast, overexpression of Gpi-anchored RNCAM results in axons that can bypass the correct target before co-converging to glomeruli located at a distance. Partial complementation of phenotypes in mice overexpressing both isoforms suggests that two distinct RNCAM isoform-dependent activities influence segregation of OR-defined axon subclasses.

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### **In vivo characterization of nasal trigeminal chemoreceptors**

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A wide variety of volatile chemicals stimulate nasal trigeminal nerve endings leading to a sensation of irritation. We have been examining possible ways that these stimuli interact with the nerve endings using pharmacological tools and multiunit recordings from the rat ethmoid nerve *in vivo*. First, we demonstrated that the neuronal nicotinic receptor (NnAChR) blockers, dihydro-beta-erythroidine hydrobromide, mecamlamine hydrochloride and guanethidine monophosphate significantly decreased trigeminal nerve responses to nicotine but not to cyclohexanone (CH). This provided evidence that alpha4- and alpha3-containing NnAChR subtypes are found in trigeminal nerve endings. Next, we showed that acetazolamide, a carbonic anhydrase inhibitor, significantly decreased trigeminal nerve responses to carbon dioxide, but not to nicotine. This suggested that CO<sub>2</sub> stimulation is mediated by intraepithelial acidification. Lastly, we demonstrated that capsazepine (CPZ), a competitive inhibitor of vanilloid receptors (VR-1) selectively decreases nerve response to carbon dioxide by ~50%, but leaves responses to nicotine unchanged. These results suggest both nAChR and VR-1 receptors are present on nasal trigeminal nerve endings. Other receptors responding to acid such as ASIC and P2X receptors may also be present to account for the 50% response to CO<sub>2</sub> which was not eliminated by CPZ. Still other receptor mechanisms must exist in order to account for responses to compounds like CH which were not affected by any of the blockers tested.

### **Molecular modelling of 17–40 olfactory receptor**

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In order to achieve deeper knowledge of the olfactory perception, the 'olfactory codex' need to be unveiled. We attempted to achieve this goal by building a 3-D model of the human OR 17–40 (Hatt *et al.*, 1999, Cell. Mol. Biol., 45: 285–291). Odorous molecules are recognized by our olfaction system, which is also able to finely discriminate them. Owing to the discovery of olfactory receptors and their functional expression, the molecular modelling, which describes the specific interactions between odorous molecules and olfactory receptor, has found an experimental confirmation. Methods for the 3-D structure prediction of a protein are based on the comparison of its amino acid sequence with that of already characterized proteins. This method makes use of databases and a standard computational program for the alignment of sequences. Once a suitable template was found, a 3-D model of the OR 17–40 was built by homology modelling. Docking, using the GRAMM program, was the next step. This program performs an exhaustive

6-D search through the relative translation and rotations of the molecules. Docking results show an odor-binding pocket on the extracellular surface, confirming the theory in accordance with the interaction between olfactory receptor and odorous molecule take place on the II and the V alpha-helices.

### **Does early experience determine mate choice in dwarf hamsters (*Phodopus campbelli* and *P. sungorus*)?**

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In Djungarian (*Phodopus sungorus*) and Campbell's hamsters (*P. campbelli*) an early social experience influences the later preference for conspecific males and their chemosignals (Vasilieva *et al.*, 2001). We aimed here to determine, in these two closely related species, whether early as well as late experiences with different species influence the choice the sexual partner. In experiment 1 juvenile males were reared from day (D) 1 to D30 either in a litter of conspecifics (C) or in a litter of a different species (cross-fosters, CF). At D60 males were tested for their preferences for con- and heterospecific stimuli: female urine, anesthetized females. C males showed strong preferences for conspecific stimuli; CF males preferred heterospecific stimuli. In experiment 2 normally reared 1.5- to 2-month-old males were paired with females of their own species (G-I) or with females of a different species (G-II). Two months later males were tested for their preferences for con- and heterospecific female stimuli. All G-I males preferred urine odor and anesthetized females of their own species; G-II males exhibited the reverse preference. The results of these two experiments demonstrate that early rearing conditions determine mate choice in Djungarian and Campbell's hamsters. However, the preference for a conspecific sexual partner may be reversed by breeding experience.

### **Characteristics of a decline in olfactory function with age measured by the 'Odor Stick Identification Test'**

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Odor identification ability, perceived intensity and pleasantness were measured by the 'Odor Stick Identification Test' for 191 participants between 20 and 86 years of age. The 'Odor Stick Identification Test' consisted of 13 odors that were common to Japanese people. We found that odor identification ability and the judgement of perceived intensity were relatively stable <60 years old but declined stepwise above 60 years old. Older people (>60 years) showed a decline in identification ability of odors and judged most odors to be weaker than younger people (<60 years). Older people also felt hedonics to weaker degrees than younger people, in particular offensive and dangerous odors in everyday

life, like 'gas for cooker', 'sweaty socks' and 'putrid smell'. On the other hand, older people showed similar familiarity judgement for these 13 odors as younger people. This showed the validity of the 13 odors as an odor identification test for different generations of Japanese people. These results indicate that there is a decline in olfactory function of older people (>60 years), consistent with prior research, and we should pay attention to the deterioration in the sensitivity of older people to dangerous odors in everyday life.

### Cross-cultural comparisons of distributions of tasters, non-tasters and supertasters of PTC and PROP

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A bimodal distribution for taste sensitivity for phenylthiocarbamide (PTC) or the related compound 6-*n*-propylthiouracil (PROP) has been found in every human population (except one) tested to date (see a review of >370 groups; Guo and Reed, 2001, *Ann. Human Biol.*, 28: 111–142). The proportion of non-tasters has varied from 1.3% (Nicaraguans) to 73% (French Caucasian women), for which there could be many possible explanations, in terms of genetics, methods of measurement and cultural influences. Using common psychometrics and stimulus delivery should add clarity when addressing the relationship between taster status, including highly sensitive individuals ('supertasters'), from which consumer and health issues arise. This paper describes research in Australia and Asia using the methodology of Bartoshuk *et al.* (1994, *Physiol Behav*, 56: 1165–1171), including the same (previously saturated and dried) filter papers for stimulus delivery and the rating scale derived from Green *et al.* (1993, *Chem. Senses*, 18: 683–702). Distributions of sensitivity in the Australian sample (adults, *n* = 577, including 312 Caucasians and 108 Asians) compared favourably with the US sample (young adults, predominantly Caucasian, *n* = 220). Australians were 13.7% non-tasters, 33.8% supertasters and 52.5% other tasters, compared with the US distribution of 16, 28 and 56%, respectively. Australian Asians were significantly different from Australian Caucasians, with fewer non-tasters and more supertasters, as did females in both groups. Groups of Asian women tested in Asia typically had fewer non-tasters and more supertasters than in the USA and Australia (% in sequence as above): Japan, *n* = 224, 3.6, 49.6, 46.9%; Indonesia, *n* = 86, 1.2, 64, 34.9%; Singapore, *n* = 123, 4.9, 46.3, 48.8%; and Hong Kong, *n* = 36, 8.3, 63.9, 27.8%. Causation of supertaster status will be discussed.

### Adult neurogenesis in the rat olfactory bulb

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In adult animals, neurons generated within the subventricular zone (SVZ), following the rostral migratory stream (RMS), reach the olfactory bulb (OB), where they apparently give rise to interneurons. In fact, there has never been a functional demonstration that these cells are true neurons. Filling this gap has been impeded

largely by the inability to distinguish between newly generated cells (NGC) in living brain tissue using conventional approaches. We have solved this problem combining retrovirus-mediated gene-transfer and patch-clamp electrophysiology. The viral vector, including a gene for GFP, was injected into the SVZ. After 2 weeks, GFP+ cells resembling periglomerular cells (PG) were observed in the OB. Using a whole-cell patch-clamp technique, we recorded action potentials from NGC in response to the injection of depolarizing currents, and we have isolated and characterized Na<sup>+</sup> and K<sup>+</sup> currents. About 95% of the NGC were PG cells; the remaining ended their migration between internal plexiform and mitral cell layers, and were identified as short-axon cells. The progressive maturation of NGC has been characterized in time and space along the RMS. When in the RMS, the cells show only a weak delayed rectifier K<sup>+</sup>-current, to which an A-current is added when the OB is reached. The mature cells lose the delayed rectifier K<sup>+</sup>-current, and only display A- and Na<sup>+</sup>-currents. Finally, by recording action potentials and excitatory synaptic currents in response to stimulation of the olfactory nerve, for the first time we have demonstrated that the NGC fully integrate into the bulbar circuitry, establishing functional synaptic contacts.

### The eye as a model for trigeminal irritation

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Using extracellular and intracellular recordings of corneal neurons, our group has shown that the eye is innervated by the peripheral axons of two main types of nociceptive neurons located in the trigeminal ganglion: mechano-nociceptive neurons, which respond only to injurious mechanical forces; and polymodal neurons, which are also excited by chemical irritants and noxious heat. Also, peripheral terminals of neurons responding to low temperatures are found in the cornea and conjunctiva, iris and sclera. Excitation of corneal neurons elicits predominantly pain sensations. However, studies of corneal and conjunctival sensitivity in humans have been limited to measurements of corneal threshold to mechanical stimulation. We studied whether different modalities of pain sensations are evoked by selective stimulation of the various types of corneal nociceptive neurons and what sensation is produced by stimulation of cold neurons. For this purpose, we developed a gas esthesiometer that permits the application of pulses of gas at controlled flow, temperature and chemical composition, to the center of the cornea in human subjects. Chemical stimulation was obtained with subthreshold flow pulses of a gas mixture containing variable concentrations of CO<sub>2</sub> and air at 33°C. Mechanical stimulation consisted in a suprathreshold flow of air at 33°C. For thermal stimulation, pulses of air at subthreshold flow value and a temperature of 5–30°C (cold) or 40–50°C (heat) were employed. The quality and intensity of the sensation were evaluated using verbal descriptors and a visual-analog scale. In young, healthy human subjects, sensations evoked by mechanical, heat (>45°C) and CO<sub>2</sub> (40–65% CO<sub>2</sub>) stimuli were reported as unpleasant, becoming overtly irritating at high intensities. The magnitude of sensation was roughly proportional to the stimulus intensity. Low temperature stimuli were identified as innocuous cooling, becoming irritating when corneal

temperatures of  $<30^{\circ}\text{C}$  were attained. Furthermore, subjects were able to distinguish the quality of the various types of stimuli, when applied in a random manner. These studies indicate that different intensities and qualities of pain sensation can be evoked by noxious stimulation of the cornea, possibly associated to the variable excitation of the different functional types of nociceptive neurons innervating this tissue. Finally, corneal neurons and nerve terminals contain neuropeptides (SP, CGRP). Our studies using corneas of transgenic mice indicate that these neuropeptides are released by noxious stimulation but not by cold, and participate in the neurogenic inflammation and perhaps also in the trophic maintenance and the reaction of tissues to injury.

### Response of trigeminal ganglion neurons to mechanical and cold stimuli

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We studied the mechanisms involved in the transduction of low- and high-intensity mechanical forces and temperature changes by primary sensory neurons. Using calcium imaging combined with cell-attach and whole-cell patch-clamp techniques, we studied the response of mice and guinea pig trigeminal ganglion neurons (TGN) in tissue culture and in the intact ganglion superfused *in vitro*, to volume changes evoked by cell perfusion with low osmolality solutions ( $-15$  to  $-45\%$ ), to temperature increase and decreases (to  $45$  or  $20^{\circ}\text{C}$ ) and to capsaicin ( $0.5\ \mu\text{M}$ ). Trigeminal ganglion neurons were distinguished based on the shape and duration of the action potential (AP) and on the presence of inward rectification (IR) as F-type (fast and short lasting AP, prominent IR) and S-type (slow rising, long duration AP with a hump in the falling phase, small IR). Moderate hypo-osmotic stretch evoked  $[\text{Ca}^{2+}]_i$  rises in 80% of TGN. In F-type TGN, the  $[\text{Ca}^{2+}]_i$  rise was rapid and prominent, while it was generally slow and less pronounced in S-type TGN. Taken together, these data suggest that a part of the F-type neurons correspond to low-threshold mechanoreceptor neurons while polymodal neurons with high mechanical threshold neurons possibly belong to the group of mechanosensitive S-type neurons. A small population (9%) of neurons responding to cold solutions with a  $[\text{Ca}^{2+}]_i$  rise were also identified in cultures of mice neurons. They exhibited short and fast APs, prominent IR and low rheobase and were sensitive to menthol, firing often in bursts. The demonstration that cold sensitivity could be induced in neurons previously unresponsive to cold by application of low doses of 4-AP suggests that the specific temperature sensitivity of cold neurons depends in part on the low expression of a slow, transient 4-AP-sensitive  $\text{K}^+$  current that normally acts as an excitability brake against the depolarizing effect of temperature decreases. In the guinea pig TG *in vitro*, cold solutions were unable to evoke impulse firing in the soma of any of the various types of TGN. However, after exposure to 4-AP, a fraction of F-type neurons developed cold-sensitivity. The firing response to cold seemed to originate in a portion of the neuron distant from the cell body. It can be hypothesized that cold-sensitivity is due, at least in part, to a particular blend of ionic channels expressed in a subset of TGN of the F-type. Propagated AP would be generated when changes in the activity of these

channels induced by cold evoke sufficient local currents to reach threshold depolarization levels, as may normally occur in the peripheral terminals of cold sensitive neurons.

### Sensitivity to odours

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For  $>20$  years, we have used standardized questionnaires in allergy centres as a help with the history in patients with allergic diseases. We frequently see patients who complain of odours, like perfume and flower scents, with symptoms from the respiratory tract, eyes, skin and more generalized symptoms, like headache and fatigue. There is an active debate in the press about problems with odours, and there has been a call for limitations of the use of perfumes in public places. Theatrical performances have even been made where no one in the audience or the actors used any cosmetics. Thus, in society, odour sensitivity is a problem. With this background, we have specially studied patients with odour sensitivity and the prevalence of this problem in different populations.

### The effects of androstadienone and estratetraenol on physiology and mood in men and women

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Two putative human pheromones, androstadienone (AND) and estratetraenol (EST), induce dissociable hypothalamic brain activation in men and women. Here we address two specific questions: (i) are there behavioral and physiological correlates to the dissociation in brain activation induced by these compounds; and (ii) what is the time-course of any effects induced by these compounds. In a within subjects, counterbalanced and double blind design, we measured the effects of AND, EST and a control compound (baking powder) on mood and physiology in 24 subjects (12 males). With regard to the first question, in men AND decreased negative (e.g. 'sad':  $t = 4.063$ ;  $P < 0.05$ ) and increased positive mood (e.g. 'amused':  $t = 2.198$ ;  $P = 0.05$ ). This mood modulation was associated with physiological changes (increase in skin temperature ( $t = 3.698$ ;  $P < 0.05$ ) and trend towards decrease in respiratory frequency (RF) ( $t = -1.912$ ;  $P = 0.08$ ). In women, AND had reverse effects on physiology (e.g. increase in RF ( $t = 2.490$ ;  $P < 0.05$ ) and decreased sexual arousal ( $t = -2.719$ ;  $P < 0.05$ ). Almost no effects of EST were observed in both sexes. Concerning the second question, while both early and late effects on mood were observed, only late effects on physiology were found. Taken as a whole, AND affected men and women differently. We did not, however, find a double dissociation of sex by compound to mirror that found with functional imaging. Our results do not support or contradict consideration of AND and EST as human pheromones at this stage.

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## Characterization of antennal lobe glomeruli and interneurons of the ipsi- and bilateral olfactory pathways of the heliothine moth

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The primary olfactory centre of the moth brain, the antennal lobe, is composed of an array of spherical neuropil structures, glomeruli, which are strikingly similar to glomeruli in the mammalian olfactory bulb. Whereas the male specific macroglomerular complex which processes pheromone information has been thoroughly studied in several moth species, the organization and function of the ordinary glomeruli encoding plant odour information are relatively unexplored. By synaptic antibody staining combined with confocal microscopy, we here present atlases of the glomeruli in male *Helicoverpa assulta* and male and female *Heliothis virescens*. The small size of the heliothine moths is advantageous for confocal microscopy since the entire brain can be visualized as a single image stack. The results show that the numbers of the identified glomeruli in the three specimens are similar (65, 66 and 62). By intracellular stainings combined with confocal microscopy, we also present three-dimensional reconstructions of single neurons constituting parts of the ipsilateral as well as the bilateral olfactory pathways in the moth brain. The stereoscopic effect is obtained by using a special tool of the data software LSM510 that imitates the phenomenon of binocular disparity.

## Functional subsets of taste receptor cells in the mouse vallate papilla during postnatal development

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Electrophysiological recordings from gustatory nerves have shown that the transduction capability of the peripheral taste system changes during postnatal development. It has been suggested that these variations are likely related to corresponding age-dependent changes in the expression and regulation of the membrane properties of taste receptor cells (TRCs), such as their complement of ion channels and receptors. However, little information is available on the membrane events in developing TRCs. We have examined this issue by studying with the patch-clamp technique the voltage-dependent  $K^+$  and  $Cl^-$  currents ( $I_K$  and  $I_{Cl}$ , respectively) in TRCs from vallate papilla in mouse pups and adults. These currents play a key role during taste transduction, especially in the event downstream of the early interaction with chemical stimuli. The analysis of  $I_K$  and  $I_{Cl}$  in TRCs from adult mice revealed a significant diversity among cells: 56% of them possessed only  $I_K$ , 13% only  $I_{Cl}$ , and 31% both  $I_K$  and  $I_{Cl}$ . That is, three functional subsets of TRCs occurred in vallate taste buds of adult mice. On the contrary, in mouse pups at postnatal day (PD) 3 and 4, the majority (>95%) of TRCs possessed only  $I_K$ , whereas the remaining 5% expressed  $I_K$  in conjunction with  $I_{Cl}$ . TRCs possessing only  $I_{Cl}$  appeared later in development, after about PD 14. Thus, there was a rearrangement in the relative occurrence of

the functional subsets of TRCs in the vallate taste buds during postnatal development. This may have a profound impact on the functioning of taste buds, including transduction processes. Our findings support the notion that functional maturation of the peripheral taste system might rely on alterations of membrane properties of taste cells, at least in the mouse.

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## Molecular mechanism of chemosensory transduction in the vomeronasal organ

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Chemosensory neurons of the vomeronasal organ (VNO) are supposed to detect pheromones related to social and reproductive behavior in most terrestrial vertebrates. Recent biochemical and electrophysiological studies indicate that pheromone signaling in VNO neurons is mediated via phospholipase C (PLC) activation. To determine whether  $G\beta\gamma$ -subunits of trimeric G proteins are functionally involved in pheromone-induced  $IP_3$  formation, a scavenger for  $\beta\gamma$  dimers was employed. A C-terminal peptide of the G protein-coupled receptor kinase 3 (GRK3ct) reduced the urine-induced  $IP_3$  formation in VNO preparations, indicating a role for  $G\beta\gamma$  in PLC activation. To address the question of which  $G\beta$ - and  $G\gamma$ -subtypes are expressed in the two populations of chemosensory VNO neurons, polymerase chain reactions as well as immunohistochemical experiments were performed. It was found that from the five known  $G\beta$ -subtypes, only  $G\beta_2$  was expressed in both  $G\alpha_i$ - as well as  $G\alpha_o$ -neurons. For the  $G\gamma$ -subtypes, experiments focusing on the spatial expression profile demonstrated that  $G\gamma_3$ ,  $G\gamma_8$  and  $G\gamma_{11}$  are expressed throughout the two neuron populations, whereas  $G\gamma_2$ -reactive cells are restricted to the apical  $G\alpha_i$ -positive layer of the sensory epithelium.  $G\gamma_2$ -specific antibodies selectively blocked the formation of  $IP_3$  induced upon stimulation with volatile urinary compounds. These data indicate that different  $G\beta\gamma$ -complexes mediate activation of PLC in the two populations of chemosensory VNO-neurons.

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## Convergence between somatic lingual sense and taste, electrophysiology and psychophysics

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We present here both psychophysical and electrophysiological data documenting trigeminal and taste interactions. For the psychophysical study, in an epidemiological study in man ( $n = 209$ ), we observed that dental deafferentations (DD) caused by dental treatments resulted in significantly elevated electrogustometric (EGM) thresholds, recorded at the tongue surface. Subjects with higher numbers of DD presented higher EGM thresholds. This increase was also related to the location of the DD, ie anterior versus posterior DD elicited respectively increased in EGM thresholds at anterior and posterior sites of the tongue. For the electrophysiological experiments, in rats, we observed that capsaicin application at the tongue surface ( $\mu M$ , 7 min) suppressed

tastant (NaCl, sucrose, quinine, glutamate, citric acid) evoked responses in NTS neurons (40% decrease,  $P < 0.001$ ,  $t$ -test). This suppression persisted after trigeminal ganglionectomy, indicating a peripheral mechanism. Furthermore, the same application of capsaicin elicited a punctate pattern of extravasation of Evans blue in fungiform papilla, suggesting localized interactions at the taste bud level. On the other hand, other experiments provide evidence for central interactions: trigeminal ganglionectomy ( $n = 12$ ) and lingual nerve cut ( $n = 6$ ) result in a significant decrease of tastant evoked responses in rat NTS neurons. In addition, proximal stimulation of the trigeminal ganglion after sectioning of its mandibular branch resulted in different pattern of effects. Both decrease and increase of the taste response have been observed. In conclusion, our data provide evidence for trigeminal and taste interactions at the peripheral and central level.

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### Lateralization processes related to olfactory/trigeminal stimuli

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There is growing evidence to suggest that the nature of the olfactory stimulus influences the processes of lateralization. An important factor could be the trigeminal component. Indeed, most odorants simultaneously stimulate both olfactory (CN I) and trigeminal (CN V) systems which differ in terms of their central projections, ipsilaterally for CN I and contralaterally for CN V. The aim of this study was to investigate variations in psychophysiological measurements between a nasal input with low (phenyl ethyl alcohol; PEA) and high (allyl isothiocyanate; AIC) intranasal trigeminal stimulation. In a first experiment (20 subjects), the intensity, hedonicity and irritation levels of stimuli were tested with a psychophysical evaluation to study the possible influences of perceptual characteristics. A second experiment (37 subjects) used bilateral electrodermal recordings and compared the skin conductance responses (SCRs) for both nasal inputs on either monorhinal and birhinal stimulations. First, the SCRs results showed no differences between the two nostrils for PEA as well as AIC, but differences in relation to the type of stimulus, e.g. higher amplitude in response to AIC versus PEA. Secondly, the results indicated bilateral differences in EDA recordings related to the nature of the stimulus and are discussed in terms of hemispheric asymmetric activation.

### Trigeminal sensitization and desensitization in the nasal cavity revealed by electrodermal measurements

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Few studies have dealt with the question of trigeminal sensitization and desensitization in the nasal cavity and generally used psychophysical measurements. The present work added SCR recordings because it was considered to be a reactivity measure in terms of

arousal and affect or basic emotion. The aim of this study was to investigate the response, acute effects and time course of sensitization and desensitization to allyl isothiocyanate (mustard oil) nasal stimuli in healthy subjects. Sixty subjects participated in the experiment using psychophysical (intensity ratings) and psychophysiological (skin conductance response) measurements. Nasal stimuli were delivered three times with different inter-stimulus intervals. The results showed that the psychophysical and psychophysiological data were correlated, and that successive nasal stimuli presented after a short period of time ( $< 2$  min) produced increased perceived intensity of irritation, whereas stimuli delivered after  $> 3$  min produced a markedly decreased perceived intensity of irritation. These findings are in agreement with those obtained with capsaicin, the most frequently used irritant molecule.

### Ligand binding properties of hamster aphrodisin

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Aphrodisin, a soluble glycoprotein released in hamster vaginal secretions, activates vomeronasal receptors and triggers the copulatory behavior of male hamsters. This protein belongs to the lipocalin superfamily. It is not yet known whether the pheromonal effect is triggered by the protein itself and/or by putative odorant molecules trapped in the lipocalin binding pocket. To better understand the binding properties of this protein, purified recombinant aphrodisin was expressed and secreted in large amounts by *Pichia pastoris*. The purified recombinant aphrodisin shared common structural properties with the natural aphrodisin. Furthermore, a binding assay demonstrated the efficient binding of odorants such as 2-isobutyl-3-methoxypyrazine, as well as two thiols present in vaginal secretions: methyl thiobutyrate and dimethyl disulfide (known to function as a male hamster attractant). The affinity constants for these ligands were measured using a fluorescent probe assay. In addition, we purified aphrodisin present in vaginal secretions. After lyophilization, the protein was denatured to release potentially bound ligands. The presence of natural ligands, detected using gas chromatography coupled to mass spectrometry, will be discussed. These experiments help to understand whether or not hydrophobic molecules bound to aphrodisin might contribute to the pheromonal activity.

### Evidence of a human odorant-binding protein in the olfactory mucus: location, characterization and potential role as an odorant carrier

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Odorant-binding proteins are small, abundantly secreted proteins belonging to the lipocalin superfamily, thought to participate in perireceptor events of odor detection by carrying and/or selecting odorants. Putative human OBP (hOBP) genes have recently been

described, but the presence of the corresponding proteins remained to be established in the human mucus. We report the first evidence of hOBP in the mucus covering the olfactory cleft, where the sensory olfactory epithelium is located. hOBPs were not observed in the nasal mucus covering the septum and the lower turbinate. A recombinant protein variant of hOBP was heterogeneously secreted by *Pichia pastoris* and thoroughly characterized as a monomer with one disulfide bond (C59–C151). By measuring the displacement of several fluorescent probes, we show that this hOBP is able to bind numerous odorants of diverse chemical structures, with a higher affinity for aldehydes and large fatty acids. A computed 3-D model is proposed and reveals that two lysines of the binding pocket may account for the increased affinity for aldehydes. The relatively limited specificity of this hOBP suggests that other human OBPs would take into account the large diversity of odorants. In addition, absence of hOBP expression or mutations altering odorant binding might affect odorant detection.

### Neural correlates of pheromonal learning in mice

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Female mice learn to recognize the urinary pheromones of the males that mate with them during a sensitive period of a few hours following mating. This pheromonal memory is vital for their reproductive success, as it prevents the oestrous-inducing effects of the mating male's pheromones from causing the abortion of his own offspring. A variety of experimental evidence implies that the neural changes underlying pheromonal recognition occur in the accessory olfactory bulb (AOB), at the first stage of processing of the pheromonal information. The AOB has a comparatively simple neural architecture involving a single class of projection neurons known as mitral/tufted cells, which receive feedback inhibition from inhibitory interneurons at reciprocal synapses. A simple hypothesis can account for pheromonal learning involving a long-lasting increase of inhibitory feedback on the sub-population of mitral/tufted cells that respond to the mating male's pheromones. This would selectively disrupt the transmission of the pheromonal signal from the mating male, whilst allowing the transmission of the pregnancy blocking signal from unfamiliar males. This hypothesis is supported by the finding that, following learning, the ratio of excitatory to inhibitory neurotransmitters in the AOB is decreased in response to the mating male's pheromones compared with exposure to pheromones from an unfamiliar male. Pheromonal learning is also associated with changes in the sensitivity of mGluR2 receptors on the granule cell interneurons and changes in local field potentials recorded from the granule cell layer of the AOB.

### Odor sensitivity and perception in pregnant women

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The focus of this presentation is on olfactory changes in pregnant women. It is known that some women claim to perceive changes in olfaction during pregnancy. In accordance with this, other changes that also relate to the chemical senses, including nasal congestion,

food perception, cravings and aversions, exist and have been documented. However, since the changes in olfactory functions have gained limited interest so far *per se*, the aim of this study was to understand these aspects more thoroughly. The study included 26 pregnant women in weeks 22–24 of pregnancy and a matched control group who were tested psychophysically and perceptually. The psychophysical part determined absolute thresholds (method of constant stimuli) and intensity ratings (method of magnitude estimation, calibrated for response behavior with the master scale principle). These measurements were done for odor intensity as well as for odor unpleasantness for a set of eight pyridine concentrations. In the perceptual part a set of 32 common odors (16 from the SOIT) were used to assess intensity and pleasantness (category rating scales), whether the odor/odorant evoked nausea, would be applied to the body or eaten, descriptions of the odor, and free and cued odor identification. The presentation will account thoroughly for the results with interpretations and a discussion with conclusions.

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### Influence of concentration on emotional responses to odorants

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The relationship between the pleasantness of odorants and their intensity has been mainly studied through subjective ratings, such as intensity and hedonic scales. However, some studies have pointed out that hedonic verbal judgements did not always reflect the unconscious physiological emotional responses, especially autonomic responses. So, the purpose of this study was to evaluate the influence of odorant concentration on the associated emotional reactivity estimated through a neurovegetative and a verbal analysis. The neurovegetative analysis was based (i) on the measurement of the variations of five autonomic nervous system (ANS) parameters and (ii) on the distribution of the basic emotions estimated from the corresponding autonomic patterns. The verbal analysis was made from odor ratings for intensity (intensity scale) and pleasantness (hedonic scale). Seventeen healthy non-smoker volunteer subjects (8 males and 9 females, mean age = 31 years) participated in the experiment. Four different odorants were chosen according to their hedonic characteristics obtained from a panel of 45 subjects. Each of these odorants were tested at a low and a high concentration: alpha-ionone (5% and pure), ethyl caproate (0.1 and 20%), menthenethiol (0.2 and 2%) and isovaleric acid (0.1% and 2%). Throughout the test, the ANS parameters (skin potential and skin resistance, skin blood flow and skin temperature, and instantaneous heart rate) were simultaneously and continuously recorded. Results showed that (i) the mean variations of each autonomic parameter did not significantly differ between the low and the high concentration of the same odorant, but (ii) differences emerged concerning the distribution of the basic emotions associated with the two concentrations in relation to their hedonic valence. Thus, the two concentrations of alpha-ionone (both rated as very pleasant) and isovaleric acid (both rated as very unpleasant) were associated with similar basic emotions, whereas the distribution of basic emotions differed between the two concentrations of the two other odorants (ethyl

caproate and menthenethiol), for which the high concentration was judged to be more unpleasant than the low concentration. It can be concluded that autonomic responses are influenced more by the hedonic valence of the odorants rather than by their concentration.

### Trigeminal mechanisms of novel spice-induced sensations

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Chemesthesis, the term denoting chemically induced sensations, encompasses a broad range of sensations. While the neural basis of some of these sensations, such as the heat and pain of capsaicin, are well understood, the basis of other chemesthetic sensations are not. Polyunsaturated alkylamides, a class of compounds found in several plant taxa, including several plants that are used as spices, cause sensations in the mouth that are distinct from those induced by capsaicin. Characterized as tingling, buzzing, cooling and numbing, these sensations appear to be the result of excitation of thermal, tactile and nociceptive afferents. By measuring changes in intracellular calcium in cultured trigeminal neurons we confirm the involvement of these classes of afferents. Moreover, we find that polyunsaturated alkylamides affect the activity of thermally and mechanically sensitive neurons by modulating the activity of specific classes of ion channels. Because of the character of sensations that are produced, unsaturated alkylamides may be useful in the study of certain types of paresthesia.

### Identification and characterization of a taste-specific regulator of G protein signaling

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G protein-mediated signaling processes are involved in sweet and bitter taste transduction. In particular, an implication of the G protein alpha-subunit gustducin in bitter taste transduction has been demonstrated experimentally. Thus, the time-course of cellular responses induced by bitter tastants is dependent on the intrinsic GTPase activity of alpha-gustducin, which determines the lifetime of the active G protein complex. In several signaling systems specific 'regulator of G protein signaling' (RGS) proteins accelerate the GTPase activity of G protein alpha-subunits. Using differential screening approaches, we have identified a novel RGS protein termed RGS21. RT-PCR, immunohistochemistry and *in situ* hybridization experiments demonstrated that RGS21 is exclusively expressed in taste tissue, where it is found in a subpopulation of sensory cells. It is coexpressed with alpha-gustducin and other elements of the bitter transduction cascade in individual taste cells. The first evidence for a functional implication is provided by *in vitro* binding assays demonstrating an activity-dependent interaction between RGS21 and alpha-gustducin. Interestingly, RGS21 was also detected in cells expressing T1R2/T1R3 sweet taste receptors. These results suggest that RGS21 is involved in bitter as well as sweet taste transduction processes.

### The bitter sides of glucose: identification of a human bitter receptor and its cognate ligands

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Bitter taste generally is aversive and protects vertebrates from ingestion of toxic substances, but bitter tastants also contribute to the enjoyment and palatability of food, influencing human nutritional habits. Although bitter taste has been extensively studied, receptor mechanisms are still poorly understood. Anatomic, functional and genetic evidence from rodents suggested, however, that the T2R genes encode a family of bitter receptors, while definite proof is missing in humans. We here report that the human *T2R16* gene is expressed in vallate papilla taste buds of the tongue and encodes a receptor for intensely bitter  $\beta$ -glucopyranosides. These phytonutrients did not activate any other human T2R. Neither the phenyl- $\alpha$ -D-glucopyranoside and  $\beta$ -D-galactopyranoside, which taste bitter only at high concentrations, nor chemically unrelated bitter compounds activated *hT2R16*. All tested  $\beta$ -glucopyranosides showed similar dose-response relations in *hT2R16*-expressing cells and human psychobiological experiments. Both, *hT2R16*-mediated cellular responses and bitter sensation in humans cross-desensitized following exposure to  $\beta$ -glucopyranosides, but not to other bitter compounds. It appears that *hT2R16* is broadly tuned. It binds bitter compounds that share only a  $\beta$ -glycosidic bond and a glucose residue. A broad tuning of T2Rs could explain how a limited number of receptors permits the perception and coding of numerous and different bitter substances. Together, our data identify the *hT2R16* as a broadly tuned, genuine human bitter receptor for  $\beta$ -glucopyranosides.

### The influence of salivary constituents and oropharyngeal performances on retronasal aroma perception

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In recent years, research on retronasal aroma perception has been dominated by 'nosespace' and 'mouthspace' analyses involving different trapping or mass spectrometric techniques. The focus was on physical release phenomena such as partitioning effects of odorants between polar and non-polar phases and air. However, it is likely that temporal resolution and intensity of retronasal aroma perception during food consumption are greatly influenced not only by food matrix composition but also by physiological factors. Plausible factors are oropharyngeal performances during mastication and swallowing, the adsorptive potency of odorants to oral mucosa or the formation of adhesive coatings by food matrix constituents on oral and pharyngeal mucosa. Also, salivary constituents can influence the concentrations of odorants in the nasal cavity by altering their release or retardation in mouth. For example, the influence of salivary enzymes has been discussed in terms of degradation of food polymers such as starch, thereby leading to odorant release from inclusion complexes. On the other

hand, recent investigations have indicated that salivary constituents affect odorant concentrations considerably when incubated with untreated whole saliva. To address the question of the influence of human oral and pharyngeal physiology on retronasal aroma perception, the mastication and swallowing process as well as the formation of matrix coatings have been visualized *in vivo* by application of videofluoroscopy and real-time magnetic resonance imaging. Consequences to retronasal aroma perception will be discussed. The influence of human salivary constituents on concentration changes of key food odorants such as esters, thiols, aldehydes, alcohols and pyrazines will also be presented. Techniques applied involve gas chromatography–olfactometry as well as stable isotope dilution assays.

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### Congruent odors and the Stroop effect

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The aim of this study was to investigate the effect of natural ‘congruent’ odors on the so-called Stroop effect. The question we asked was whether a congruent (yellow or green smelling) odor enhances or delays the recognition of a color word written in its own or another color. In the first pre-study we asked people what smells blue, red, green and yellow. Twenty-two percent said yellow means lemon, ~40% said green means grass. Taking in to consideration the literature, we chose citral for lemon and 1-hexanal for grass. In our second pilot study we tested the reverse association. About 53% said citral smells yellow and 32.5% said hexanal smells green. The Stroop test in the main experiment was delivered by the ERTS software. We had three treatment groups, 1% citral (3,7-dimethylocta-2,6-dien-1-al, *cis/trans*; Aldrich), 20% citral and 0.5% 1-hexanal (Sigma), and a control group (prop.gly.). One odor or the odorless control was sprayed on a sheet of paper. This lay on the table beside the subject during the whole session. Each subject was tested once under one experimental condition. The data were analyzed by a multivariate analysis of variance. The results did not confirm the hypothesis of a color-specific effect. Tendencies show a longer recognition time in the presence of an odor (especially hexanal) regardless of color, in contrast to the no-odor condition. Effects could be stronger if we could use odors which are much more strongly associated with a single color.

### Toward objective indices of chemesthetic stimulation from airborne agents

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As perceiving beings, we know that chemesthetic stimulation can trigger automatic responses, such as blinking, producing tears,

sneezing, runny nose, and so on. Such objective manifestations can have scientific value for perceptual measurement when closely linked to what people actually feel from the stimulation. To have maximum value, the responses should have comparable sensitivity to the psychophysically measured response. Research has now shown that some functional responses have the requisite sensitivity, at least when elicited by a stimulus switched on rapidly. To wit, even in the probabilistic zone between no detection and perfect detection, the frequency of eye blinks increases commensurately with probability of detection of vapor stimulation to the eye. The activity of the orbicularis oculi muscle under the eye increases commensurately with probability of detection of chemesthetic stimulation of the nasal passage. Similarly, a neurophysiological response, the NMP, manifests itself and increases in amplitude in that probabilistic zone of psychophysical detection. It would be of interest to discover whether these various responses also correspond with the time-course of chemesthetic sensations. In this respect, it is encouraging to note that rate of secretion of mucus in the nasal passage increases commensurately with the cumulative amount of irritation perceived from inhalation of particulate matter in exposures that lasted 20 min or longer. The presentation will explicate the objective measures studied in this laboratory and will seek to project their usefulness into the future.

### Toward an index of ability to transmit semantic information regarding odors

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The purpose of the study was to develop a performance-based scale of semantic information regarding odors. Over 100 subjects sought to identify common odorants by smell. Subjects received the odorants twice, on different days. Experimenters recorded responses, latency of responses and subjects’ confidence in correctness. Two experienced judges rated the quality of responses on a scale of 1–5, agreeing in the overwhelming majority of cases and averaging the ratings when in disagreement. In one analysis, an author scored responses as correct or incorrect according to a strict criterion (e.g. only ‘coffee’ for ground coffee). Subjects emitted correct responses more quickly, with greater confidence and with greater consistency (tended to apply the same label in both sessions) than incorrect responses. Logistic regression combined latency, confidence and consistency into a value of ‘strength of evidence’ that separated correct labels from incorrect ones with an accuracy as high as 85%. Another analysis compared strength of evidence to ratings of ‘correctness’. Strength of evidence predicted ratings of correctness quite well. A scale based on latency, confidence and consistency both predicted correctness according to a strict criterion and rated correctness quite well. It may prove possible to determine how well subjects can transmit semantic information about odors without knowing which odorants they received or what labels they applied.

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## Processing of oral chemical irritation in trigeminal subnucleus caudalis

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Oral irritant sensations associated with spicy foods and carbonated drinks are an important and increasingly popular component of flavor. Oral irritation is elicited by chemicals such as capsaicin in red chili peppers or CO<sub>2</sub> in carbonated drinks. These irritant chemicals excite trigeminal nerve endings in the oral cavity, in some cases by binding to specific molecular receptors. The trigeminal nerve endings convey signals to the trigeminal nuclear complex in the brainstem, with an important relay in trigeminal subnucleus caudalis (Vc). Vc neurons in turn send signals to higher centers involved in sensation and perception. We have recently compared the temporal pattern of Vc neuronal activity with that of chemically evoked irritation. Repeated application of capsaicin to the human tongue elicits irritation that grows in magnitude across trials (sensitization). Following a rest period, reapplication of capsaicin initially elicits much less irritation (self-desensitization), but eventually evokes a progressive rise in irritation (stimulus-induced recovery, SIR). We observed similar patterns of Vc neuronal firing in rats. Repeated or continual application of capsaicin (100 p.p.m.) induced a progressive increase in firing. After a rest period, reapplication of capsaicin was initially ineffective but eventually evoked a progressive increase in firing like SIR. Similarly, citric acid (250 mM) and NaCl (5 M) elicited sensitization psychophysically as well as in Vc neuronal firing. In contrast, repeated application of nicotine (in tobacco) to the human tongue evokes irritation, the magnitude of which decreases across trials (desensitization). Similarly, nicotine initially excites Vc neurons, followed by a decrease in firing rate despite the maintained presence of nicotine. Menthol and mustard oil similarly elicited a declining pattern of irritation and Vc firing. Nicotinic irritation and excitation of Vc neurons is reduced by local application of mecamylamine, and thus may involve neuronal nicotinic acetylcholine receptors expressed in nociceptors. Carbonated water also elicits an oral tingling sensation and excites Vc neurons in a manner that is significantly reduced by carbonic anhydrase inhibitors, suggesting that the fizzy sensation of carbonated drinks is due to acidic stimulation of trigeminal nociceptors. That a given irritant elicits similar temporal and/or pharmacological profiles for perception and neuronal activity supports a role for Vc neurons in the mediation of oral irritation.

## Desensitization of oral irritation by sequentially applied mustard oil and reciprocal cross-desensitization with capsaicin

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The magnitude of oral irritation elicited by pungent chemicals may either increase or decline across trials, phenomena called sensitization and desensitization, respectively. We tested whether mustard oil (allyl-isothiocyanate) elicits a sensitizing or desensitizing pattern of oral irritation, and whether it exhibits cross-desensitization with capsaicin. This was assessed by obtaining

successive ratings (using a bipolar category scale) of the intensity of irritation elicited by sequential applications of mustard oil (0.125%, 40 µl) 10 times at 1 min intervals to one side of the tongue. After a 10 min break, either mustard oil or capsaicin (10 p.p.m., 15 µl) was applied bilaterally and in a two-alternative forced choice (2-AFC) procedure subjects chose which side had more intense irritation and rated irritant intensity on both sides. Ratings significantly declined across trials (desensitization). In the 2-AFC procedure, subjects consistently chose the side not previously receiving mustard oil as more intense for both mustard oil and capsaicin, and assigned significantly higher intensity ratings to that side. Capsaicin exhibited sensitization and cross-desensitization of irritation elicited by mustard oil. Sequential application of mustard oil at shorter (20 s) intervals initially evoked a sensitizing pattern followed by desensitization. The temporal patterns of oral irritation exhibited by mustard oil, and its reciprocal cross-desensitization with capsaicin, are similar to those of menthol and nicotine.

## From taste to flavor: insights from neuroimaging data

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Food choices mainly rely on flavor perception, which includes gustatory, lingual, somatic and retronasal olfactory sensations. Recent neuroimaging techniques and functional magnetic resonance imaging in particular have allowed the identification of areas critical to flavor information processing. This presentation will review studies investigating the cortical substrate of flavor, including studies of cortical activations in response to taste alone or in combination with a lingual somatic component and in response to retronasal olfactory stimulation, in the light of other findings from animal research, clinical observations and neuroimaging techniques, including positron emission tomography and magnetoencephalography. Discussion will focus on the role of the insula in taste information processing, with converging findings pointing to the dorsal part of the insula as a primary focus of gustatory input and other evidence suggesting a more integrative role of the inferior insula, possibly combining taste and smell information into flavor.

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## Influence of verbal-cognitive strategy on odour identification depends on odour familiarity and not on perceived intensity

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Odour identification commonly involves sniffing, then mentally searching for a word, and is a difficult task in terms of speed and accuracy. It has been shown previously on 650 human subjects (Bell and Paton, 2000, *Aroma-Chology Rev.*, 9: 3–9) that finding the descriptor for an odour can be made more accurate by use of

one cognitive strategy (think of possible descriptive words then sniff) rather than another (sniff then think of possible words). Such strategic options face people wishing to maximize perceptual accuracy in judgement of complex aromas. The odours were not identified with the same errors. Lemon was most accurately identified, followed by mint, rose, burnt, then almond. This raised the question of whether odour familiarity might determine whether a particular cognitive strategy is more effective. Five odours, varying in familiarity, were given to 55 adults in tasks similar to the previous study. The first strategy ('think then sniff') was more effective than the second ('sniff then think') only when the least familiar odours were presented. Another 80 adults then performed the tasks with two odours (one highly familiar and one of low familiarity) at two different perceived intensities (high and low). There was no advantage in either strategy for the high or low intensities of either odour. Sensory mechanisms defining familiarity appear to be independent of stimulus intensity and to involve enhancement of retrieval of information from verbal memory. The 'think then sniff' strategy offers the opportunity for people with little training in odour recognition to improve their effectiveness in identifying unfamiliar odours.

### The smell of emotion: human olfactory communication of emotions

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Olfactory communication of emotions, particularly of sexual arousal and fear, is widely reported in animals. In previous studies, we discovered that humans can detect emotional odors. When given a choice, both men and women discriminated odors collected from the same person when they were in a neutral or an emotional state, and correctly identified the emotional source of the odor. The purpose of this study is to extend our previous findings to further examine sex differences in this ability. In addition, because experience enhances sensitivity for non-social odors, we hypothesized that frequent interactions with an emotionally intimate partner might enhance discrimination and identification of emotional odors. We collected sweat from the underarm and areola regions on gauze pads from 20 pairs of sexually active heterosexual young adult partners on three occasions while they watched a neutral video followed by an emotional video. The videos had been tested previously to respectively produce fear, sexual arousal, happiness and neutrality. Without knowing their source, each participant judged in counterbalanced order odors collected from his/her partner and from another person matched in age, gender and ethnicity as the partner. We found that intimacy enhances women's performances on the discrimination but not the identification tasks. Women discriminated their partner's neutral odors from their odors of sexuality, happiness and fear but not those of an unfamiliar man. They performed equally well at identifying fear odors of their partners and an unfamiliar man. Men, in contrast, discriminated between neutral and emotional odors better when the odors come from an unfamiliar woman, e.g. sexual arousal and happiness. They could also identify the odor of happiness from their partner. Implications of the findings for understanding olfaction as a channel of communication will be discussed.

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### Development of a food volatiles extraction method

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Food volatiles have been investigated for fundamental chemicals of their aroma characteristics. They are very complex and have differences in aroma corresponding to the type of characteristics. Research on food flavor by general measurement methods such as headspace analysis or solvent extraction often fails to grasp certain quantities of constituents. Above all, these methods have problems such as each constituent's volatility or solubility. The aim of this study was to develop a novel measurement method of volatiles and to identify volatile food components that feature aroma characteristics. We developed a new volatile extraction method based on the vaporization of constituents under varying levels of reduced pressure. Food samples were placed in a decompressed sample chamber. The aroma compounds were volatilized under a reduced pressure atmosphere. The food volatiles were then collected into a canister with slow stream of inert gas. Finally, they were analyzed by the cold trap dehydration method using a preconcentrator gas chromatograph/mass spectrometer. Contrary to common static headspace data, we successfully observed quite a few volatiles in p.p.b. concentrations with sufficient accuracy. The results clearly show that this method could be applied to various matrices that include different vapor pressures and different polarities while eliminating the pyro-degradations. We also succeeded in observing the sensory data of an entire aroma that was extracted by this extraction method. The details of the results will be discussed.

### Taste and speak: when do experts generalize?

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Previous work showed that experts are better at discriminating and describing familiar chemosensory stimuli than novices. Our aim was to determine whether this superiority holds true for stimuli on which experts have not been trained. We trained 19 subjects to become experts at detecting and identifying off-flavors in beers and at describing general beer characteristics. After 69 h of training we compared experts' and novices' sensory performance on 12 familiar beers (six commercial beers and six beers supplemented with an aroma) and six new supplemented beers. A same/different task showed that, as expected, experts outperform novices for familiar beers. However, because no difference between experts and novices was observed for unfamiliar beers, we concluded that experts are not able to generalize their discriminative abilities to new stimuli. In contrast, a matching task showed that they are able to generalize their descriptive abilities. In this task experts and novices had to describe 18 other beers (six familiar commercial beers, six familiar supplemented beers and six unfamiliar supplemented beers) and then to match the 18 descriptions, written either by an expert or a novice, with the beers. For both familiar and unfamiliar beers, experts performed better than novices when the descriptions were

written by an expert. No difference was observed between experts and novices when a novice wrote the descriptions. An analysis of the descriptors generated by both experts and novices showed that experts use more precise terms than novices.

### Are supertasters also superfeelers?

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The trigeminal component of the lingual nerve is specialized for tactile information processing. Damage to this nerve is prominently manifested as a loss in taste and touch. To investigate loss in tactile function, accurate spatial measurements are required. The conventional method, two-point discrimination, yields variable results and does not control for non-spatial cues. This study evaluated a novel neurosensory test for assessing spatial resolution acuity in individuals differing in taster status. An up-down tracking procedure was used to estimate the threshold height for the recognition of embossed letters examined by the anterior tongue tip (Essick *et al.*, 1999). The 56 stimuli consisted of Teflon strips, one side of which bore a letter (A, I, J, L, O, T, U, W) 2.5, 3, 4, 5, 6, 7 or 8 mm in height. Eighty-seven subjects (nontasters,  $n = 21$ ; medium tasters,  $n = 29$ ; supertasters,  $n = 37$ ) participated. Supertasters exhibited a significantly lower ( $P \leq 0.001$ ) letter recognition threshold than nontasters, i.e. supertasters could accurately identify letters at a lower letter height than nontasters. The results might be explained by the fact that supertasters have significantly more fungiform papillae than nontasters (Barthoshuk *et al.*, 1994; Chopra and McGlone, unpublished data). The higher density of these chemoreceptive units might be paralleled by a higher density of mechanoreceptive units. Alternatively, elevated sensory cognitive processing in supertasters might extend to tactile discriminative performance.

### Influence of language and culture on odour mental representation

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Previous work suggests that the more relevant dimensions underlying odour categorization are linked to hedonism and intensity. Our goal was to evaluate if odour categorization is also based on the quality of odours. In a first experiment, three groups of 30 subjects from three cultures (French, American and Vietnamese) sorted 40 stimuli according to: (i) odour similarities; (ii) odour name similarities; and (iii) imagined odour similarities. Multi-dimensional scaling analyses of these data showed that, in all countries, the psychological odour space was divided in relatively well-separated categories. A consensus emerged between the three cultures regarding three of these categories, 'sweet', 'floral' and 'unpleasant' smells, but we also found, between countries, a certain variability that we attributed to food habits. A comparison with the odour name and imagined odour spaces suggests that these categories are made not strictly on a linguistic basis but also on the perceptual quality of odours. In order to evaluate the typicality of each member for these categories, 30 students of each culture

performed a typicality task on the same odour set. Fruit and flower categories revealed common prototypes across cultures (i.e. melon and lily of the valley). This result suggests that typicality of odours might not be entirely dependent on culture.

### Identification of novel taste-specific genes using differential screening approaches

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Identification of genes specifically expressed in taste tissue is supposed to provide new insight about the molecular mechanisms underlying recognition and transduction of gustatory signals in lingual sensory cells. Towards this goal we have performed differential screening approaches using taste tissue versus non-sensory lingual epithelium; three subtractive cDNA libraries were prepared from isolated foliate and fungiform taste buds. The isolated cDNA clones were partially sequenced and by searching the relevant databases known genes as well as redundancy were eliminated. Differential RT-PCR experiments with cDNA from sensory and non-sensory epithelium were performed as a second step to determine those genes which are exclusively expressed in taste sensory tissue. Subsequent *in situ* hybridization studies allowed the definition of the topographic expression patterns on circumvallate, foliate and fungiform papillae; depending on the gene type, labelled cells were found in all or in subsets of the papillae. The sequenced full-length clones were assessed by diverse bioinformatic tools towards a characterization of the encoded proteins. So far, a novel taste-specific regulator of G protein signaling termed RGS21 has been identified and several other transcripts are currently under investigation. These results indicate that differential screening approaches can lead to the identification of novel taste-specific genes.

### Perinatal plasticity of olfaction and adaptive responses in two mammalian newborns

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The foremost challenges of mammalian newborns (N) are breathing and ingesting milk to obtain both energy and immunity. These adaptive responses involve directional, coordinated motor actions on the part of neonates. N from a range of mammals have evolved reliance on odour cues to complete their locatory task. Behavioural strategies to reach that aim involve either inborn attraction to predisposed signals or plastic responses to odorants acquired *in utero*. This second hypothesis was assessed here in the rabbit and human N in studying the impact of prenatal odour experience on postnatal orientation responses. When simultaneously exposed to amniotic and colostrum odours, rabbit pups are equally attracted to both, suggesting a chemical overlap between them. Manipulations of the odor environments of the foetus and the pup confirmed that notion: foetal rabbits born to cumin-fed females exhibit as N a selective attraction to the odour of placenta

or colostrum from pregnant/lactating females having the same diet. Thus, the foetus memorizes odours and the pup is subsequently able to extract those same odours from the perinatal fluids. Finally, when the olfactory overlap between both perinatal substrates is disrupted, the pups' performance to locate a nipple and gain colostrum is reduced. Similar experiments with human N will be discussed. To sum up, the foetal specification of odour information might improve the adaptative responses of the N to the immediate postnatal environment.

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### Perceptual, cognitive and psychosocial factors in trigeminal experience

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Although the experience of chemosensory irritation in the ocular or nasal mucosa is elicited by stimulation of the trigeminal nerve and can be potentiated by subsequent inflammatory response, an individual's subjective experience is often the product of those sensory or physiological signals and psychological processes involved in perception, memory and judgement. To evaluate how these processes mediate between sensation and subjective experience, our research on trigeminally mediated irritation is guided by an information-processing model of chemosensory perception. The model incorporates (i) antecedent, subject-based factors such as personality or mental models of risk perception; (ii) situational factors such as instructional manipulations or social cues that may alter attention; and (iii) processing factors, such as attributions of causality that can promote misinterpretation of symptom sources. The utility of adopting such a conceptual framework resides in the ability to explicitly manipulate and evaluate the role of cognitive and social factors in studies that measure the detection or perception of odors and trigeminal irritants. Consistent with cognitive models of pain and symptom perception, studies done in this framework reveal that trigeminal experience can be attenuated or enhanced by such factors as expectancies, about the chemical and exposure situation prior to exposure, and personality type.

### Fos expression in rat piriform cortex after retrieval of an olfactory discrimination task

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Acquisition, consolidation and retrieval are basic stages underlying the formation of long-term memory. Identification of brain areas activated by recall of a behavioral task is a crucial point since it could visualize the location of meaningful cue storage. The piriform cortex (PCx), which is the main area of the primary olfactory cortex, has close relationships with limbic structures and is assumed to play an important role in olfactory mnemonic processes. In this experiment, we mainly investigated the involvement of the PCx in the recall of a two-odor discrimination task by using immunocytochemical detection of the Fos protein as a marker of cellular activation. Olfactory operant conditioning was performed in a four-arm radial maze. Three groups of rats were used: trained (T), pseudo-trained (P) and control (C). The C rats enabled

us to determine the baseline level of Fos. T and P rats were water-deprived. The T rats learned to discriminate between two odors. Conditioning consisted of 20 trials per day. Discrimination was considered as acquired when the animals chose the rewarded odor in 80% of trials. After an interval of 10 resting days, rats were placed again in the maze to check the level of retention of the task. They were then sacrificed in order to perform Fos immunocytochemistry. Pattern of Fos labeling in the olfactory learning group was compared with that elicited in the P rats, which were randomly rewarded with water. Counting of Fos-immunoreactive cells was performed at eight levels of the PCx to assess the level of activity across the rostro-caudal subdivisions of this cortex. Analysis is in progress to determine whether the PCx is involved in contextual retrieval of olfactory cue. Attention will also be paid to limbic areas known to participate in motivational aspects of memory formation.

### Neonatal learning of natural and artificial odours associated with the maternal breast

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Human newborns have been shown to be attracted either to odour cues naturally emitted by their mother's breast or to artificial odours experimentally introduced in the nursing context. It remains to be answered whether natural and artificial odorants simultaneously associated with breast-feeding will elicit differential responses when presented pairwise outside the feeding context. Double-choice tests were conducted in 3- to 5-day-old breast-fed newborns exposed or not to an artificial scent (S) carried in a cream prescribed for nipple protection. Odour S was contrasted with either (i) a blank stimulus or (ii) the milk odour (M) of the infant's own mother. The duration of nose orientation to either odorant was coded from the videotaped tests. The results indicated that: (i) odour S (when paired with a blank) was detected by, and attractive to, infants regardless of their previous experience with it; and (ii) in tests opposing M and S, S-exposed infants oriented their nose for equal durations to S and M, indicating that the natural and artificial odours became equally reinforcing to them. In contrast, S-naïve newborns tended to turn longer to M than to S. These results suggest that infants may assign similar affective value to a species-specific odorant, milk, and to a species-atypical odorant acquired in the highly reinforcing context of nursing.

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### Antennal feedback loop regulates pheromone release in beetles

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Regulation of pheromone release is critical for intraspecific communication and avoidance of predators or parasites dependent on such messages. Pheromone production is under endocrine control in insects (Vanderwel, 1994, Arch. Insect Biochem. Physiol., 25: 347; Tillman *et al.*, 1999, Insect Biochem. Mol. Biol.

29: 481). However, mechanisms controlling the amount of pheromone released are poorly understood. I report a potential feedback system in Colorado potato beetles (CPB) that may enable CPB to measure and regulate pheromone release. Male CPB release quantities of an aggregation pheromone, (S)-CPB I, undetectable by gas chromatography (Dickens *et al.*, 2002, *J. Exp. Biol.*, in press). Release of (S)-CPB I is enhanced by excision of one or both antennae and topical application of juvenile hormone III (JH III). Excision of both antennae and JH III treatment result in synergistic amounts of pheromone that are sustained for days. These results with CPB confirm our earlier study with boll weevils in which the feedback system was first discovered (Dickens *et al.*, 1988, *J. Entomol. Sci.*, 23: 52). In other Coleoptera, JH III may stimulate corpora cardiaca to release a brain hormone (BH) that induces pheromone biosynthesis (Hughes and Renwick, 1977, *Physiol. Entomol.*, 2: 117). Since JHA increased pheromone levels and decreased sensitivity of olfactory receptors in boll weevils, JH may regulate pheromone release by modulating olfactory sensitivity. Thus, a negative feedback loop may exist comprising neural and hormonal signals; it includes pheromonal stimulation of antennal receptors that leads to decreased pheromone release.

### Orientation to the nest by smell in rabbit pups

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Rabbit pups are born into a fur-lined nest and as they are not brooded or retrieved, the nest is essential for their early survival. We asked whether they can orient to it by smell. Pups removed from the nest on the day of birth ( $n = 22/12$  litters) and placed for 6 min in an arena with nest material beneath the floor at one end, spent more time at the stimulus than at the non-stimulus end ( $81.1 \pm 21.1\%$ , mean  $\pm$  SD). Control pups ( $n = 19/14$  litters) tested in an arena without nest material showed no side preference ( $50.7 \pm 27.4\%$ ). Pups removed from the nest as they were born ( $n = 17/8$  litters) and tested in the arena without postnatal experience of a natural nest also spent more time at the stimulus end ( $69.7 \pm 20.4\%$ ). Specificity of the response to nest material was confirmed when newborn pups ( $n = 18/8$  litters) tested in the arena with orange essence as stimulus tended to avoid it, spending somewhat less time at the stimulus end ( $43.1 \pm 34.5\%$ ). An ANOVA followed by post-hoc Fisher's PLSD tests showed the scores for the two experimental groups tested with nest material to differ significantly from the control groups tested without nest material or with novel orange essence ( $P < 0.05$ ) but not from each other ( $P = 0.18$ ). Thus, these results suggest that rabbit pups can orient to the natural nest by smell without postnatal experience.

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### Successful odor identification in the honeybee *Apis mellifera* occurs within an odor-independent time window

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To understand the functioning of the olfactory system of the

honeybee, it is important to know about its temporal and qualitative resolution capacities. When closer to the limits of their olfactory resolution, bees might need more time to discriminate similar odor mixtures and concentrations, or might commit errors doing so. The observation of these details could be invaluable for answering questions concerning odor processing in general. In an adaptation of the experiment done by Laska *et al.* (1999), free-flying honeybees were faced with 24 odors, consisting of two odorants, linalool and nonanol, in log-step concentrations (0.025 to 25%, v/v), as well as their mixtures. Bees which had been differentially conditioned to a certain odor (S+) were confronted with a wooden rack containing the rewarded and the remaining 23 similar odors. The time they spent at the different odors while searching the S+ was recorded and evaluated. We found that: (i) bees could be differentially conditioned to discriminate every odor; (ii) bees did not generalize from odor mixtures to their components; (iii) bees learned to differentiate different concentrations of the same odor. Generalization to lower or higher concentrations did not happen; (iv) the time needed for successful identification was the same for all used odors, independent of their concentration or complexity; and (v) odor identification occurs between 160 and 690 ms after exposure to the odor.

### Olfactory dysfunction in patients with mild cognitive impairment

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A large number of studies have examined olfactory function in patients with dementia of Alzheimer's type (DAT) and demonstrated a variety of deficits, but the findings have not always been consistent. However, there has been little evaluation of olfaction in patients with mild cognitive impairment (MCI). Patients with MCI represent a heterogeneous group; some of the patients in this group are in the earliest stages of DAT. The aim of this study is to provide a broad assessment of olfactory function in patients with MCI. We tested patients with MCI and healthy normal control (NC) subjects, matched for age, gender, education and smoking status. Olfactory evaluation consisted of tests of detection threshold (single staircase procedure), quality discrimination and identification. We also report neuropsychological test results in these two groups of subjects. Patients with MCI show specific neuropsychological deficits restricted to memory (short- and long-term) and confrontational naming. We found that patients with MCI have higher detection thresholds than NC subjects. Furthermore, they have a deficit on odor quality discrimination and odor identification. However, when we remove the variance due to olfactory sensitivity, deficits on odor identification and quality discrimination are no longer significant. Our results show clear impairments in olfactory sensitivity, discrimination and identification in patients with MCI. We conclude that their difficulties in olfactory discrimination and identification are secondary to their deficit in olfactory sensitivity, and propose that high detection threshold may be useful as a possible early indicator of DAT.

## Neural basis of olfaction in the *Drosophila melanogaster* larva

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*Drosophila* larvae possess a simple chemosensory system, capable of perceiving both volatile and non-volatile gustatory stimuli. Chemosensation, before metamorphosis, is thought to be accomplished by several chemosensory sensilla distributed over the body of the larva: the dorsal organ (DO), the terminal organ (TO), the ventral organ (VO), the ventral pits (VP) and other internal sensilla. Anatomical studies have led to the hypothesis that the DO mediates olfaction while TO, VO and VP mediate taste. The DO is a multiporous structure, ideal for volatile reception, in contrast with the single pore structure of the VO, TO and VP, ideal for contact reception. However, no functional studies to date have shown the exclusive requirement of the DO for olfaction. Here we provide functional evidence that the DO neurons are required for olfaction but not for taste. We used a genetic approach to selectively silence the neurons that innervate the DO and tested the larvae for olfactory- and taste-driven behavior. The larvae whose DO neurons were silenced had defective olfactory driven behavior while their wildtype siblings and parental lines had normal olfactory-driven behavior. Taste-driven behavior in these manipulated animals was unaffected. Future experiments will examine the molecular basis of odor perception in *Drosophila* larvae, and how these odors are represented in the larval brain.

## Bitter taste and food choices

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Individual differences in sensitivity to bitter taste have been linked with a specific pattern of food likes and dislikes. Sensitivity to 6-*n*-propylthiouracil (PROP) has been variously linked with heightened taste responses to some bitter phytochemicals, sugar and salt, trigeminal irritation by alcohol, and the oral perception of emulsified fat. The question we asked was whether PROP sensitivity is associated sufficiently strongly with food choices to predict the risk of obesity and diet-associated chronic disease. Tracking causal associations between taste and food preferences, eating patterns, and disease risk is the domain of nutritional epidemiology. Population-based studies on PROP, food choices and disease risk should therefore rely on standard methods of dietary intake assessment and make use of the available array of epidemiological tools, including logistic regression analyses and adjustment for unequal distribution of covariates. Epidemiological studies also use categorical variables to a greater extent than studies in taste psychophysics, where the use of continuous scales has been the norm. If taste research is to make a contribution to public health, it must follow established rules for demonstrating causal associations between health-related variables such as diet and disease risk.

## Investigating associations between genetic variation in taste, diet and cardiovascular disease risk

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Studies from our laboratory have examined relationships between two markers of genetic variation in taste [perceived bitterness of 6-*n*-propylthiouracil (PROP) and fungiform papilla number (FP)], diet and cardiovascular disease (CVD) outcomes. Although those who taste PROP as intensely bitter tend to have high FP number, the two markers are probably under different gene control and dissociate when factors influencing taste nerves alter taste but not FP number. We have hypothesized that individuals who differ in PROP bitterness or FP number also vary in oral sensations from foods/beverages, in what they like to eat and choose to eat, and ultimately in their risk of chronic diseases such as CVD. Risk factors of CVD (e.g. dyslipidemia, hypertension, obesity) are elevated by excessive intakes of alcohol, fat and sodium, and inadequate intakes of complex carbohydrates, fruits and vegetables. Through careful psychophysical methods that permit valid comparisons of sensory intensity across individuals, we have found that adults who taste PROP as more bitter also find alcohol as more bitter/astringent and consume alcoholic beverages less frequently. Those who taste PROP as more bitter or have a higher FP number also report more creamy sensations from foods/beverages high in fat, prefer these foods/beverages less and have diets lower in fat. Individuals who taste PROP as less bitter also tend to report more frequent addition of salt to foods and more frequent intake of vegetables. Using PROP bitterness and FP number as taste genetic markers appears to enhance the ability to see the taste genetic–diet relationship. This relationship may vary with sex and age. The findings summarized above warrant investigations on differences in CVD risk with variation in PROP bitterness and FP density.

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## The influence of oral and product temperature on the sensory perception of texture and flavor attributes in custard desserts and mayonnaise

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In order to unravel the effects of oral and product temperature on odor, flavor and texture sensations of semi-solids, we have independently modulated both temperatures to determine their relative effects. A trained panel of 20 adults assessed 20 odor, flavor and texture attributes in one high-fat and one low-fat product of both custard dessert and mayonnaise. The oral temperatures were manipulated by 5 s mouth rinses performed prior to the assessments, resulting in oral temperatures of 27, 35 and 43°C. The products were evaluated at 10, 22 and 35°C. All combinations of product, product temperature and oral temperature were evaluated. The results show that the effect of oral temperature on the perception of sensory attributes was small, but present, while

the product temperature influenced the ratings greatly. Nine of the rated attributes, including odors and flavors, were significantly influenced by product temperature. There were large differences between the products within one type, where the difference between the high- and low fat products was more prominent in mayonnaise than in custard. These findings will be discussed in terms of physiological effects and physico-chemical properties.

The present project was started in 1999 and is funded by a consortium of academic, industrial and governmental partners.

### The vomeronasal epithelium in the mid-term human foetus: lectin binding

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A histochemical study was conducted to explore potential functionally related properties of the membrane glycoconjugates in prenatal accessory olfactory structures. The vomeronasal organ (VNO) in the nasal septum was examined for lectin histochemistry in second trimester fetuses obtained from historic museum collections. Age estimates were taken from modal values of crown-rump, head-circumference (95–240  $\mu\text{m}$ ) and foot-length measurements. Histology of 10  $\mu\text{m}$  sections established the maximum epithelial thickness along the major axis of the organ. From this zone lectin affinities (UEA1; VVA; BSA1; SBA and DBA) were determined with DAB reactivity. The VNO was unequivocally found in all fetuses, ages (weeks) and sex were: 11.5, m; 14, f; 16.5, m; 18, f; and 22.5, f; length changes over this uterine period fitted established growth curves. Positive reaction products for L-fucose and galactose (nGalNAc) residues identified two ORN-specific glycoproteins in the organ's (neuro)epithelium; these were evaluated following a protocol applied to the main olfactory epithelium in man. UEA and SBA bound at the luminal surface, in upper dendritic and basal zones. The luminal binding patterns showed slight but differential age-related gradient of intensity for each lectin. The results suggesting some mid-term alterations in the membrane properties of neuron-like VN elements (VNRs?), attributable to a lower and more delayed pattern of neurogenesis than in the MOE. Findings were interpreted against the ontogeny of the neurophyllic gonadotrophin cell line. The extent of human early VNO/AOB connectivity remains conjectural, as does that of any extrabulbar pathways.

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### Multimodal monosodium L-glutamate sensitivity in humans

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Monosodium L-glutamate (MSG) taste (so-called 'umami') is not clearly described by every subject. Several experiments are reported in order to document a recently found ageusia to L-glutamate (Lugaz *et al.*, 2002). The individual relative sensitivity to both MSG and NaCl was evaluated at the threshold level ( $n = 171$ ) and at a suprathreshold level ( $n = 109$  of the initial population). A

multigaussian analysis showed several modes in the MSG threshold distribution. Twenty-seven percent of subjects (Ss) could be classified as glutamate hypotasters. Time intensity profiling illustrated the difference of sensitivity in hypo- and normotasters. Finally, 3.4% of Ss could not discriminate between MSG and 29 mM NaCl in 30 successive triangle tests and were considered to be non-tasters. Of 350 Ss, 3.5% screened in a public scientific exhibition again failed to prove they perceived any different taste in MSG versus NaCl solution after three different tests. A familiarization experiment with 5'-GMP, a compound sharing the specific 'umami' taste of MSG, distinguished two groups in a population of 30 Ss. Eighteen Ss perceived at first  $2.7 \pm 0.6$  mM 5'-GMP isointense to the 29 mM NaCl reference. This isointense 5'-GMP concentration was reduced to  $0.48 \pm 0.14$  mM after four learning sessions. Twelve Ss never learned and showed stable isointense perception for equivalent amounts of sodium ions in both NaCl (29mEq) and disodium 5'-GMP (22 mEq Na in 11 mM 5'-GMP) solutions. 'Umami' hypogeusia and ageusia point to multiple receptors and individual genetic differences for MSG (and related compounds) taste sensitivity. Finally, 6-*n*-propylthiouracil and MSG hypogeusias were shown to be independent.

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### Modulation of taste sensitivity with familiarization to novel stimuli

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Mild conditionings may modulate and shape taste information in everyday life. Quantitative psychophysics, cerebral imaging and taste nerve electrophysiological recording were used to try to identify features of the modulation of taste sensitivity with familiarization to novel tastes. At detection threshold and suprathreshold levels, human taste sensitivity to novel stimuli increased during the first 3–4 sessions. After such training, individual data were reproducible over time (weeks or months) and the standard deviation reduced. Five subjects performed three functional magnetic resonance imaging experiments with four stimuli before and after the first psychophysical session, then after 15 days of psychophysical training. A significant evolution of the percent of activated pixels was observed in taste cortical areas, either increasing or decreasing, depending on stimulus and subject, between the first and the second experiment ( $P < 0.001$ ,  $\chi^2$ ), suggesting plasticity of taste coding. Psychophysical results showed a concomitant evolution of the hedonic assessment for the corresponding stimulus and subject ( $P < 0.003$ ,  $\chi^2$ ). Hamster whole nerve chorda tympani responses were recorded for a series of 21 unfamiliar taste stimuli, repeated six times. In the control group, response amplitudes increased significantly for all stimuli across repetitions, but in groups previously exposed to either dulcine, potassium glutamate or guanosine 5'-monophosphate, responses did not increase for the familiar stimulus specifically. Taste learning may occur both through central plasticity and peripheral effects

such as induction of chemoreceptor synthesis or transductional coupling facilitation.

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### Spontaneous preference for the odours of eugenol and cineol in male C57BL/6J mice

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In the course of a study on learning and memory formation in mice we developed an olfactory recognition test which does not require positive or negative reinforcement. Since a priori odour preferences/aversions must be taken into account, we initially screened eight odorants in a preference test. We formed four odorant pairs: (i) (–)-carvone versus isoamylacetate; (ii) anethol versus amylpropionate; (iii) cineol versus allylcapronate; and (iv) eugenol versus linalool, and tested them in male C57BL/6J mice. Three 1.5 ml Eppendorf cups, two of them containing 2 µl of different odorants, were fixed to the floor of a test arena (39 × 23 cm). Mice were allowed to adapt for 5 min, then the upper half of all cups were cut open for the odour to evaporate, and mice had 5 min to explore. The total time investigating each cup was analysed for the first 180 s. No significant differences between cups were found for the first two odorant pairs (ANOVA for repeated measures,  $df = 2, 19$ ,  $n = 20$ ). Of the last two pairs, cineol ( $F = 6.25$ ,  $P = 0.005$ ) and eugenol ( $F = 13.03$ ,  $P < 0.000$ ) were inspected significantly longer than the empty and the other scented cup. An additional test with naive mice revealed no preference between allylcapronate versus linalool, but there was a preference for eugenol versus cineol ( $F = 7.55$ ,  $P = 0.002$ ). That the non-preferred odorants were indeed perceptible at the concentrations used could be shown by recognition tests. Currently we are using such pairs of non-preferred odorants to investigate the time course of consolidation of olfactory memory.

### Synapse number and olfaction sensory coding in *Drosophila*

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We have addressed the role of synapse number in sensory perception by means of studying behavioral responses of genetically modified flies to defined stimuli. The genetic modifications included the threefold increase in synapse number resulting from the gigas mutant phenotype, and the functional inactivation of identified subset of sensory neurons caused by the directed expression of tetanus toxin. Under these conditions, we analyzed the motor reaction to quantitatively controlled stimuli of various sensory modalities (odors and light). On the other hand, we evoked adaptation to selected odorants and measured the volume of olfactory glomeruli, and estimated the number of synapses. The data from these experimental approaches indicate that the number of synapses is a significant parameter that determines the sensitivity of perception. If the number increases, the 'excess' of sensory input is accepted and processed by the central nervous system. Sensory specificity, on the other hand, is maintained through changes in synapse number and only the type of cell seems

critical for the quality of stimulus. The selective inactivation of identified sensory input becomes an additional strategy to decipher the sensory coding used in olfaction.

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### Effects of cyclic nucleotides in pheromone transduction of the hawkmoth *Manduca sexta*

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In extracellular tip recordings of pheromone-stimulated trichoid sensilla on the antennae of male *Manduca sexta* moths, we investigated the effects of cyclic nucleotides on pheromone transduction. A bombykal stimulus of 10 µg dose and 50 ms duration was applied every 5 min during 3 h of recording. After inclusion of 10 mM 8-bromo cGMP (8bcGMP) or 20 mM tetraethylammonium in the tip recording electrode, the action potential frequency was continuously reduced, the latency of the first action potential was prolonged, while the sensillar potential remained unchanged. Furthermore, in the presence of 8bcGMP the stimulus-correlated reduction of the action potential amplitude was less pronounced. When 8bcGMP was injected into the hemolymph the amplitude of spontaneous action potentials increased. Both effects indicate an 8bcGMP-dependent change of conductance. To examine which ion channels are affected by 8bcGMP we performed patch clamp recordings of cultured olfactory receptor neurons. Application of 8bcGMP affects three types of K<sup>+</sup> currents: a delayed rectifier and a Ca<sup>2+</sup>-activated K<sup>+</sup> current were blocked, while a slowly inactivating delayed rectifier current was activated. At least two types of non-specific cation currents were affected: an apparently PKC-dependent current was blocked, while a medium-sized cation channel was more frequently observed after addition of 8bcGMP. We hypothesize that cGMP selectively adapts the action potential response by closure of K<sup>+</sup> channels during this non-adapting stimulus protocol.

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### Molecular assembly of cAMP-mediated olfactory signaling pathways via scaffolding proteins

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Odor detection via odorant receptors of chemosensory neurons in the main olfactory epithelium induces cellular responses by stimulating adenylyl cyclase, leading to a rapid and transient elevation of the cAMP concentration which triggers the opening of cyclic nucleotide-gated ion channels. The rapid kinetics of the olfactory signaling cascade suggests that the functional elements are organized in multiprotein complexes by scaffolding proteins via their multiple docking sites for signaling proteins. A-kinase anchoring proteins (AKAPs) function as scaffolding proteins for the assembly of complexes involved in other cAMP-mediated signaling pathways. It therefore seems conceivable that AKAPs may also play an important role in olfactory signaling. Molecular cloning strategies as well as approaches based on specific probes and antibodies demonstrate that several distinct AKAPs are

expressed in the olfactory epithelium. In biochemical experiments the AKAP-derived peptide HT31 increases the activity of olfactory adenylyl cyclase and phosphodiesterase. These results indicate that AKAPs are involved in the assembly of olfactory signaling complexes.

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### Trigeminal ERP: relation to stimulus duration and concentration

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While widely used in studies on intranasal trigeminal sensitivity few data are available on potential effects of stimulus duration on the electroencephalogram-derived trigeminal event-related potential (ERP). The aim of the study was the development of a mathematical model which allows the relation between the ERP component and both stimulus duration and stimulus intensity to be described. Further, the study was designed to learn more about the characteristics of individual ERP components. Twenty healthy subjects participated (10 male, 10 female, 18–38 years, mean age 25.2 years). Trigeminal ERPs were recorded after stimulation with CO<sub>2</sub>. Five different CO<sub>2</sub> concentrations (45, 50, 55, 60 and 65% v/v) and five different stimulus durations (100, 150, 200, 250 and 300 ms) were used. To reduce the duration of the experimental sessions, nine of the 25 possible combinations were selected for each subject. Within the trigeminal ERP amplitudes and latencies of both, early (N1) and late components (P3) were measured. The relation between stimulus duration, intensity and amplitudes or latencies of trigeminal ERP components could be described as a model of power, following the formula  $P = b + k * I_{g1} * D_{g2}$ . A linear relationship was found between stimulus concentration, amplitude N1 and amplitude P3, and between stimulus duration and amplitude P3, respectively. Further, a linear relationship was seen between intensity ratings, stimulus duration and stimulus concentration, respectively. These data indicate that the different ERP components encode different stimulus characteristics. Specifically, later components of the trigeminal ERP not only encode stimulus intensity, but, other than earlier ERP components, also encode stimulus duration. Thus, later ERP components seem to reflect the integration of stimuli over relatively long periods of time, which appears to be of high significance to the functioning of the intranasal chemosensory systems.

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### Dynamics of odor representations in the olfactory bulb

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We analyzed the processing of odor-evoked activity patterns in the olfactory bulb (OB) by comparing patterns across the input units [olfactory receptor neurons (ORNs) and glomeruli] and output units [mitral cells (MCs)]. Experiments were performed on the intact zebrafish brain using 16 amino acid stimuli (10 μM). ORN

and MC responses were recorded electrophysiologically; glomerular activity patterns were measured optically after selective loading of sensory axons with a calcium indicator. At all levels, odor identity was encoded combinatorially by overlapping patterns of active units. Across ORNs and glomeruli, chemically related stimuli evoked similar activity patterns that were clustered in neural coding space. Imaging revealed a hierarchical chemotopic organization of glomerular activity patterns. During stimulus presentation (2.4 s), patterns were almost constant. Across MCs, however, activity patterns changed gradually during the initial 800–1000 ms of the odor response. At response onset, MC activity patterns reflected largely their inputs. Patterns evoked by related odorants were similar and clustered in coding space. Subsequently, however, initially similar activity patterns became decorrelated and clusters disappeared. This decorrelation reduced redundancy and enhanced the discriminability of related compounds. The underlying mechanism is not a sharpening of each MC's tuning, but a reorganization of population activity. A crucial process involved appears to be the dynamic redistribution of inhibition in the network. These results indicate that the circuitry in the OB actively transforms odor representations over time from one population code into another, more reliable, one. Time thus serves for the evolution of a network towards an increasingly informative state.

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### Olfactory information restructuring by projection neurons visualized with genetically expressedameleon in *Drosophila melanogaster*

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The axons of olfactory receptor neurons expressing a given receptor gene converge onto glomeruli in the mammalian olfactory bulb and the insect antennal lobe (AL) alike. Since different receptor neurons have characteristic odour–response profiles, the input to the AL results in a topographical mapping of these response profiles. In this study, we sought whether the output of the AL is also spatially organized. To this end, we selectively expressed the calcium sensitive reporter proteinameleon in a large subset of projection neurons in *Drosophila melanogaster*, and measured the glomerular responses. Odour elicited characteristic and overlapping response profiles. The target areas of the projection neuron axons are the lateral horn and the mushroom bodies (MB). Is the spatial pattern present in the AL preserved on its way to the MBs? Using the same reporter gene in the same population of projection neurons, we found that odour-elicited activity patterns were strongly overlapping, indicating that the separation in the AL is not entirely maintained in the target area. Nevertheless, different odours elicited different and characteristic activity patterns in the MBs, clearly showing some spatial segregation. This reshuffling of AL-output information in the MB may be the substrate for processing the highly multidimensional

olfactory information encoded in combinatorial glomerular patterns at the level of the ALs.

### How specific and unspecific inhibitory connections shape the olfactory response profile of identified glomeruli

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Odors are represented by specific spatio-temporal activity patterns in the olfactory bulb of vertebrates, or its insects analogue, the antennal lobe (AL). Inhibitory circuits are involved in the processing of odors to shape the olfactory code. To investigate these processing strategies, we simultaneously measured the receptor neuron input and projection neuron output responses of identified glomeruli to odors spanning 7 log units of concentration using calcium imaging in the honeybee AL. Increasing odor concentration led to stronger responses and more glomeruli being excited over the AL. Stimulus intensity may thus be encoded as overall excitation. Dose–response functions of the most-responsive glomeruli were sigmoidal and comprised dynamic ranges of 3–4 log units for both input and output neurons. Glomeruli with weak and intermediate responses in the input had reduced responses in the output, leading to lower numbers of activated glomeruli and contrast-enhanced odor representations. Odors can be separated to much lower concentrations in the output neurons than in the input. We also investigated the functional role of inhibitory connections by applying the two inhibitory transmitters GABA and histamine, and the GABA-antagonist picrotoxin. Taken together, the AL network optimizes odor representation by expanding the dynamic concentration range, improving concentration-invariance while maintaining intensity information, and increasing stimulus-uniqueness.

### Voltage-dependent Na<sup>+</sup> and K<sup>+</sup> currents in supporting cells of the mouse vomeronasal organ

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The vomeronasal organ (VNO) is a chemosensory structure involved in the detection of pheromones in most mammals. The VNO sensory epithelium contains both neurons and supporting cells. A lot of data collected on vomeronasal neurons suggest that these cells represent the pheromonal transduction site, whereas scarce information is available on the functional properties of supporting cells in the VNO. To begin to understand their role, we have characterized with patch-clamp recording techniques the electrophysiological properties of these cells isolated from mouse VNO. Vomeronasal supporting cells were distinguished from neurons by their typical morphology and by the lack of immunoreactivity for the olfactory G-protein  $\gamma$ -subunit, G $\gamma$ 8, and the olfactory marker protein (OMP), two specific markers for vomeronasal neurons. Results showed that, like neurons, supporting cells possessed voltage-dependent, tetrodotoxin (TTX)-sensitive Na<sup>+</sup> currents ( $I_{Na}$ ), and voltage-dependent, tetraethylammonium (TEA)-sensitive K<sup>+</sup> currents ( $I_K$ ). However, these

currents exhibited biophysical and pharmacological properties that differed markedly between the two cell types. In fact,  $I_{Na}$  was less sensitive to TTX in supporting cells than in neurons ( $IC_{50}$ : 520 nM and 15 nM, respectively) and was completely inactivated at  $\sim -80$  mV. By contrast, in VNO neurons complete inactivation could be obtained only with voltage  $> -40$  mV. In addition,  $I_K$  was more sensitive to TEA in supporting cells than in neurons ( $IC_{50}$  = 0.35 mM and 3.39 mM, respectively). Our findings indicate that supporting cells in the mouse VNO express voltage-dependent channels similar to those described in other types of glial cells, such as astrocytes.

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### Characterization of elements of the cyclic nucleotide signaling pathway in olfactory organs: $I_h$ -channels from the spiny lobster, *Panulirus argus*

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The second messengers cAMP and cGMP play an essential role in visual and olfactory signal transduction in both vertebrates and invertebrates. In contrast to vertebrates, where cyclic nucleotide-gated (CNG) ion channels are the main targets for cAMP and cGMP, relatively little is known about the targets of these signals in arthropods. We used RT-PCR to discern the presence of ion channels that are gated or modulated by cyclic nucleotides in lobster olfactory receptor neurons (ORNs), and found the cells express homologs of members of the  $I_h$  (also called HCN or HAC), CNG and EAG families of ion channels. We focused on the  $I_h$  channels that are activated by both hyperpolarization and cyclic nucleotides since an  $I_h$  current occurs in lobster ORNs. A full-length cDNA (PAIH) coding for an  $I_h$ -channel was cloned from the lobster olfactory organ. Recombinantly expressing the PAIH in HEK 293 cells gave a slowly activating, non-inactivating inward current under whole-cell voltage-clamp to hyperpolarizing voltage steps, the amplitude and activation rate of which increased with increasing hyperpolarization. The ion channel was preferentially selective for potassium ions. Intracellular cAMP (1 mM) shifted the activation curve of the whole-cell current up to 40 mV from  $-119$  to  $-79$  mV to more positive values within the normal resting potential range. cAMP and cGMP also accelerated the kinetics of channel opening and increased the deactivation time of the channel. A current with similar general properties, e.g. ion selectivity, also occurred in cultured lobster ORNs. cAMP (1 mM) shifted the half-maximal activation of the current from  $-110$  to  $-81$  mV. An antibody raised against PAIH detected the protein in Western blots of olfactory sensilla, as well as to the olfactory organ itself, brain and eye. The physiological properties of the channel, and its presence in the transduction zone, suggest this channel may serve a role in transduction in lobster ORNs.

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## Odorant detection and screening by engineered yeasts operating as reporter systems

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The ligand preferences of olfactory receptors are still largely unmapped. Yeasts have the potential to provide a convenient functional system for searching 'odor space' to match odorant agonists with otherwise orphan receptors.

Taking advantage of the parallel function between yeast and mammalian GPCR signalling pathways, *S. cerevisiae* were engineered to bypass the alpha-factor signalling pathway, so that activation of either Ste2p or a recombinant heterologous olfactory receptor will no longer induce cell-cycle arrest but stimulate a reporter activity. Odorant receptors and cognate Galpha-proteins have been expressed from plasmid constructs to enable ligand-induced receptor activation of luciferase placed under the control of the *FUS1* promoter. Luciferase activities may be quantified with respect to control and odorant exposure. Alpha-factor stimulation of the endogenous Ste2 receptor provides an internal control, and dose-dependent activation of the somatostatin receptor a control of receptor coupling. Expression of olfactory or somatostatin receptors in response to galactose induction from the Gal1/10 promoter is monitored at the level of mRNA and with antibodies against the receptor protein. Experimental conditions for luciferase level measurements will be presented to evaluate the feasibility of high-throughput screening of odorants for a given olfactory receptor. The response kinetics of the luciferase reporter after the initial stimulation, and the influence of exposure with respect to signal intensity and odorant discrimination will be reported. The efficiency of the various Galpha-proteins used for coupling (Gpa1, Golf, Galpha15) will be compared.

## Odour specificities of olfactory receptors in *Drosophila*

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We are investigating the ligand-specificity of individual odour receptors in *Drosophila*. A new approach allows us to map a receptor to a neuron and thereby determine its ligand-specificity from physiological recordings. We use the promoter of an olfactory receptor gene to drive expression of green fluorescent protein (GFP) via the UAS-GAL4 system, and then we record physiologically from GFP-labeled sensory hairs. These recordings, along with recordings from similar constructs driving expression of the cell death gene *reaper*, allow us to correlate individual receptors with neurons and with the ligands to which they respond. As a second means of defining a ligand for a receptor we have created a deletion mutation of one *Or* gene. We observe a loss of odor sensitivity in one particular class of olfactory receptor neurons (ORNs). Specifically, electrophysiological recordings of action potentials elicited by a panel of odours showed that the ORNs in the maxillary palp can be grouped into six classes according to their response profiles. One of these classes, pb1B, is narrowly

tuned to 4-methylphenol and some structurally related phenols. Deletion of the *Or71a* gene causes loss of response to these phenols in pb1B neurons. Re-insertion of the gene into the mutant flies restores sensitivity. This suggests that the specificity for 4-methylphenol in these neurons is conferred by *Or71a*. Using similar molecular tools we show that a second member of the *Or* gene family, *Or22a*, is responsible for the relatively broad odour profile of a class of ORNs in the antenna. We are now driving expression of other odour receptors in the 'empty' neurons of the mutant flies to examine the specificity conferred.

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## Expression and function of odor receptors

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We are investigating the odor specificity of individual olfactory receptors in *Drosophila*. To visualize in which sensilla a receptor is expressed *in vivo*, we use the promoter of an *Or* gene to drive expression of green fluorescent protein via the UAS-GAL4 system. Electrophysiological recordings of action potential responses from green fluorescent sensilla allow us to associate a receptor with a functional type of sensillum. Subsequently, in order to localize a receptor to one of the neurons within the sensillum, we record from flies in which the *Or* promoter drives expression of the cell death gene reaper. Death of a neuron correlates it with the *Or* gene. We note that the other neuron responds to odors despite the loss of its partner. Thus we have found that *Or71a* is expressed in a class of palp neurons (pb1b) narrowly tuned to 4-methylphenol. In addition, we have created a deletion mutation in this gene and observe loss of response following stimulation by 4-methylphenol. Notably, sensitivity to 4-methylphenol can be rescued by supplying a wild-type copy of the gene in mutant flies. We are now trying to map the full repertoire of *Or* genes expressed in the palp to the six functional classes of palp neurons. We are also investigating the molecular mechanisms by which ORNs select the olfactory receptors they express and have identified a DNA sequence, the 'MP Dyad', which lies upstream of all maxillary palp *Or* genes and which appears necessary for their selection by maxillary palp ORNs.

## Noxious chemical stimulation of rat mucosa activates a parasympathetic reflex to increase meningeal and cortical blood flow

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Hot spices, such as chilli peppers containing capsaicin, activate mucosal trigeminal afferents and cause a burning sensation that can be accompanied by facial flush and lacrimation. Similar vegetative symptoms are found in some forms of primary headaches, e.g. cluster headache, indicating the involvement of the cranial parasympathetic system. On the other hand, several lines of evidence indicate that the trigeminovascular system is involved in

all types of vascular headaches characterized by changes in cranial blood flow and the release of calcitonin gene-related peptide (CGRP), a potent vasodilatory neuropeptide of afferent nerve fibers. To study the mechanisms that may link the activity of mucosal afferents with intracranial parasympathetic efferents in the control of meningeal blood flow and lacrimation, we developed an animal model to measure changes in blood flow caused by noxious chemical stimulation of rat mucosa with and without blocking the activity of the parasympathetic system. Using laser Doppler flowmetry, we recorded blood flow in the oral mucosa and in arteries of the exposed parietal dura mater encephali and the cerebral cortex. Oral blood flow was increased by 7–53.8% on average when the oral mucosa was rinsed with solutions of capsaicin ( $10^{-6}$ – $10^{-4}$  M). Stimulation of oral or nasal mucosa with capsaicin ( $10^{-5}$ – $10^{-3}$  M) also caused increases in dural and cortical blood flow ranging from 4.2 to 15.7% on average. These increases in flow were abolished by i.v. injection of hexamethonium chloride (20 mg/kg) and by local administration onto the dura of atropine ( $10^{-3}$  M) or [Lys1,Pro2,5,Arg3,4,Tyr6]-VIP ( $10^{-4}$  M), an antagonist of vasoactive intestinal polypeptide (VIP) receptors, i.e. by pre- or postsynaptic blockade of parasympathetic transmission. The involvement of the parasympathetic system was confirmed by an increased lacrimation induced by mucosal capsaicin application, which was also reduced by hexamethonium. VIP topically applied to the dura turned out to be very effective in increasing meningeal blood flow. Furthermore, increases in dural flow caused by nasal capsaicin stimulation could also be blocked by the CGRP receptor antagonist CGRP8-37 ( $10^{-4}$  M) administered to the dura. To study this interaction, we used an *in vitro* preparation of the hemisectioned rat head to directly measure CGRP release, which was significantly increased by VIP at a concentration of  $10^{-5}$  M and by acetylcholine ( $10^{-4}$  M). We conclude that noxious chemical stimulation of mucosal tissues activating trigeminal afferents causes vasodilation and increased blood flow in the meninges and the cerebral cortex via a parasympathetic reflex. The vasodilatory effect depends on the release of acetylcholine and VIP from parasympathetic efferents innervating intracranial blood vessels. VIP additionally stimulates perivascular meningeal afferents to release CGRP. This trigemino-parasympathetic reflex may contribute to the development of headaches resulting from infections of oral or nasal mucosa. In a more general way it seems likely that this coupling of extrameningeal trigeminal afferents and meningeal parasympathetic efferents contributes to headaches involving diseases of cranial tissues.

### Chemosensory changes with age and their effects on food intake and nutrition

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On average, elderly have an impaired taste and smell perception compared with young adults. Elderly also have higher optimally preferred flavour concentrations in various foods. These higher optimal flavour concentrations are probably related to a higher preference to foods with added monosodium glutamate (MSG), a flavour enhancer. In addition, elderly show a lower degree of sensory specific satiety and a higher resistance to monotony. These

changes in preferences with age can be applied to influence food intake. In a recent 16 week parallel study in a nursing home, we daily sprinkled 1 g of flavour powder (containing MSG plus appropriate flavour) to the warm meal of an experimental group of 36 subjects (mean age = 85 years). No changes were made in the control group. Mean weight change in the experimental group was +1.1 kg, whereas mean weight change in the control group was –0.3 kg. These results suggest that the use of flavour enhancers in the cooked meal was an effective way to improve dietary intake and body weight in nursing home elderly.

### Bimodal effects of oral chemesthetic and bitter stimuli across the tongue

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Although oral chemesthesia and bitter taste are both assumed to signal the presence of potentially dangerous oral stimuli, the two modalities are most often studied as independent sensory channels that respond to different stimuli. This approach is exemplified by the use of stimulus prototypes that are believed to stimulate one or the other modality selectively. For chemesthesia, the best known prototype is capsaicin; for bitter taste, numerous chemicals have been used, including quinine, urea and 6-*n*-propylthiouracil. Evidence from a handful of previous studies suggests, however, that capsaicin can affect the sensitivity to some bitter tastes, and that some bitter stimuli can excite the trigeminal nerve. The results of psychophysical experiments will be described that show capsaicin (and menthol, another common chemesthetic stimulus) can induce bitterness in some individuals, particularly when applied to the circumvallate papillae, and that urea and magnesium chloride (another common bitter stimulus) can induce sensory irritation when applied to the tongue tip but not when applied to the circumvallate (CV) papillae. In addition, on the tongue tip the bitterness of quinine and the bitterness, stinging and burning of urea and magnesium chloride were cross-desensitized by capsaicin, whereas in the circumvallate region pre-treatment with capsaicin failed to reduce the bitterness of any of the tastants, including capsaicin itself. Thus it appears that virtually all sensory irritants may be capable of stimulating the gustatory system, particularly on the back of the tongue, and that some bitter tastants can stimulate ‘capsaicin-sensitive’ somatosensory neurons, primarily on the front of the tongue. These spatial dependencies imply that multimodal oral stimuli are processed differently by the chorda tympani and trigeminal nerves than they are by the glossopharyngeal nerve.

### Investigations of nasal respiratory system blocking through continuous nasal airflow

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Exposures to airborne chemicals have been associated with adverse symptoms in susceptible persons with a variety of conditions. Whether an interaction with the nasal respiratory system is an

integral part of the pathway leading to symptoms has often been unresolved, in part because of the difficulty of isolating the nasal system in controlled experiments: orally inhaled gas diffuses into the nasal cavity through the retropharyngeal path, even when the nares are physically occluded. Our approach to nasal blocking involves flowing a stream of purified air into both nares throughout the respiratory cycle in order to impede retropharyngeal diffusion during oral breathing and allow blinded exposures of the lower respiratory system. Although conceptually simple, few studies describe any implementation of this method, and quantitative validation has not been addressed. In our experiments, blocking levels are measured through doping of the orally inhaled airstream with a tracer gas and detection of that tracer in the nasal cavity using an atmospheric-sampling mass spectrometer. A laboratory experiment has been used to pilot these techniques prior to human trials. A model of the upper respiratory system was constructed and outfitted with sampling probes to examine the level of tracer gas reaching various parts of the nasal cavity. Nasal isolation was measured at different sites as a function of nasal blocking and oral flow rates. System design, initial results, plans for human studies, and applicability to other areas of research will be discussed.

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### Sensory axon targeting and synapse specificity in olfactory glomeruli

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Olfactory sensory neuron (OSN) axons converge on glomeruli where they synapse with subsets of projection neurons and interneurons. There is accumulating evidence that odor receptors are expressed by the OSN axons and that they play an important role in discriminating between small subsets of neighboring glomeruli during targeting. It is less clear that they influence synapse formation/stability or the long-distance targeting required of OSN axons. Pursuing the hypothesis that multiple molecular mechanisms may influence these processes, perhaps hierarchically, we have been exploring the expression of alternate candidates in the spatio-temporal frameworks within which the projections of the OSN axons are established. We will first review the data that bear on the expression of cell surface molecules that may influence cell:cell adhesion and axon targeting in the olfactory pathway. We will then present evidence that members of the cadherin family, and their intracellular binding partners the catenins, are differentially expressed within the olfactory pathway. Localization of specific cadherins and gamma-catenin to the nerve layer suggests a role in axon extension while the presence of cadherins and beta- and delta-catenin in glomeruli suggests a role in synapse stability. Finally, we will show Nogo-A and Nogo receptor in the olfactory pathway where they may establish an inhibitory spatio-temporal gradient consistent with a role in the development of the olfactory pathway.

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### SURE, why not? The SUBstitution-REciprocity method (Olsson and Cain, 2000) for measurement of odor quality discrimination thresholds: replication and extension to nonhuman primates

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There is no natural physical continuum for odor quality along which an odor quality discrimination (OQD) threshold can be measured. Recently, Olsson and Cain (2000) introduced a psychometric method which, for the first time, allows the standardized determination of OQD thresholds. The method defines a threshold value that is an average fraction by which one odorant has to be substituted with another to reach a criterion level of discrimination. This measure of discrimination is reciprocal in the sense that it is a result of two separate psychometric functions involving two different standards but the same comparison stimuli. Using the same odor stimuli as Olsson and Cain with six human subjects, we were able to replicate their finding that a change of approximately one-third in stimulus content was needed to reach the OQD threshold for eugenol and citral. As the SURE method is based on discriminatory responses, it should also be applicable with nonhuman species which can be trained to give unequivocal discriminatory responses at the behavioral level. Using an olfactory conditioning paradigm, we therefore trained four squirrel monkeys to discriminate between exactly the same pairs of odor stimuli as our human subjects. We found the psychometric functions and the resulting OQD threshold value of the monkeys to be very similar to those of the human subjects. Our results show that the SURE method can successfully be employed with nonhuman primates and thus allows direct across-species comparisons of OQD and thus of similarities and differences of the odor spaces between species.

### Chemo-perception and behaviour in *Drosophila* larva and adult is controlled by the pan-neural gene *prospero*

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The way by which genes control complex behaviors is still a matter of a debate but the availability of numerous mutant genes in *Drosophila melanogaster* has allowed to start unravelling the role of some of these genes on behavior, and specially on olfaction and gustation.

*prospero* (*pros*) is a neurogenic gene highly conserved from flies to humans. *pros* is involved in different various aspects of nervous system development. More specifically, it is required to specify the cellular type during the embryonic formation of central nervous system, and in the development of larval and adult peripheral nervous system (PNS). *pros* also controls various neural aspects like cell cycle division, dendrite outgrowth. We characterized an enhancer-trap strain (Voila1) containing a PGal4 transposon inserted nearby the 5'-UTR of *pros* (Grosjean *et al.*, 2001). Voila1 is exclusively expressed in the nervous system, and in the PNS it quasi-specifically labels the gustatory system of larvae and adults (Balakireva *et al.*, 1998, 2000). Voila1 homozygous larvae show two

main defects: (i) they have an abnormal taste response and (ii) they very rarely survive until adulthood. The clean excision of the transposon simultaneously rescued both characters. The other alleles resulting of imprecise excision produced new *pros* alleles that were useful to explore the relationship between larval taste response and the requirement of *pros* in the neural regions relevant for this behavior. We focused on the antennomaxillary complex which prefigures and contains the terminal organ and the dorsal organ, which are the two main chemosensory larval organs. Our results together suggest a relationship between the level of *Pros* in the nervous system and the degree of behavioral alteration in larva.

### Influence of foreign residence on perception of everyday odors: a German–Japanese cross-cultural study

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An increasing number of studies suggest that learning plays a major role in shaping the way odors are perceived. The goal of the present study was to assess the influence of migration on cultural differences in odor perception. Thirty Germans (average age 30.2 years) and 28 Japanese (average age 27.8 years) emigrants, all living in Germany, were exposed to eight everyday odors (four typical Japanese and four typical Western odors) in a standardized randomized order. To analyze the degree of experience-dependent differences in odor perception, subjects were asked to rate the intensity, pleasantness, familiarity and edibility of the odors, and to name them or to describe the evoked associations. Data analyses revealed some significant differences between the two populations for descriptors and edibility judgements of the odors as well as for ratings of pleasantness, intensity and familiarity. The Japanese provided significantly more correct descriptors for all Japanese odors than the Germans, whereas less significant differences were found for the Western odors. In the Japanese group no relationship was found between time subjects had spent in Germany (mean = 6.1 years, SD = 10.3 years) and most of the assessed variables. This suggests that eating habits/olfactory experiences learned in early childhood might be very resistant to later change.

### Decreased trigeminal sensitivity in anosmia

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The present study aimed to investigate intranasal trigeminal sensitivity in a large sample of patients with anosmia due to different etiologies. We investigated trigeminal detection threshold for formic acid in healthy controls ( $n = 96$ ) and patients with anosmia due to head trauma ( $n = 18$ ) or sino-nasal disease ( $n = 54$ ). Anosmics exhibited higher thresholds compared with normosmics ( $P < 0.001$ ). In addition, thresholds were found to be higher in patients with posttraumatic anosmia compared with anosmics with sino-nasal disease ( $P < 0.001$ ). The data indicate that (i) loss of olfactory sensitivity in humans may be associated with a decreased

sensitivity towards trigeminal stimuli; and (ii) alteration of intranasal trigeminal function is stronger in patients with posttraumatic anosmia compared with patients with sino-nasal disease. This may have implications for the medico-legal investigation of anosmic patients where trigeminal stimuli are frequently used to assess the patient's response bias.

### Functional characterization of binding proteins for pig appeasing compounds and molecular cloning in the vomeronasal organ

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We have used tritiated analogues of appeasing compounds to characterize their binding proteins in the pig vomeronasal organ (VNO). Three lipocalines from the VNO extracts bind the appeasing compounds with different affinities. In Western-blot experiments, these proteins showed cross-reactivity with antisera raised against porcine odorant-binding protein (OBP), salivary protein (SAL) and von Ebner's gland protein (VEG). These proteins have been previously localized in the respiratory mucosa (OBP, SAL, VEG), the saliva (SAL, VEG) and tears (VEG). To confirm this unsuspected site of production, we have isolated the cDNAs encoding these proteins from VNO extracted RNA. The three amino-acid sequences showed 100% identity with the OBP, SAL and VEG sequences already obtained from other porcine tissues. These data suggest that OBP, SAL and VEG are involved in the detection of appeasing compounds in the VNO. Heterologous expression is ongoing and will contribute to understand the role of each protein in the molecular recognition of these compounds.

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### Olfaction in neurodegenerative disease

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There has been a gradual increase of interest in olfactory dysfunction since it was realized that anosmia was a common feature of idiopathic Parkinson's disease (IPD) and Alzheimer-type dementia (AD). It is an intriguing possibility that the first sign of a disorder hitherto regarded as one of movement or cognition may be that of disturbed smell sense. In this review of IPD, parkinsonian syndromes, essential tremor, AD, motor neuron disease (MND) and Huntington's chorea (HC) the following observations are made: (i) olfactory dysfunction is frequent and often severe in IPD and AD; (ii) normal smell sense in IPD is rare and should prompt review of diagnosis unless the patient is female with tremor dominant disease; (iii) anosmia in progressive supranuclear palsy and corticobasal degeneration is rare and should likewise provoke diagnostic review (iv) hyposmia is an early feature of IPD and AD, and may precede motor and cognitive signs respectively; (v) subjects with anosmia and one ApoE-4 allele have an ~5-fold increased risk of later AD; (vi) impaired smell sense is seen in some

patients at 50% risk of parkinsonism; (vii) smell testing in HC and MND is not likely to be of clinical value; and (viii) biopsy of olfactory nasal neurons reveals non-specific changes in IPD and AD, and at present will not aid diagnosis.

### Oral mucosal blood flow in patients with burning mouth syndrome

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Pathophysiology of the burning mouth syndrome (BMS) is largely unknown. The aim was to study oral mucosal blood flow (mBF) in BMS patients using laser Doppler flowmetry (LDF) Thirteen BMS patients (11 female, 2 male; mean age  $\pm$  SD =  $64.3 \pm 7.9$  years, mean disease duration =  $18.9 \pm 6.2$  months) and 13 healthy non-smoking controls matched for age and gender (11 female, 2 male; mean age =  $64.7 \pm 8.1$  years) were investigated. Using LDF, mBF was measured at the hard palate, the tip of the tongue, on the midline of the oral vestibule and on the lip. Measurements were made at rest and over 2 min following dry ice application of 10 s duration using a pencil shaped apparatus. In addition, blood pressure (BP), heart rate (HR), peripheral cutaneous blood flow and transcutaneous  $pCO_2$  were continuously recorded. mBF increased at all measurement sites in response to dry ice application ( $P < 0.001$ ), with peak flow at 0.5–1.5 min after stimulation onset. During the following 1.5–2 min, blood flow decreased at all sites, with a tendency to return to baseline towards the end of the observation period. Except for BP and peripheral blood flow, all of the cardiovascular changes exhibited significant changes during the observation period; no differences between groups were detected. When compared with healthy controls, BMS patients generally exhibited larger changes in mBF. These changes were significant for recordings made on the hard palate [ $F(1,24) = 13.9$ ,  $P < 0.001$ ]. Dry ice stimulation appears to be an effective, non-invasive and reasonably tolerable means to investigate mBF at different mucosal sites. In general, vasoreactivity in BMS patients was higher than in healthy controls. BMS patients exhibited a greater response on the hard palate compared with controls. These changes in oral blood flow appear to be specifically related to BMS symptoms, indicating a disturbed vasoreactivity.

### Zinc gluconate in the treatment of dysgeusia: a double blinded controlled study

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Albeit not life threatening, dysgeusia affects the patient's well being tremendously. However, no efficient therapy has been reported so far. In a randomized manner, zinc gluconate (140 mg) and placebo were assigned to 50 dysgeusia patients. Twenty-four subjects received placebo (age range = 47–78 years); 26 subjects received zinc gluconate (mean age = 61 years). Among other variables,

gustatory function, depression scores and a mood scale were evaluated before and after therapy, which lasted for 3 months. Results were analyzed by means of SPSS 10.0 for Windows. For normalization, differences were computed between data obtained after and those obtained before therapy. Analysis was performed using *t*-tests for independent samples. In addition, correlations were computed between the variables of interest. When compared with placebo, therapy with zinc significantly improved gustatory function ( $P < 0.001$ ). In addition, following therapy, patients in the zinc group were less depressed, as indicated by changes of the BDI score ( $P = 0.012$ ) and the mood scale ( $P = 0.039$ ). Patients with zinc also found suprathreshold tastants to be less unpleasant than patients in the placebo group which indicates resolution of dysgeusia ( $P = 0.046$ ). No significant group differences were seen for other investigated parameters with the exception of salivary calcium levels, which were significantly higher in zinc patients as compared with controls ( $P = 0.41$ ). Interestingly, treatment with zinc had no significant effect on levels of zinc measured in serum ( $P = 0.46$ ) and saliva ( $P = 0.65$ ). Zinc is useful in the treatment of dysgeusia in terms of (i) improvement of general gustatory function, (ii) improvement of dysgeusic sensations and, consequently, (iii) improvement of general mood scores.

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### Odor intensity ratings in response to ortho- and retronasal stimulation

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Retronasal olfaction has received less attention than its orthonasal counterpart. The aim of this study was to investigate the perception of odor intensity following ortho- and retronasal presentation of olfactory stimuli using air-dilution olfactometry. A total of 23 subjects (11 women, 12 men, age 19–45 years) were investigated. In addition to examination of orthonasal olfactory function using 'Sniffin' sticks' (threshold, discrimination and identification), the subject's retronasal olfactory function was assessed psychophysically using an identification test consisting of 20 aromas applied orally (Heilmann *et al.*, 2002, Arch. Otorhinolaryngol. Head Neck Surg., 128: 414–418). Odor intensity ratings were recorded in response to ortho- and retronasal stimulation using air-dilution olfactometry. For retronasal stimulation, odorants were applied via tubing placed below the lower turbinate under endoscopic control. Odorants were thus released in the epipharynx. For olfactory stimulation, phenylethylethanol and hydrogen sulfide were used in two different concentrations. Subjects participated in two sessions separated by at least 1 day. There was no significant difference in the intensity of odors presented ortho- or retronasally. However, the interaction between factors 'odor concentration' and 'stimulation site' [ $F(1,16) = 6.80$ ,  $P = 0.019$ ] indicated that subjects were better in the discrimination of odor intensities when odors were presented retronasally compared with presentation through the orthonasal route. This interaction was only observed for the first half of the first session; it was not seen during other periods of the experiments. These results indicate that there are subtle differences in the perception of odors presented ortho- or retronasally. While there are no general differences in intensity perception, different

odor concentrations are better discriminated when odors are presented retronasally. However, probably due to desensitization, these differences disappear with repeated stimulation.

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### Dynamic regulation of olfactory bulb mitral cell activity by metabotropic glutamate receptor mGluR1

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In the main olfactory bulb (MOB), mitral cells (MCs) express high levels of the group I metabotropic glutamate receptor, mGluR1. We explored the functional role of mGluR1 in regulating MC activity in rat and mouse MOB slices. The selective group I agonist DHPG, and not group II or III agonists, induced potent, dose-dependent and reversible depolarization, and increased firing of MCs. These effects remained undiminished in the presence of fast synaptic blockers, indicating that they are due to direct activation of mGluRs on MCs. MC excitatory responses to DHPG were absent in mGluR1 knockout mice, but persisted in mGluR5 knock-out mice. The results indicate that excitatory responses of MCs to mGluR agonists are primarily, if not entirely, mediated by the mGluR1 subtype. Application of mGluR antagonists (MCPG, LY341495, LY367385) regulated two key features of MC activity. They modulated the periodicity of membrane potential bistability, i.e. the duration of the up- and down-states, and rendered MCs less likely to generate action potentials. They also reduced the response probability to olfactory nerve stimulation and increased the latency of the olfactory nerve-evoked response. These findings suggest that endogenous, synaptically released glutamate tonically modulates MC excitability and hence the operation of the MOB circuitry via activation of mGluR1. Activation and inactivation of mGluR1 dynamically regulate MC responsiveness to synaptic input, including olfactory afferent input, possibly to modulate lateral inhibition and to increase contrast in the MOB network.

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### Specificity of sweet-best or bitter-best fibers in rhesus monkey chorda tympani does not change with stimulus concentration

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Previously we demonstrated that information from only sucrose-best (S) fibers in chorda tympani (CT) is sufficient to separate sweeteners from compounds of other taste qualities. The purpose of this study was to test whether the S as well as other fibers maintain their specificity when stimulated with higher concentrations. Recordings were obtained in eight rhesus monkeys during stimulation of the tongue with four different concentrations of NaCl, citric and ascorbic acids, quinine (QHCl), denatonium, sucrose and SC45647. Using a multi-unit spike separation technique, we isolated 143 CT fibers. In agreement with our previous observations, the S fibers were most narrowly tuned. Furthermore, the specificity of all S fibers did not change during stimulation with

any concentrations of stimuli belonging to other taste qualities. Thus 19 out of 27 S fibers responded only to the sweeteners and did not respond to any concentration of NaCl, acids and QHCl and denatonium. Even in the remaining eight S fibers, the responses to non-sweet stimuli were smaller than those to sucrose or SC45647. Among 52 QHCl-best fibers, 25 responded only to the bitter stimuli. In 27 QHCl-best fibers, however, acids at high concentrations also elicited strong responses. Some NaCl-best (15%) and acid-best (17%) fibers maintained their specificity at all concentrations of all stimuli used. The S fibers of rhesus CT remain narrowly tuned in spite of changing stimulus concentrations. This suggests that S fibers play a determining role in coding of the sweet taste.

### Behavioral and receptor cell responses to high intensity sweeteners and enantiomeric sweetener pairs in flies

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Sweet taste transduction in vertebrates may involve a cAMP pathway for natural sweeteners and an IP<sub>3</sub> pathway for high intensity sweeteners. To investigate these mechanisms in *Phormia regina*, we studied behavioral proboscis extension responses (PER) and recorded receptor cell action potentials from single sensilla in isolated proboscis preparations. First, sucrose, aspartame, sodium saccharin, sodium cyclamate and the halogenated sugar, sucralose, were tested across concentrations known to be effective in humans (Schiffman and Gatlin, 1993). Flies that gave a PER to 64 mM sucrose were tested with each concentration of a high-intensity sweetener (nine flies per sweetener), but none gave a PER to the other sweeteners. There was a typical concentration–response function for sugar cell firing to sucrose, but not for other sweeteners. These data suggest that a high intensity sweetener/IP<sub>3</sub> pathway is not excitatory for the sugar receptor cell and are consistent with the suggestion of Ozaki *et al.* (1992) that IP<sub>3</sub> is involved in adaptation of the fly response to sucrose. Next, enantiomeric pairs of pentose and hexose sugars were tested to determine whether sweet stimulation is receptor-mediated. There were differences in firing rates to enantiomeric pairs of both pentose and hexose sugars ( $P < 0.015$ ), but PER differences only to the pentose pairs ( $P = 0.003$ ) (repeated measures, two-way ANOVAs). These results support a protein receptor–ligand interaction over a purely physicochemical process.

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### Chemical markers that determine the olfactory response from manure sources

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Determination of human olfactory response to livestock odours by instrumental means has been investigated to simplify on-site measurement and reduce cost. A new approach has been developed

using volatile components to identify the olfactory response from different manure types. The chemical concentration of the odorants from pig manure as well as chicken manure composted with straw was determined in the headspace. Thermal desorption–gas chromatography–mass spectrometry was used to find marker compounds indicative of the olfactory response. The major odorous compounds, selected as those significantly greater than their odour threshold values, were used to develop models to explain the olfactory response of a trained odour panel. An equation for each manure odour was determined, which was able to predict the odour concentration. The approach gave good correlation ( $R^2 = 0.76$ ) for the predictive odorants for odour from pig manure, which were  $H_2S$ , 4-methyl phenol and acetic acid.  $H_2S$ , dimethyl sulphide (DMS), butanoic acid, methanethiol and trimethylamine were the major odorants emitted from chicken manure. The odour response from composting chicken manure was best described as the sum of the combined concentrations of  $H_2S$  + DMS ( $R^2 = 0.95$ ). Multiple linear regression and interpolative neural network approaches were used and the merits of both are discussed.

### Change and recovery of taste in result of middle ear surgery

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The extent of taste disorders following middle ear surgery (mes) depends on the grade of chorda manipulation. There is a lack of systematic investigations on this topic. The aim of this study was to evaluate the taste disorders after mes by anamnesis, by chemical stimulation and electrogustometry preoperatively, 2–3 weeks and 6 months after mes, respectively. There is a total number of 158 patients. Of these, 118 were operated on for the first time. In 54 out of the cases the manipulation was classified as minor manipulation, 14 as strong manipulation and 50 as chorda cutting. Interestingly, the most complaints were observed in those patients with strong chorda manipulation followed by those patients with chorda cutting. But there were also single cases of permanent complaints after minor chorda manipulation. The recovery of the taste sense was superior after strong chorda manipulation. The recovery after chorda cutting was incomplete, and these patients suffered more than the two other groups from a lack of taste sense of the ipsilateral anterior two-thirds of the tongue. The investigation shows a great discrepancy about the results of the anamnesis, chemical stimulation and electrogustometry. The most sensitive method to verify the taste loss after chorda cutting is the electrogustometry.

### The relationship between nasal anatomy and olfactory ability

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The clinically important relationship between nasal anatomy and olfactory ability was explored using three different approaches. (i)

When nasal dilators were used to temporarily increase the size of the nasal cavity, subjects perceived olfactory and trigeminal stimuli as being more intense, and they were better able to identify these smells. Additionally nasal dilators were shown to increase a number of sniff characteristics including flow rate and duration. Besides providing some potentially clinically important observations about the relationship between olfaction and nasal anatomy, these data suggest that the sniff itself may, at least in the short term, be hard wired. (ii) By repeated measurements of the airflow through the two nostrils, a correlation was found between the nasal cycle and subject handedness. It was observed that for right-handed subjects the right nostril was more often the dominant (more open) nostril, whereas for left-handed subjects the left nostril was dominant more often. Accounting for handedness may help remove some of the variability seen in nasal cycle studies. (iii) Regardless of handedness, in the more open nostril, water-soluble odorants were shown to make a bigger contribution to an odorant mixture as compared with the contribution they make in the less open nostril. By comparing the binasal response to these uninasal responses, it was possible to generate models which suggest how the central nervous system deals with disparate nostril information. These models may be helpful in explaining the clinical impact that single nostril malfunctions have on odor perception.

### Sensory determinants of one-trial odor learning in newborn rabbits

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Rabbit pups are nursed only once a day for 3–4 min, and depend on a pheromone on the mother's ventrum for the release of nipple-search behavior and sucking. However, if the mother's ventrum is perfumed before nursing, pups tested 24 h later on a fur scented with the odorant demonstrate nipple-search behavior while naive control pups do not. In a previous study (Hudson *et al.*, 2002, *Devl Psychobiol.*, in press), behavior of the doe, milk consumption and arousal during searching were found not to be major reinforcers, but attachment to and sucking nipples were. In a first examination of the role of perioral stimulation in this early learning, we examined the role of the whiskers; pups have ~40 per side, 4–17 mm long. Pups with whiskers cut on day 2 ( $n = 16/9$  litters) and placed on the ventrum of a perfumed doe in a standardized conditioning procedure did not differ significantly (mean  $\pm$  SD, t-tests) in search or sucking performance from uncut littermates: latency to attach to nipples,  $7.4 \pm 8.1$  versus  $8.0 \pm 12.9$  s; time spent attached to nipples,  $8.2 \pm 3.0$  versus  $8.6 \pm 2.9$  s. In the recall test on the perfumed fur 24 h later, no significant difference was found between de-whiskered and control pups in time spent searching in response to the perfume ( $18.1 \pm 14.8$  versus  $15.5 \pm 8.4$  s) or in the length of search bouts ( $1.4 \pm 0.6$  versus  $1.5 \pm 1.0$  s). Thus, at least at this early age, sensory input from the whiskers seems unimportant in rabbit pups for learning odors in the suckling context.

## Experience-dependent modulation of olfactory function in the newborn rabbit

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It is becoming increasingly clear that to understand olfactory function we need to understand olfactory learning and the manner in which experience shapes individuals' neural and behavioral responses to the odorous world. In mammals, however, it is usually difficult to find the well-defined and readily elicited behaviors typically sought by neuroethologists. Odor-guided suckling in rabbits is an exception, and provides an unusually good opportunity to study the neural basis of pheromone perception and olfactory learning in a natural context. Rabbit pups are nursed only once a day for 3–4 min, and depend on a pheromonal map on the mother's ventrum for the release of the stereotyped nipple-search behavior and suckling. This response is mediated by the main, not the accessory, olfactory system, and requires somatosensory input from the muzzle for its full expression. Although the pheromonal response is present at birth, pups can learn to respond to a novel odorant painted on the mother's ventrum with suckling behavior in just one 3 min nursing episode. This rapid learning is restricted to a sensitive period during the first few postnatal days, depends on the reinforcing properties of intraoral stimulation rather than on the ingestion of milk, and is characterized by a delay in its full expression of several hours. Both the pheromonal and postnatally learned odor cues appear to be neurally encoded in a distributed manner since lesioning large and seemingly arbitrary areas of the olfactory bulb does not impair behavioral responding.

## Development of a questionnaire for the assessment self reported olfactory functioning

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In recent years several olfactory tests have been published to evaluate olfactory dysfunction. However, to the best of our knowledge, no psychometric sound assessment tool for the evaluation of smell-related problems in patients with a smell disorder is available. The goal of the present study was the development of a questionnaire to assess self reported olfactory functioning. In the study were included 128 normal controls screened for intact olfaction and cognition. The age range was from 18 to 82 years. Nine patients with a smell disorder (anosmia) were also included. All subjects included were evaluated by means of the Wiener Olfaktorische Testbatterie (WOTB) for olfactory dysfunction. The WOTB is a multiple-choice testing procedure to evaluate olfactory identification ability using common natural odorants. In an initial step 30 medical students assessed their olfactory functioning by means of a 17-item Likert scale questionnaire. After statistical analysis, the 5-item self-reported smell related problems scale (SRP) and 6-item olfactory related quality of life (ORQOL) scale emerged. The internal consistency reliability using Cronbach alpha was 0.87 for the SRP scale and 0.89 for the ORQOL scale. Empirical validity of the SRP scale and the ORQOL scale was assessed by comparing patients with smell disorder and healthy

controls. Both scales significantly discriminate between healthy controls and patients with anosmia ( $P < 0.001$ ). The 5-item SRP and 6-item ORQOL scale possess very good psychometric properties and may be useful in evaluating patients with smell disorder. The application for the two scales in the clinical setting will be discussed.

## Impaired trigeminal function in olfactory loss: a study based on lateralization of chemosensory stimuli

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A number of studies reported indications for dysfunction of intranasal trigeminal sensitivity in patients with olfactory loss. The aim of this investigation was to compare anosmic and hyposmic patients to healthy controls. A total of 64 subjects participated; 37 subjects had olfactory dysfunction (15 patients with functional anosmia; 22 hyposmic patients; age range = 28–69 years, mean age = 55 years), 27 subjects were normosmic (age range = 29–95 years, mean age = 48 years). Olfactory dysfunction was assessed using the 'Sniffin' Sticks' test kit, which includes tests for phenyl ethyl alcohol odor threshold, odor discrimination and odor identification. The subjects' ability to lateralize odors was investigated for benzaldehyde and eucalyptol. Using a hand-held stimulator, both odors were applied 40 times each to the left or the right nostril, while the contralateral nostril received the same volume of odorless air (15 ml of odorized or odorless air; stimulus duration ~500 ms). The subjects' performance was scored as the number of correct identifications of the stimulated nostril. Hyposmic subjects had significantly lower lateralization scores for both stimulants ( $t > 4.5$ ,  $P < 0.001$ ). Seemingly, this was not related to the likely cause of olfactory dysfunction (upper respiratory tract infection, trauma, sino-nasal disease, idiopathic) as all of the four subgroups had lower lateralization scores than controls ( $P < 0.05$ ). Hyposmic subjects did not differ from subjects with functional anosmia in terms of lateralization ( $P > 0.29$ ). For eucalyptol there was a tendency towards a positive correlation between duration of olfactory loss and localization of this stimulant ( $r_{37} = 0.32$ ,  $P = 0.054$ ). Further, in healthy subjects there was a negative correlation between age and the ability to localize eucalyptol ( $r_{27} = -0.54$ ,  $P = 0.004$ ). In conclusion, these data add to previous work indicating that olfactory loss results in decreased trigeminal sensitivity.

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## Studies of umami synergism using ionotropic and metabotropic glutamate receptor agonists

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We have already shown that the responses for both monosodium glutamate (MSG) and a MSG–inosine 5'-monophosphate (IMP) mixture can be classified into three types (transient inward current,

sustained inward current and outward current), and that the amplitudes of two of the types of inward currents were much larger in the mixture than in the MSG alone. We present here which types of glutamate receptors are involved in these inward currents. We examine here responses of mouse taste cells to some types of glutamate receptor agonists and nucleotides: ionotropic glutamate receptor agonists, NMDA and KA; the metabotropic glutamate receptor agonist, *trans*-ACPD; and the nucleotide, IMP. The response was measured under whole-cell patch clamp configuration. The concentration of each of the glutamate receptor agonists was 1 mM and that of IMP was 0.5 mM. The amplitudes of the transient currents were almost identical with both the NMDA alone stimulation and the NMDA–IMP mixture stimulation. KA also induced transient inward and outward currents in the taste cells, while the KA–IMP mixture induced the outward current. Only the outward current was induced by *trans*-ACPD. However, inward as well as outward currents were observed by the *trans*-ACPD–IMP mixture. These results suggested that ionotropic glutamate receptors are not involved in umami synergism transduction. And it suggested that the *trans*-ACPD–IMP mixture could activate the postulated umami receptor, though *trans*-ACPD alone could not because it is a weak umami substance.

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### Does androstenone care whether it attracts gay or straight people?

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Some studies indicate that women prefer seats sprayed with androstenone whereas males are rather repelled by this odour. The aim of this study was to examine the importance of sexual orientation in this context. Moreover, we wanted to investigate whether the motivational state modulates the behavioural effects of androstenone. Forty homosexual males and 39 heterosexual females participated in this study. In the experimental session the subjects, who were not aware of the purpose of the study, had to wait in a room. In the waiting room the subjects had the possibility to choose between two chairs on the left and two on the right side. In a pre-test without androstenone 13.6% of 81 subjects chose the left side. In the main experiment this side was treated with 0.078 mg androstenone. Half of the subjects were asked to arouse themselves sexually before entering the waiting room. In the control group the subjects were asked to develop an appetite for food. The sitting-distribution did not differ between the four experimental groups. Across all groups 27.8% of the subjects chose the treated chair group ( $P = 0.001$ ). The results show that androstenone has a spatially attracting effect not only on heterosexual females but also on homosexual males. However, this effect was not modulated by the motivational state of the subjects. The effect in homosexual males could provide a hint that the response to androstenone is learned rather than innate.

### Pheromone receptor cells: density of receptor molecules and dissociation constant of the pheromone-receptor complex

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Modeling of perireceptor and receptor events (Kaissling, 2001, Chem. Senses, 26: 125–150) based on morphometrical, radio-metrical, electrophysiological and biochemical data reveals estimates of the density  $d_R$  of receptor molecules R within the membrane of pheromone (bombykal) receptor cells of the male silkworm *Bombyx mori* and of the dissociation constant  $K_d$  of the pheromone–receptor complex PR. We found tentative values of  $d_R = Q \cdot U_{\text{sat}} \cdot T \cdot V \cdot A / (p \cdot a) \geq 4400$  receptor molecules per  $\mu\text{m}^2$  of membrane area and  $K_d = k_{-1}/k_1 = k_{-1} \cdot U \cdot T / k_{\text{fall}} \geq 35 \mu\text{M}$ .  $Q = 0.25$  is the fraction of stimulus molecules producing responses at low stimulus intensities.  $U_{\text{sat}} \geq 30 \mu\text{M/s}$  is the uptake of pheromone by the sensillum (olfactory hair) for maximum occupation of the receptor molecules R by pheromone molecules P.  $T = 1/k_{-1} + k_2/(k_{-1} \cdot k_2) = 153$  ms is the lifetime of the PR complex, where  $k_1 = 0.209/(\text{s} \cdot \mu\text{M})$  and  $k_{-1} = 7.9/\text{s}$  are the rate constants for the reaction  $P + R \rightleftharpoons PR$ , and  $k_2 = 16.8/\text{s}$  and  $k_{-2} = 98/\text{s}$  are the rate constants for the reaction  $PR \rightleftharpoons PR'$  ( $k_{-1}, k_2, k_{-2}$  from Minor *et al.*, 2001, Chem. Senses 26: 792).  $R'$  is the activated receptor which triggers intracellular transduction.  $V = 264 \mu\text{m}^3$  is the volume of the olfactory hair.  $A$  is the Avogadro constant.  $P = k_2/(k_2 + k_{-1}) = 0.69$ , the probability of activation when a complex PR is formed.  $a = 60 \mu\text{m}^2$  is the membrane area of the dendrite of the bombykal receptor cell.  $k_{\text{fall}} = 1/\text{s}$  is the rate constant of stimulus deactivation.

### Olfactory receptor subtypes expressed in individual OR37 neurons

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The mammalian olfactory system not only detects but also discriminates between thousands of small, structurally diverse volatile compounds. This enormous discriminatory power is supposed to be mediated by distinct subpopulations of olfactory sensory neurons (OSNs) responding to an odorant. The response spectrum of an individual cell is supposed to be determined by the selective expression of one distinct olfactory receptor (OR) type; however, it is unclear, if this one-receptor-per-cell paradigm is valid for all olfactory cells. *In situ* hybridization experiments revealed that most ORs are expressed in one of the broad nonoverlapping zones extending along the anterior–posterior axis of the olfactory epithelium. Cells expressing the same OR subtype appear to be randomly distributed within one zone. One particular OR family, the OR37 family, displays a unique clustered expression pattern: OSNs expressing an OR37 subtype are located exclusively in a small, particularly exposed epithelial area which overlaps with several expression zones. The observation that zonal receptor types are uniformly expressed in this area—which in addition comprises a high density of cells expressing OR37 receptors—leads to the question, if OR37 cells express additional olfactory receptors. In

order to investigate defined OR37 cells, neurons from transgenic mice were employed. In these mice OSNs which express a defined OR37 subtype also express green fluorescent protein (GFP); the appropriate cells can thus be identified in the epithelium due to their green fluorescence. Pieces of the olfactory epithelium were subjected to gentle dissociation procedures resulting in cell suspensions where individual OR37 neurons could be visualized by fluorescence; they were collected by means of microcapillaries or contactless laser pressure catapulting techniques. Collected individual cells were then analyzed by RT-PCR techniques. These approaches led to the identification of one or a few additional receptor types in OR37 cells. The receptor subtypes identified were all members of the mammalian class II ORs. Individual cells showed different receptor combinations; however, in some cells the same type of receptor was found.

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## Measuring odour emissions from landfill sites

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Eighty per cent of the UK population live within 2 km of either an active or a closed landfill site. About 100 million tonnes of waste from England and Wales is disposed of in 1500 landfill sites each year. The major fraction, roughly two-thirds, of this waste is biodegradable organic matter from households, businesses and industry. By the time it reaches the landfill site the degradation processes have already begun and have left their fingerprint. The odours associated with the discharge and disposal operation and the subsequent landfill gas that is generated from the later methanogenic biodegradation of organic wastes can lead to significant impact near landfill sites. Measuring these emissions and evaluating their nuisance factor remain challenges. This paper examines odour generation processes, ties together some of the experience of UK operators in managing landfill malodour, and describes methods used to characterize and measure odour and gas emissions from landfills.

## Encoding of perceptual differences visualized by brain imaging techniques

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Encoding of olfactory signals takes place at different levels within the olfactory pathways. Here we want to review some of the findings that have been obtained using newly developed imaging techniques in man. Several technologies are currently available for brain activation studies. Here functional magnetic resonance imaging (fMRI) and magnetic source imaging (MSI) results were compared for the identical stimuli. FMRI experiments were performed with a 1.5 T and a 3 T magnet. MSI experiments were performed with a 74- and a 122 channel system. All experiments were performed using an OM4b olfactometer. A birhinal olfactory stimulator, crucial to obtain very controlled chemical stimuli,

controlled in onset and offset with no additional mechanical and thermal sensation. As a general finding we can state that en(de)coding of the olfactory information can be found in various areas of the brain. However, activities of the olfactory bulb could not be successfully recorded so far. Activated areas that could be obtained include the piriform cortex, the limbic system, parts of the frontal cortex and the insula, as well as premotor areas and the supramarginal gyrus. There is evidence that some of these areas are specialized for the emotional aspects of the presented odorant, such as the hippocampus or the orbitofrontal cortex. Others are active in situations where information about odorants is retrieved and attention is directed. The cingulum is a good example for such a type of processing. Additionally, we found that during the presentation of an odorant as well as during recalling or imagining an odorant, the supramarginal gyrus is activated demonstrating an unexpected close relationship of olfactory processing to speech.

## Expression patterns of T1R3 and various taste perception related genes

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The analysis of the patterns of receptor expression provides important insight into our understanding of chemosensory discrimination. We have reported the identification of a novel sweet taste receptor, T1R3, expressed in a subset of taste receptor cells. Here we examined the expression pattern of T1R3 in relation to the taste perception-related genes encoding gustducin, T1R1, T1R2, T2Rs, ENaC and so on in various taste papillae of mouse using two-color fluorescent *in situ* hybridization. Recently, T1R1/T1R3 were reported to respond to umami taste and T1R2/T1R3 were to sweet taste. In our study, T1R3 was partially coexpressed with T1R1, which was weakly expressed, and overlapped with T1R2 expression in circumvallate papillae. T1R1 was partially coexpressed with gustducin, although T1R3 was mostly segregated with it. Interestingly, some parts of T1R3-positive cells also expressed ENaC, an amiloride-sensitive Na<sup>+</sup> channel in circumvallate papillae. These results implied that T1R3-expressing cells could be classified into several functional groups, which may explain the relationship between sweet and other taste (for example, umami or salty) perception at the taste cell level.

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## Insights into fat perception using PROP taster status

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Previous research has shown fat perception may vary with individual sensitivity to 6-*n*-propylthiouracil (PROP), which may be related to human anatomical and functional variations.

Understanding how PROP sensitivity affects perception and overall product liking is important for developing and testing flavor systems for fat-containing foods. However, conflicting results on this relationship are prevalent. Two studies were conducted to further investigate the role of PROP sensitivity on flavor and texture perception in dairy products. In the first study, 10 non-tasters (NT) and 10 supertasters (ST) rated nine dairy products using free-choice profiling (FCP). FCP is a type of descriptive analysis that allows semi-trained panelists to rate products based upon individually created lists of descriptors using a 15 cm line scale. Generalized Procrustes Analysis captured ~67% of the variance in fat perception for both groups in three dimensions. The major underlying dimensions were similar for the NT and ST, and were related to (i) dairy-related texture and flavor attributes; (ii) sweet and sour taste; and (iii) foamy/airy texture. However, the ordering of the first two dimensions was reversed for NT and ST, and NT used fewer textural terms throughout. In the second consumer research study, 63 NT and 51 ST evaluated the same nine dairy products for acceptance (9-point hedonic scale) and creaminess intensity (9-point category scale). Consumers were also presented with a list of the appearance, flavor and texture terms derived from the FCP study, and checked the ones that applied to their perception of the products. Across all products, ST rated the creaminess intensity higher and used more terms to describe their perceptions than did NT. These data suggest that NT and ST use different cues to judge fat in dairy products with ST relying more heavily on texture attributes. However, both groups seemed to ultimately arrive at the same overall integrated sensation of fat in these products.

### Experiments on atopi, non-IG hyperresponsiveness and other susceptibility factors in relation to irritation

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Causality is difficult to elucidate in epidemiological studies and it needs to be supported by studies on the linking of exposures to symptoms and to (patho-)physiological responses in the relevant organs. Also, identification of specific responders to the exposures will add to the understanding. The study reported here used three groups of subjects (nasally hyperreactive to histamine, hayfever patients and healthy nasally non-reactive). They were exposed to clean air, resuspended office dust, and the same dust with added glucane or aldehydes in a cross-over type climate chamber experiment. Symptoms, and measurements of inflammatory responses and susceptibility factors in the eyes and the nose, were measured both before and after the 4 h exposure. Both types of spiked dust showed significantly increased interleukin-8 response in nasal lavages and an increase in the thickness of the mucosa measured by volume changes using acoustic rhinometry. Furthermore, the two physiological responses were correlated within exposures. Nasal symptoms, like irritated dry nose, were increased at the end of exposure within the same exposure groups and showed correlation in the expected direction, although not significant. There were no differences between the three subject groups, but this may be caused by too low statistical power.

### A new rapid procedure for the assessment of olfactory threshold with 'Sniffin' Sticks'

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The assessment of olfactory function with 'Sniffin' Sticks' involves three tests addressing olfactory threshold, odor discrimination and odor identification (TDI test). A single staircase procedure is used for the measurement of olfactory thresholds. Three sticks are presented in a randomized order, with two containing the solvent and the third the odorant at a certain dilution. Altogether 16 dilution steps are implemented. The procedure starts with the lowest concentration of the odorant (*n*-butanol). Once the odorant is detected in two consecutive trials, the sequence is reversed and the next presentation uses the lower concentration, i.e. the next higher dilution step. If the odor is not detected, the sequence is reversed again towards higher concentrations, and so on. Seven reversal points are needed with the last four averaged in order to guarantee reliable and reproducible threshold estimates. This makes it a time-consuming procedure of up to 20 min, which generates problems in the light of human resources within a clinical environment. The aim of this study was to develop and validate a new and faster but no less accurate procedure for the assessment of olfactory threshold. Again a single staircase procedure was found to be the most appropriate, and again three pens were randomly presented. But this time, instead of only one pen, two pens might contain the odorant in a randomized way. The rest of the testing procedure remained the same. This technique reduced the probability of guessing the correct answer from 1/9 to 1/36. Thirty subjects were tested, and the results of olfactory thresholds obtained with the new method correlated significantly with those obtained with the old procedure. Using the third reversal point for calculation revealed a coefficient of correlation of  $r_{30} = 0.87$ . The use of only three reversal points instead of seven decreased the time consumption of the test to 10 min. The correlation coefficient for test and retest was  $r_{30} = 0.67$  (compared with 0.61; Hummel *et al.*, 1997). These data suggest the usefulness of the new rapid method for the assessment of olfactory thresholds.

### Collection of chemosensory event-related potentials with real-time stimulus monitoring

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In 1993, Evans *et al.* proposed suggestions for collection and reporting of chemosensory event-related potentials (CSERPs). In these suggestions, there was description about stimuli rise-time, as follows. 'Reliable CSERP have been collected with this technique (embedding chemosensory stimuli in a constant flow) using chemosensory stimuli with rise-times less than 50 ms to 70% of maximum concentration.' Two methods for the observation of replacement from air to odorant were also suggested. One method was the usage of photo-dense aerosol instead of direct odorant and the other was that of temperature change. These methods, however, did not directly measure the change between air and chemosensory

stimuli. Additionally, ordinal thermistors and thermocouples do not trace millisecond change, because of the first-order time lag in thermal conduction. The other observation method, using aerosol, would usually pollute one of the odorant lines of olfactometer, which decreases the possible odorant variations. In order to solve these problems, we have developed an ultrasonic gas meter. This gas meter can directly monitor the real-time change of odorant stimuli concentration every 0.5 ms (sampling rate 2 kHz), which is rapid enough to observe the stimulus rise time. Using this equipment, we have measured the CSERPs, with simultaneous observation of changes between chemosensory stimuli and air.

## Olfactory event-related potentials in children

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Registration of olfactory event-related potentials (ERP) in adults has become a routine procedure in specialized centres. However, so far, olfactory ERP have never been studied systematically in children. In this study results of olfactory ERP in children are presented for the first time. Twelve children (six girls, six boys, aged 3.5–10 years) participated in this study. H<sub>2</sub>S was used for olfactory stimulation (odorant presented to the left nostril, stimulus duration 200 ms, interstimulus interval 30 s), with phenylethylalcohol being additionally used in some children. EEG recordings from Fz, Cz, Pz, C3 and C4 were referenced to A1/A2 (band pass 0.2–30 Hz; sampling frequency 250 Hz). In some children the ability of the children to discriminate different odorants was investigated using the 'Sniffin' Sticks' test battery. Children were divided into three age groups ( $\leq 4$  years, 5–7 years, 8–10 years), with four children per group. The largest amplitudes of PIN1 and NIP3 were seen in the youngest children. With increasing age, peak latencies increased, which was most important for P3 [ANOVA:  $F(2,11) = 3.96$ ,  $P = 0.058$ ]. These results indicate that olfactory ERP can be obtained in children. Additionally, based on electrophysiological data, the present results suggest that important changes in the processing of olfactory information do occur during early childhood.

## The role of enhanced aroma in the acceptance of a snack product among elderly and young

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A yogurt-like fermented oat bran product was flavored with regular and heightened concentrations of redcurrant aroma, and tested in two tasting sessions (side-by-side) and in a 6 day home-use trial (monadic testing daily, 3+3 packages of the snack) by elderly ( $n = 50$ , age range 63–85 years) and young ( $n = 75$ , age range 18–34 years). The stimuli were rated for odor and flavor intensity and pleasantness. In the home-use, subjects also reported the consumed quantity of the snack. Subjects conducted an olfactory test (TOE) consisting of 16 common odors, to be detected (among

four stimulus vials) and identified (with aid of four-alternative verbal labels). In the elderly, the mean detection score was 14.6 and the identification score was 10.0. For the young, these scores were 15.8 and 13.4, respectively. TOE scores did not predict the perceived difference in odor or flavor intensity of the two samples, although there was a slight trend to that direction. In both age groups, the difference in perceived odor and flavor intensity of the two samples was larger in tasting sessions than in home-use. Although the heightened aroma samples were initially rated less pleasant in both age groups, the pleasantness ratings of the elderly increased during the home-use, while in the young they decreased. In both age groups, the intake of heightened aroma sample was lower than that of regular and remained similar over the 6 day home-use.

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## Identification of candidate olfactory receptors of the moth *Heliothis virescens*

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In the antennae of moths, which have been proven as invaluable model for studying principles of odor perception, recognition and processing of olfactory signals is accomplished by antennal sensory neurons located in sensillar hair structures. Pheromones and odorants are supposed to be recognized by seven transmembrane domain receptor proteins in the dendritic membrane of these cells; however, the molecular identity of moth olfactory receptors is still elusive. We have assessed a genome database of the moth *Heliothis virescens* for sequences which may encode heptahelical receptors and employed exon-specific probes to screen an antennal cDNA library of the moth. Analysis of the isolated cDNA clones led to the discovery of a divergent gene family encoding putative seven transmembrane domain proteins. The notion that they may encode candidate olfactory receptors of the moth is supported by the finding that several subtypes are specifically expressed in the antennae. Moreover, *in situ* hybridization experiments revealed that they are indeed expressed in antennal sensory neurons. By means of double-labeling *in situ* hybridization studies, it was demonstrated that each receptor subtype appears to be expressed in a distinct population of sensory cells.

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## Induction of emotions and motivations by odours

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Contrary to former studies (Steiner, 1979; Tassinary, 1984) in induction of emotion and motivation by odours, self-report and

facial expression (for emotions and curiosity) were measured at the same time and analysed completely (FACS). A repeated measures design was used. In the first part of the study happiness, disgust, anger, hunger, thirst and curiosity were induced. In the second part of the study the relaxation effect of three odours was tested. Therefore at first stress was induced and then the performance of the odours should induce relaxation. For odour performance a cotton bud was dipped in the odour liquid until it was saturated. Analysis of variance for repeated measures was performed. The hypotheses were tested by contrasts against a neutral odour (dipropylene glycol). Induction of hunger worked well with baked apple and lemon. Thirst could not be induced at all by baked apple or methylsalicylic acid, but there was a slight induction by lemon. Disgust and anger could be induced for both methods in the easiest way (by methyl methacrylate, propionic acid and other odours). Happiness could not be analysed using the self-report measurement because reliability was too low. Also happiness was not shown when emotions were measured by facial expression. Relaxation was induced by all the odours used, but in different intensities. The strongest effect was induced by heliotropine (piperonal), followed by menthol and mint. This means that menthol is the effective essence of mint. Against the hypothesis, neutral odour showed the highest values of curiosity. But all values for curiosity were very high.

### The nature of synergism in monosodium glutamate–inosine 5′-monophosphate mixtures

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Enhanced receptor output points to a peripheral first cause of monosodium glutamate (MSG)–inosine 5′-monophosphate (IMP) synergism. There is some doubt about the central nature of this taste synergism, particularly whether it is primarily an intensity- or hedonic effect. A split-tongue technique was used to distinguish psychophysically between peripheral and central contributions to the synergism: conditions with components mixed on the tongue were compared with conditions where the components were on different anterior tongue sides. A significant peripheral synergistic effect was found. In a second experiment, the hypothesis was tested that the synergism consists of increased palatability rather than intensity. Stimuli were beef bouillon enriched with different MSG–IMP mixtures. Subjects tasted and swallowed samples in alternating intensity- and palatability sessions. Before participation, the subjects went through a screening experiment to ensure their ability to distinguish between palatability and intensity. The results clearly showed synergism in the palatability- but not in the intensity condition. We conclude that the peripherally enhanced receptor output translates into central palatability rather than intensity. In the past, 'subjective intensity' may in fact have been palatability. The nature of the synergism might not have revealed itself, if the subjects had not explicitly been instructed to distinguish between pleasantness and intensity.

### Chemosensitivity of non-innervated taste bud cells in mouse fungiform papillae

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Taste substances increase the cytosolic calcium concentration ( $[Ca^{2+}]_{in}$ ) in taste bud cells (TBCs) for releasing neurotransmitter. Although each taste bud in mouse fungiform papillae contains ~50 cells, only a few TBCs have synaptic contacts with taste nerves. We investigated whether the non-innervated TBCs were chemosensitive with a calcium indicator, calcium green-1, under an in-situ optical recording condition. The application of 0.2 M NaCl on receptor membranes increased  $[Ca^{2+}]_{in}$  in 13 TBCs in a single taste bud, indicating that the number of TBCs that increased  $[Ca^{2+}]_{in}$  in response to NaCl was larger than that having synaptic connections with taste nerves. These TBCs were arranged close to each other in a taste bud. In the electrophysiological study, the application of 0.1–1.0  $\mu$ M amiloride not only on receptor membranes but also on basolateral membranes decreased NaCl-induced inward currents. The application of 0.1  $\mu$ M amiloride on basolateral membranes also blocked NaCl-induced increase in  $[Ca^{2+}]_{in}$ , suggesting that amiloride-sensitive channels of basolateral membranes modify the taste response in the mouse taste system. We suspect that non-innervated chemosensitive TBCs together with innervated TBCs form cell-networks with chemical synapses and/or electrical synapses.

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### Human genomic variability of normal traits: olfaction as an example

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The olfactory pathway, which mediates the sense of smell, harbors receptors for practically any volatile organic compound. This is afforded through the expression of a repertoire of >1000 olfactory receptor (OR) genes in specialized sensory neurons. Work in our laboratory has led to the elucidation of the entire superfamily of OR-encoding genes in the human, mouse and dog. More than six hundred ORs in the human have become pseudogenes in recent primate evolution, constituting a system of hundreds of natural gene knockouts. Additional inactivation events are sought in single nucleotide polymorphisms at functionally important residues. We currently use this genomic resource in order to obtain a better definition of relationships between human genotypes and specific anosmia phenotypes (Menashe *et al.*, 2003, *Chem. Senses*, 28: abstract). In parallel, we are studying the molecular genetic basis of congenital general anosmia (CGA), in which humans are born without a sense of smell. This condition is presumed to arise from mutations in transduction components, e.g. the olfactory G-protein and cation channel. For these studies, we have identified more than 150 Israeli CGA cases, one-third of which are familial, the largest systematic study done so far. A combination of linkage

and association analyses is performed to identify the causative mutation. We hope to thus enhance the understanding of the molecular basis of olfaction.

### Olfaction: an epidemiological study on 1046 subjects

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The aims of the study were to look for a possible correlation between self-olfactory evaluation and two olfactory test methods, to evaluate the importance of olfaction among the senses and the impact of an imagined olfactory loss on the patient's quality of life. People were asked to give sex, age, nasal and general disease state, and to name their preferred odour. On a visual analogue scale (VAS), people were rating their olfactory performance and the severity it would represent to lose the sense of smell. They were tested by a five-item orthonasal ('Sniffin' Sticks') and a five-item retronasal olfactory identification test, and were asked to establish a 'hit parade' of the five senses (vision, audition, taste, touch, olfaction). A total of 1046 people (658 women and 388 men) were included in the study. No correlation between smell scores and self-rating performance of olfaction was found. Most people rated themselves as slightly better than the average. Almost all people considered the loss of smell as a severe disability. Despite this result, olfaction was rated by most people as the least important sense. There was no difference between orthonasal and retronasal olfaction test scores. Interestingly, the most frequent answer to the favorite odor was 'no preferred odor'. The second favorite odor was vanilla, followed by rose and cinnamon. Our data confirm that olfaction seems to be a neglected sense for most people. Olfaction was rated the least important sense and most people could not name their favorite odor. These data suggest that olfaction is a very unconscious sense as they stand in opposition to data concerning people losing their sense of smell who report considerable loss of quality of life (Miwa *et al.*, 2001, *Arch. Otolaryngol. Head Neck Surg.*, 127: 497–503).

### The significance of behavioral analysis of olfactory function, or: there is more to olfaction than genes, second messengers and ion channels!

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Enormous progress has been made in recent years with regard to our understanding of chemosensory function at the genetic and molecular levels. However, these new findings, for example concerning the repertoire of genes coding for olfactory receptors (ORs) or the mechanisms underlying the olfactory primary process, raise new questions which can best, if not exclusively, be addressed at the behavioral level. Thus, in addition to their more obvious and traditional role in studying the biological significance of intra- and interspecific chemical communication, cognitive functions such as odor learning and memory, and the capabilities and limits of a species' olfactory system in general, behavioral

assays gain new importance as they also allow us to test hypotheses generated by findings that employ genetic or molecular approaches. Odor structure–activity relationships, arguably one of the central topics in olfactory research, are a good example: the finding that the mammalian genome codes for ~1000 different types of ORs allows us to test whether the number of functional OR types is a determinant of a species' olfactory performance, and its capabilities to discriminate between structurally related odorants in particular. The finding that ORs are proteins, that is, chiral structures, sheds new light on enantioselectivity of olfaction. To this end, appropriate behavioral assays need to be developed—a challenge for behavioral biologists.

### Metallic taste of ferrous sulfate: a case of retronasal smell and gustatory referral?

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Hettinger *et al.* (1990, *Chem. Senses*, 15: 755) showed that qualitative reports of metallic taste from solutions of FeSO<sub>4</sub> decreased when the nose was pinched shut, implicating a retronasal olfactory origin of the apparent metallic taste. We replicated this study with psychophysical scaling and found decreased ratings of metallic intensity of FeSO<sub>4</sub>, but not CuSO<sub>4</sub>, when the nose was occluded, confirming Hettinger *et al.*'s observation but allowing for the possibility of another type of metallic taste stimulation from copper sulfate. Sniff analysis of the headspace over FeSO<sub>4</sub> solutions indicated no smell (as compared with water control) in either a same–different paradigm or in rated intensity. This result suggests that whatever volatile substance is responsible for the metallic taste may be formed from reactions in the mouth or released more efficiently when sipped. However, some subjects in the same–different task could discriminate ferrous sulfate from water when tasted, even with the nose pinched (significantly higher frequency of 'different' judgements for FeSO<sub>4</sub>/water pairs compared with water/water pairs). The origin of this cue is unclear, but could include either a true taste or a mouthfeel sensation.

### Odor identification in patients with mild cognitive impairment and Alzheimer's disease

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It is becoming increasingly clear that olfactory dysfunction is an initial symptom of patients with Alzheimer's disease (AD). In mild cognitive impairment (MCI), which progresses to AD in a large proportion of patients, the degree of olfactory dysfunction is unclear. The goal of the present report was to study odor identification ability in patients with MCI and AD using the Wiener Olfaktorische Testbatterie (WOTB). The WOTB is a multiple-choice testing procedure to evaluate olfactory identification ability using common natural odorants. All patients visiting the Memory Clinic at the Neurological University Clinic complaining of memory problems had a complete neurological assessment and underwent neuropsychological testing. One hundred and three healthy controls in the age range 50–84 years, 42 patients with MCI in the age range 51–83 years and 23 patients with AD in

the age range 53–88 years were included in the study. Statistical analysis (ANOVA) revealed a significant group effect ( $P < 0.001$ ). *Post hoc* tests showed that the three patient groups differed significantly. Our data indicate that olfactory functions are impaired early in the disease process. Thus, the assessment of olfactory functions as a marker for subclinical AD will be discussed.

### T1R taste receptors

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Several G-protein-dependent transduction cascades have been implicated in sweet and umami taste, but many of the molecular details of taste signaling remain obscure. The mammalian genome contains three T1Rs, C-family GPCRs that are selectively expressed in taste receptor cells. Our recent research indicates that T1R2 functions in combination with T1R3 to recognize diverse natural and synthetic sweeteners. T1R1 functions in combination with T1R3 to recognize the umami taste stimulus L-glutamate, and this response is enhanced by 5'-ribonucleotides, a hallmark of umami taste. Provocatively, the close correspondence of T1R2/T1R3 ligand specificity to sweet taste and T1R1/T1R3 to umami taste suggests the possibility that these receptors mediate all sweet and umami taste.

### Characterization of voltage-dependent and odor-activated currents in isolated olfactory receptor neurons of sea lampreys (*Petromyzon marinus*)

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In sea lampreys the olfactory system plays a key role in controlling a variety of behaviors, including feeding, migration and reproduction. Migrating adults, for example, appear to use unique bile acids, excreted by larval sea lampreys, to find suitable spawning streams. Sexually mature males, and probably females, release pheromones that aid in the attraction of mating partners. We used electrophysiological methods (patch-clamp whole cell recordings) to investigate the properties of voltage-dependent ion channels as well as responses to behaviorally relevant stimuli. We used freshly dissociated receptor neurons from migratory stage animals and from larvae. Cells displayed spontaneous action potentials, as well as action potentials in response to injection of current and application of odorants. A number of voltage-dependent currents were observed and characterized. Pheromone- and amino acid-induced responses of olfactory receptor neurons from both migratory stage animals and larvae could also be observed. We found that while petromyzonol sulfate, a lamprey-specific bile acid, led to depolarization due to activation of a cation conductance in both larval and migratory stage animals, arginine elicited activation of a cation conductance in adult animals but generated an inhibitory response in larvae. These results indicate that physiological changes on the level of the olfactory receptor neurons occur during metamorphosis.

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### The influence of training on chemosensory event-related potentials and interactions between the olfactory and trigeminal systems

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It is not possible to accurately predict the perceptual response to odorants and odorant mixtures without understanding patterns of suppression and facilitation that result from interactions between the olfactory and somatosensory (trigeminal) systems. The current study extends previous findings by exploring the effect of intensive training on the interaction between these systems and also by using a different mixed chemosensory stimulus in order to examine whether the principles established in earlier studies generalize to different odorants. Stimuli were chosen so as to selectively activate the olfactory (hydrogen sulphide, H<sub>2</sub>S) and trigeminal (carbon dioxide, CO<sub>2</sub>) nerves. In addition, linalool, a stimulus that activated both systems, was included. Thirty-five participants (19 men, 16 women; age range 18–44 years, mean age 23.7 years) rated the intensity of each stimulus when presented both alone and in binary mixtures (linalool + H<sub>2</sub>S, and linalool + CO<sub>2</sub>). Chemosensory event-related potentials (CSERPs) were obtained from five recording positions. Strong and specific training effects were observed with the odorant used for testing, but not with a different training odor. These effects were reflected in both psychophysical ratings and in the amplitudes and latencies of 'exogenous' and 'endogenous' CSERP components. Linalool was perceived as being significantly less intense than the other stimuli when presented alone. In binary mixtures, intensity estimates of linalool were unaffected and CO<sub>2</sub> was slightly, but not significantly, suppressed; however, H<sub>2</sub>S was strongly suppressed by linalool. One week of intensive odor training produced significant and specific reductions in the intensity of linalool and H<sub>2</sub>S both alone and as a mixture; however, training with a different odor (champignon) had no effect. CSERP data confirmed previous findings showing changes in topographical distribution that reflected the degree of trigeminal activity. Binary mixtures generally produced larger amplitudes than single stimuli. Latencies clearly differentiated between the three single stimuli and the binary mixtures. Both early 'exogenous' and later 'endogenous' CSERP components reflected the rated training effects. After linalool training, H<sub>2</sub>S and mixture amplitudes and latencies changed to reflect those of linalool (N1 and P2). Following linalool training only, changes were observed at the frontal site with 'cognitive' component P3, which reflected the changes in stimulus ratings.

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### Conditioning of emotional responses to odours

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Despite claims of a special association between odours and emotional responses, there is surprisingly little direct evidence of such an association, and less evidence that these associations can be learned through experience. The studies described here aimed to

create a learned association between a unique and unfamiliar odour and an emotional response (relaxation), as reflected by relaxation ratings and measures of physiological (autonomic) arousal. The general design involved participants being involved in three sessions. In the baseline session the variables to be measured were recorded. During the learning session relaxation or anxiety inducing stimuli were paired with an odour (or not). In the final test session, the same variables were measured either in the presence of the same or a different odour, or in the absence of an odour. In a final study, the effectiveness of odour to induce a learned relaxation response was compared with that of similar visual and auditory stimuli (in which temporal and spatial cues were minimized). Results indicated that both arousal and relaxation responses can be successfully conditioned to odours, and that this change can be successfully recorded by cognitive and physiological measures. These changes were not found in the absence of odour, nor did they generalize to odours not present during conditioning. Results also suggested that, while they may not necessarily be relaxing or arousing on their own, some odours possess a greater potential to be associated with emotional responses. It is suggested that this potentiation may itself be a result of prior learning (a release from inhibition) or of some inherent character of the odour. However, a second study showed that, while odours were more effective than a no stimulus control and ambient colours in reinstating relaxation, they were no more effective than a simple tone and less effective than music.

### CO<sub>2</sub> stimuli delivered to the nasal mucosa: thresholds and just noticeable differences at different stimulus durations

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Most odors excite both the olfactory and trigeminal systems in the nasal cavity. Gaseous CO<sub>2</sub> delivered within a constant stream of humid warm air (8 l/min, 80% relative humidity, 36°C) to the nasal cavity has become an established method to study nasal trigeminal sensitivity (Kobal, 1985). In a randomized cross-over study in 12 healthy volunteers, we wanted to study the effects of stimulus durations (200, 400, 800 or 1600 ms) on the perception of CO<sub>2</sub> stimuli. For determining perception and pain thresholds we used triple-forced choice staircase paradigms. Just noticeable differences (JNDs) were assessed at three distinct concentrations (50, 60 and 70% v/v CO<sub>2</sub>), employing also a triple-forced choice staircase paradigm. Detection thresholds of the CO<sub>2</sub> stimuli were 9.4 ± 4.8, 6.9 ± 4.2, 6.4 ± 2.6 and 4.1 ± 2.1% v/v CO<sub>2</sub> for stimuli of 200, 400, 800 and 1600 ms duration, respectively. The stimulus duration had a significant effect on the detection threshold ( $F = 7.04$ ,  $P = 0.001$ ). The stimuli became painful at 48.6 ± 9.1, 47.7 ± 9.1, 45.8 ± 9.1 and 42.8 ± 10% v/v CO<sub>2</sub> for stimuli of 200, 400, 800 and 1600 ms duration, respectively. However, the stimulus duration had no effect on pain thresholds. The JNDs ranged from 1.9 ± 0.9% v/v CO<sub>2</sub> for stimuli of 50% v/v at 200 ms duration to 5.5 ± 2% v/v for stimuli of 70% v/v at 1600 ms duration. The influence of the stimulus concentration on the JNDs was highly significant ( $F = 40.22$ ,

$P < 0.001$ ). JNDs increased with the stimulus intensity, with all three concentrations differing from each other in alpha-corrected post-hoc *t*-tests. The influence of the stimulus duration on JNDs was comparatively lower ( $F = 3.32$ ,  $P < 0.05$ ), and alpha-corrected post-hoc *t*-tests revealed no difference between specific durations. The results provide a basis for experiments that employ CO<sub>2</sub> stimuli with clearly distinct painfulness.

### The relationship between food preferences, stress, introversion and taster status

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For some individuals, stress can influence eating. The way in which food preferences are affected remains unclear, with some studies suggesting that low caloric density foods are preferred (Willenbring *et al.*, 1986) and others suggesting that high caloric density foods are preferred (Oliver *et al.*, 2000). In addition, genetic 6-*n*-propylthiouracil (PROP) taster status can also affect food preferences (Bartoshuk, 2000). The hypothesis of the present study is that food preference is affected by stress and taster status, and that this effect will be enhanced in introverts, who are often more reactive to stress than others. To that end, participants completed three questionnaires (NEO-PI, SCL-90-R, Eating Questionnaire), then rated the intensity of a PROP sample. Results were subjected to a regression model, which showed that, in addition to a main effect of emotional stability ( $P = 0.015$ ), two first-order interactions, extraversion × stress ( $P = 0.036$ ) and taster status × stress ( $P = 0.016$ ), were also significant in predicting food density preference. Our results agree with those of Willenbring *et al.* (1986) in that stress was associated with preference for foods lower in caloric density, though its only impact was in association with other variables. When the impact of either the interaction between extraversion and stress, or taster status and stress, is considered, the effect is a preference for foods high in caloric density.

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### Electronic sensing in the pharmaceutical, food, beverage and cosmetic industries

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Sensor array systems have been evaluated as fast, reliable and simple instruments for quality control for the last few years. This presentation will introduce the latest developments in E-nose and E-tongue technologies. The aim of the study was to demonstrate that both the electronic nose and electronic tongue systems are correlated with human sensory panel for various applications such as the development of liquid oral dosage (in pharmaceutical), the verification of incoming raw materials to your gold standard, and the detection of taints and off-odors (in the food, beverage and cosmetic industry). Measurements were conducted using an Electronic Nose Fox with 18 sensors/Electronic Tongue Astree instrument with seven sensors and an expert sensory panel. Good results/correlations were obtained for the various examples used to demonstrate the use of electronic noses and tongues in the

pharmaceutical, food, beverage and cosmetic industries. Both qualitative and quantitative applications were reliably solved using electronic sensing technology where objectivity, reproducibility and rapidity were very important.

### Lingual somatosensory and taste interactions: simultaneous recordings of the chorda tympani (VIIth) and trigeminal (Vth) nerves of the hamster

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Simultaneous electrophysiological recordings of the VIIth and Vth nerves were undertaken to evaluate their relative contribution in taste and somatosensory stimuli. Whole nerves were recorded in anaesthetized male hamsters ( $n = 11$ ) using Ag–AgCl 200  $\mu\text{m}$  diameter electrodes. The VIIth nerve was laid on a hook and the Vth was transfixed. A collection of 60 stimuli, including 41 acidic stimuli (pH 1.9–5.6, step 0.5), was tested every 2 min 15 s during 270 min. Responses of the VIIth nerve were pH-dependent and tenfold those of the Vth nerve. Repeated acidic stimulations induced a desensitization of the VIIth nerve. A series of different types of responses could be observed, including activations and inhibitions on either nerve or both. Out of 600 runs, 463 elicited a response on the VIIth (mean latency =  $3.6 \pm 2.5$  s) and 315 on the Vth nerve [mean latency =  $6.0 \pm 5.1$  s;  $P(t) < 0.001$ ]. A secondary response possibly appeared at  $25.4 \pm 10.7$  s on the Vth nerve and at  $32.2 \pm 15.1$  s on the VIIth nerve [ $n = 94$  and  $56$  respectively;  $P(t) = 0.01$ ]. The observation of the order of secondary responses (Vth nerve first) occurring after the stimulus rinse together with the desensitization of the VIIth nerve by strong and repeated acids suggest modulation of the VIIth neural activity by the Vth nerve. Nerve sections are in progress to check for a peripheral or a central origin of this interaction.

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### Psychological effects of subthreshold exposure of 4,16-androstadien-3-one on women

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The aim of the current study was to investigate the replicability of mood effects of a subthreshold concentration of the putative human pheromone 4,16-androstadien-3-one (androstadienone) in women by comparing a new experiment (experiment 1) with a previously reported experiment (experiment 2; Lundström, *et al.*, 2001). In experiment 1, 38 women participated in a double-blind, counterbalanced, between-groups experiment. Participants were exposed to either a solution consisting of 250  $\mu\text{M}$  androstadienone in mineral oil, masked with 1% Eugenol, or to a solution of mineral oil and Eugenol only. Mood was measured before and 20 min after exposure onset. Among the nine mood variables, there was a significant (positive) change only in participants' feeling of being focused ( $P = 0.01$ ). In experiment 2, 37 women participated in an experiment almost identical to experiment 1. Differences were that experiment 2 utilized a within-groups design, the participants were tested on days 12–14 in their menstrual cycle and propylene

glycol was used as the solvent. Again, a significant and positive change of the participants' feeling of being focused was observed ( $P = 0.004$ ). In both experiments, sensory detection of androstadienone was rigorously controlled for. It could therefore be ruled out that the observed effects were mediated by conscious experience. The test–retest reliability between the two experiments and across the nine mood variables was assessed with correlational analysis ( $r = 0.77$ ,  $P = 0.01$ ). Altogether, the results suggest that mood effects of androstadienone in women are weak but persistent.

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### Early discrimination of odour hedonics in preterm newborns

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Are preterm newborns able to process the hedonic value of odorants in the same way as full-term newborns? Infants born at 28–30 gestational weeks ( $n = 25$ ) were tested in their incubator during episodes of calm ( $n = 9$ ) or active sleep ( $n = 16$ ). At  $10 \pm 4$  days after birth, they were exposed to low intensity, isointense, pleasant and unpleasant odorants (vanilla, V; butyric acid, B), and a scentless control (water). Each odorant was presented in counterbalanced order under the infant's nose on a cotton swab for 10 s. Changes in facial responses and respiratory rhythm were measured. No reactivity to either stimulus was elicited during calm sleep. However, during active sleep periods, the respiratory rhythm was altered by both odorants, indicating reliable odour detection. The respiratory rate differentiated V and B, which elicited increasing and decreasing variations, respectively. At the facial level, V induced more mouthing actions (i.e. licking, sucking, chewing) than B, while B released more frequent negative facial actions (nose wrinkling, upper lip raising) than did V. These results demonstrate that the olfactory system can detect and discriminate distinct olfactory qualities at least 2.5 months before term. They also reveal that smell does not appear to be turned off during sleep in the immature newborn. It might thus provide valuable environmental information to the brain of preterm infants, at least during phases of active sleep.

### Fat taste and metabolism in humans

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The prevailing view holds that fat is detected by textural cues. However, dating back to Aristotle, there have been claims of a taste component, and recent electrophysiological studies, animal feeding trials and human psychophysical and physiological assessments provide increasing support for this view. The latter studies reveal that oral exposure to dietary fat elevates the postprandial triacylglycerol (TAG) concentration in humans by ~40% and prolongs its elevation for up to 2 h. Fat-free foods and fat replacers do not augment the TAG response. The failure of foods with similar and dissimilar textures to promote the TAG rise indicates

the oral signal is not based on texture. Olfactory stimulation alone with a high-fat food is ineffective whereas oral stimulation with the food while the nose is open or closed leads to a comparable rise. This is consistent with a taste cue. There are conflicting data concerning the efficacy of fatty acids varying in saturation as signals. Some work indicates long-chain, *cis*-polyunsaturated fatty acids are the most effective stimuli whereas other evidence reveals monounsaturated fatty acids are also detectable by gustatory cues. The mechanism whereby dietary fat augments the postprandial TAG concentration presumably entails a neurally mediated influence on lipid absorption, endogenous synthesis and/or reduced clearance. Recent evidence that the TAG rise only occurs when there is lipid in the gut and that oral exposure enhances the release of stored lipid from the previous meal implicates pre-absorptive factors. Other potential sites of action have yet to be examined. Further studies are required to confirm the hypothesis that fat is a basic taste and to determine its health implications.

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### **PROP taster status: dietary modifier, marker or misleader?**

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Bitterness is generally considered a negative food attribute that prompts avoidance or masking through dietary modification. Thus, sensitivity to bitterness may be an important contributor to individual variability in food choice. Recently, particular attention has focused on the associations between sensitivity to the bitterness of 6-*n*-propylthiouracil (PROP), food acceptance and dietary intake. Two hypotheses have emerged, one that posits there is a causal association between bitterness perception and food choice, and the other holds that bitter sensitivity is only a marker for other influences on ingestive behavior. The premise of the former hypothesis is that individuals who are inherently sensitive to the bitterness of PROP will also perceive higher levels of bitterness in foods containing bitter compounds and find them less acceptable. Although intuitively enticing, this view is based on a number of questionable assumptions, including: (i) sensitivity to PROP is related to sensitivity to bitter compounds actually in foods; (ii) taste sensitivity and intensity are reliably correlated with food acceptance; (iii) bitter notes are detectable and undesirable in foods as prepared and consumed; (iv) taste is a strong independent determinant of intake; and (v) bitter foods are disliked or avoided. Moreover, there are data conflicting with this hypothesis; for example, taster status is stable over most of the lifecycle, but preferences change, and there are mixed data on the reported association between PROP taster status and body weight or body mass index. Alternatively, PROP taster status may just be a marker of influences on eating behavior. PROP taster status is variably and non-specifically correlated with selected personality traits and eating attitudes. It is also related to sensory ratings for non-food items. Taken together, the literature provides limited support for a causal relationship between PROP taster status and food choice. There is evidence, albeit weak, consistent with a view that PROP taste sensitivity is a marker for food acceptance. At present, knowledge of PROP taster status offers limited insight for dietary assessment or therapeutic modification.

### **Stimulus response functions of avian olfactory bulb neurons**

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Recent findings in this laboratory indicate that the physiological characteristics of avian olfactory bulb (OB) neurons closely resemble those of other vertebrates, exhibiting variable spontaneous temporal firing patterns with mean firing rates between those reported for mammals and reptiles. Here we investigated avian olfactory processing by examining the responses of single avian OB neurons to two biologically important gases over appropriate concentration ranges. Recordings of extracellular spike activity were made from 80 single units in the left OB of 11 anaesthetized adult hens (*Gallus domesticus*) using glass-insulated tungsten microelectrodes. Spontaneous firing rates ranged from 0.07 to 47.28 spikes/s. Single units were tested for their response to an ascending concentration series of either ammonia (2.5–100 p.p.m.) or hydrogen sulphide (1–50 p.p.m.), delivered directly to the olfactory epithelium via a purpose-built gas delivery system. Gas stimulation resulted in modification of spontaneous activity causing inhibition (47% of units) or excitation (53%) of firing. Units were responsive to small (<5 p.p.m.) step changes in stimulus concentration, allowing stimulus–response curves for both inhibited and excited units to be constructed. These were variable and not monotonic, with regression analysis revealing units best-fitting linear, log and power response functions. This is the first study to present stimulus response curves for single avian OB neurons and is novel in its use of biologically relevant stimuli over ranges likely to be experienced by the animal.

### **Response characteristics of avian nasal trigeminal nociceptors**

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Although the chemoreceptive properties of the nasal trigeminal system are well known, reported trigeminal nasal thresholds to chemical stimulation and stimulus response curves have been based on integrated multiunit or whole-nerve recordings. In particular, the physiological characteristics of single nasal nociceptors responding to both mechanical and chemical stimulation have not been well described in any species. In this study, the responses of single nasal trigeminal units recorded from microdissected twigs of the ethmoidal nerve of anaesthetized hens (*Gallus domesticus*) were investigated. Using a mechanical search stimulus, slowly and rapidly adapting nasal mechanoreceptors were identified, which exhibited a range of von Frey thresholds. Some slowly adapting units also exhibited chemical sensitivity when exposed to ammonia gas, acetic acid vapour or carbon dioxide. The spike durations observed indicated that most of the polymodal afferents were probably unmyelinated C fibres, with some fibres in the A-delta range. Stimulus–response profiles were constructed and subjected to analysis by linear regression. No single function type described all the curves, with different units best-fitting linear, log and power relationships. These results demonstrate that polymodal nociceptors are present in avian nasal mucosa and represent the first

attempt to quantify the responses of single polymodal nasal nociceptors to a range of concentrations of noxious airborne chemicals.

### Segregating pseudogenes: the genetic basis of human olfactory variability

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Specific anosmia and hyperosmia, the widespread inter-individual variations in human olfactory thresholds to particular odorants, have often been rationalized in terms of genetic polymorphisms in individual olfactory receptor (OR) proteins. Experimental evidence for this hypothesis requires a demonstration that the genome of each individual contains a different assortment of intact and disrupted ORs. It is known that the human OR repertoire has at least 60% pseudogenes. If some of these inactivated genes still segregate between intact and pseudogenized forms, they might underlie the variable olfactory phenotypes. We launched a whole genome search for such segregating pseudogenes (SPGs) by two strategies: (i) studying 32 of the ~70 reported OR pseudogenes with a single sequence disruption. These are likely to be evolutionarily recent, and hence prone to display polymorphisms; and (ii) genotyping 18 potential SPGs from the Celera Single Nucleotide Polymorphism (SNP) database. We used high-throughput mass-spectrometry SNP scoring (Sequenom) to genotype the mutated positions in 190 individuals from heterogeneous origins. A total of 24 OR SPGs were revealed. For 10 SPGs, <10% of the individuals have a homozygous disruption, consistent with the typical prevalence of specific anosmia. In contrast, for one SPG, 87% of the population are similarly affected, potentially underlying the less studied specific hyperosmia. Importantly, nearly every individual has a unique repertoire of intact ORs, constituting a genetic basis of human olfactory diversity and providing a basis for future genotype-phenotype correlations.

### Sensitivity for capsaicin inhalation

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Inhalation with capsaicin has been widely used as a cough test for evaluation of anti-tussive drugs. Capsaicin stimulates the C-fibres of the sensory nerve system, and such nerves are found in the respiratory tract, especially in the larynx, as a defence system to prevent foreign material from reaching the lower airways. So, when these nerves are stimulated, a strong cough reflex is initiated. The sensitivity for capsaicin inhalation has been found to differ between various patient groups. Patients with odour sensitivity and multiple chemical sensitivity are very sensitive in comparison to healthy subjects and to patients with asthma. Also, allergy and smoking habit seem to affect the sensitivity for capsaicin. The capsaicin inhalation test has been found to be useful for objectively verifying sensitivity to various chemicals.

### Applications and limitations of electronic noses

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In this talk we assess the concept of electronic noses by looking at a typical application (quality control in food industry). Electronic noses are put in context with competing analysis methods, and their advantages and limitations are discussed. Research on electronic noses has been going on for almost 15 years. Commercial products became available in the early 1990s. Only in a few cases have electronic noses found their way into practical use. A general acceptance of this tool as an analytical method has not been achieved yet. An electronic nose is a device that uses an array of chemical sensors to measure a gas composition. The individual sensors of the array are not specific to a single analyte, but show sensitivities towards classes of gas molecules. By using sensors with overlapping sensitivities and subsequent pattern analysis, this kind of device can be used to perform analysis of even very complex gas mixtures. The analogy to the biological nose, where an array of receptors is connected to a neural network, has led to the name 'electronic nose'. Both, the receptors/sensors and the subsequent data treatment, however, are very different to the mammalian odour recognition system. Therefore electronic noses cannot be regarded as objective odour measurement devices. Only by careful calibration and validation can electronic noses be used for quality assessments related to odour. Calibration and validation of the electronic nose must be performed for every analysis task the system is to perform. This calibration very often requires extensive analysis with reference methods (i.e. human odour panels, analytical chemistry). Only by comparing the reference data with the output of the electronic nose can a valid statement about the sample under investigation be given. As pattern recognition is a statistical approach, the number of calibration measurements is relatively large. The intention of the electronic nose, to be an easy method that can be used by non-experts, is only valid once the application has been solved. Minor changes of the application (e.g. changes in the production process under investigation, a change of the product under investigation) regularly lead to the necessity of a re-calibration, which often can only be performed by experts acquainted with electronic noses.

### Gustatory evoked cortical activity in humans studied by simultaneous recording of EEGs and MEGs

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Evoked potentials are used widely in the clinical field for the objective evaluation of sensory disturbances in humans. However, gustatory evoked potentials (GEPs) have not been studied extensively without agreement on the waveform among investigators. In this study GEPs and gustatory magnetic fields (GEMfs) in response to 0.3 M NaCl were recorded simultaneously from five subjects to establish GEPs as an objective gustatometer. Each subject received 240 stimulus presentations in total during six

sessions. Three GEP components (P1, N1 and P2) were observed and correlated to the corresponding equivalent current dipoles (ECD1, ECD2 and ECD3, respectively). ECD1 was localized to area G in all subjects, P1 being the indicator of intact gustatory projection to area G, while ECD2 and ECD3 were localized to various cortical structures, including the inferior insula and the superior temporal sulcus, indicating that N1 and P2 reflect higher-order gustatory functions.

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### Detection mechanism of MSG and 5'-GMP in mouse non-dissociated taste receptor cells

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To examine electrical responses induced by monosodium glutamate (MSG) and its enhancement with 0.1 mM guanosine 5'-monophosphate (5'-GMP) in mouse taste receptor cells (TRCs), we applied the perforated patch clamp method and the localized stimulating procedure only to the receptive membrane of non-dissociated TRCs in mouse fungiform papillae. Outward current responses in 20% of TRCs, inward current responses in 30% of TRCs and no response in residual TRCs were induced at a holding potential of -80 mV by apically applied MSG (0.03–0.3 M). Outward current responses were not affected by 0.1 mM 5'-GMP. In contrast, 50% of inward current responses were potentiated by 0.1 mM 5'-GMP. An antagonist of NMDA receptor (NMDAR) suppressed neither inward nor outward current response, but metabotropic glutamate receptor type 4 (mGluR4) antagonists only suppressed outward current responses. The brain-derived mGluR4a was immunohistochemically detected in almost all cells in a fungiform taste bud, but several subtypes of NMDAR were not identified. These results suggest that only inward current responses, which are mediated by different receptors from both mGluR4a and NMDAR, are the main part of umami response.

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### Synapses among mouse taste bud cells

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Our previous studies suggest that taste bud cells have synapses among them and form cell networks. We looked for electric synapses by monitoring dye-couplings, and chemical synapses by monitoring electric or Ca responses to neurotransmitters with taste buds in peeled epithelia. Half of the examined cells showed dye-couplings. The number of coupled cells was at most five, but usually two, including injected ones. This was the first observation

of dye-couplings among mammalian taste bud cells, and showed cell networks in taste buds using electric synapses/gap junctions. On the other hand, 0.1–100  $\mu$ M 5-HT applied to the basolateral membranes of taste bud cells elicited oscillating currents. Since 10  $\mu$ M ketanserin reversibly blocked the oscillating currents, expressed receptors may belong to 5-HT<sub>2</sub> subtypes. [Ca<sup>2+</sup>]<sub>in</sub> investigated with the fluorescence of calcium green-1 showed that 5-HT applied to the basolateral membranes increased [Ca<sup>2+</sup>]<sub>in</sub> in a [Ca<sup>2+</sup>]<sub>out</sub>-dependent manner. The number of cells that increased the fluorescent intensity by >1% was about three per taste bud. Such 5-HT-sensitive cells tended to occur in the peripheral part of taste buds. Neighbouring taste bud cells or innervating taste nerve fibres appear to release 5-HT. These electric and chemical synapses contribute to form cell networks integrating taste information or to regulate the turnover of taste bud cells.

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### Incidental and intentional memory for uncommon odors in young and elderly subjects

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Incidental and intentional memory for uncommon odors were tested in 39 young (age = 25.6  $\pm$  4.3 years, mean  $\pm$  SD; 21 female, 18 male) and 41 elderly (age = 66.7  $\pm$  6.1 years; 29 female, 12 male) subjects who also rated 40 odors on pleasantness, familiarity and intensity. To verify that they had not developed a repeatable name for the odors, they tried to name the odors in a random series in which the same odors appeared twice. In the incidental memory test they received 10 out of the 20 odors which they had rated the previous day interspersed with 10 new odors, and were asked to indicate which of these odors they had smelled before. The same procedure was followed for the intentional learning procedure with 20 new odors, but this time they were instructed to remember them. Different subgroups learned different sets of odors under both conditions. Results show that incidental odor memory is as good in the elderly as in the young, but that the young do much better on the intentional test, whereas the elderly do not improve. Contrary to results with known odors, the elderly show better recognition in incidental learning for less familiar odors than for more familiar ones, and in both learning conditions the elderly remember less pleasant odors better than pleasant ones. In the incidental learning condition, they recognize less pleasant odors better than the young. No gender effects are found. The difference in intentional learning between age groups cannot be explained by better verbal mediation in the young subjects.

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### Targeting olfaction

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An olfactory sensory neuron expresses selectively one member from a repertoire of ~1000 odorant receptor (OR) genes and projects its axon to a specific glomerulus in the olfactory bulb.

Both processes have been recapitulated by minigenes for the *MOR23* and *M71* OR genes, introduced into mice. Minigenes of 9 kb and as short as 2.2 kb are selectively expressed by populations of neurons that do not co-express the endogenous gene but co-project their axons to the same glomeruli. The *MOR23* minigene contains a 395 bp upstream region essential for expression. In this region we recognize sequence motifs conserved in many other OR genes. Transgenic lines expressing the OR in ectopic epithelial zones form new, ectopic glomeruli. These new glomeruli also receive specific input from neurons expressing the cognate endogenous receptor, suggesting a recruitment through homotypic interactions between OSNs expressing the same OR.

## Sensory memory and food perception

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Memory of previous encounters plays a role in food expectations and in food choice. How accessible and accurate is the implicitly acquired and stored product information? To answer this question two experiments are carried out. In the first experiment stimuli varying in texture and flavour were used. In the second experiment the memory for variations in texture alone was tested. In both tests, the subjects (69 and 76 respectively, age 19–59 years) came to the institute without prior eating, where they received a breakfast of several items (including breakfast drinks, biscuits and pâté/breakfast drinks, biscuits and yoghurt respectively). Subsequently during the day they answered questions about their hunger feelings every hour and returned for a taste experiment at the end of the day. Confronted with a series of multiple samples of respectively five and four texture variations of each of the breakfast items mentioned above, they had to indicate which of these variations they had eaten at breakfast. In the second experiment the subjects received the stimuli again to judge them on liking and to compare them with the food they ate in the morning regarding liking and sensory aspects. Signal detection measures showed that incidentally experienced foods can be remembered, but memory for fat content is weak. *t*-tests showed that experiences with food are subjected to changes in memory, while men and women differ in the ways in which they remember certain food products.

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## In search of the exocrine origin(s) of the rabbit mammary pheromone

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Newborn rabbit pups are directed to their mother's nipples by specialized odour cues. Previous investigations indicated that these cues are released from the doe's abdominal surface, but no precise, anatomically based data exist so far on their source. We furthered these studies using a behavioural assay which consisted

of presenting pups with samples of various cutaneous tissues or fluids collected from lactating females. Three experiments were conducted: (i) surface sampling: skin samples collected at increasing distance from the nipples were assessed for their ability to elicit searching and oral grasping responses in pups; (ii) deep sampling: dermal and mammary tissues taken below the nipple were tested in the same way; and (iii) fluid sampling: milk collected at different levels of the mammary pathway were tested. The surface sampling revealed that the efficient odour cues were released from the nipples of lactating does. The other tissues of epidermal, dermal or mammary origins were behaviourally inefficient. The lacteal fluids became behaviourally active only after they had flown through the nipple. These results suggest dual exocrine sources of active factors in the nipple of lactating rabbits: surface cues that are distributed over the nipple epidermis and cues released within the nipple that render milk reactogenic. Histological analyses on nipples from lactating females revealed sebaceous structures opening to the surface. Additional analyses are underway to characterize the exocrine structures eventually lining the lumen of the lactiferous ducts.

## Candidate bitter and sweet receptors in human and mouse

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The gustatory system of mammals can sense a variety of chemicals as having either bitter or sweet tastes. Numerous studies indicate that the detection of sweet and bitter tastants by taste receptor cells in the mouth involves G proteins. Previous genetic studies in mice and humans identified chromosomal loci that govern sensitivity to certain bitter or sweet compounds. Using publicly available human genome sequence data, we asked whether genomic regions corresponding to these loci contain genes encoding novel G protein-coupled receptors (GPCRs). By focusing first on the region of the human genome that is syntenic to a mouse bitter taste locus, *SOA*, we identified a novel family of candidate bitter receptors, the *T2Rs* (TRBs). The size and diversity of this receptor family are consistent with an ability to detect the variety of chemically compounds perceived as bitter. We found *T2R* genes at specific sites on human chromosomes 5, 7, and 12 that are implicated in bitter taste sensitivity. Using mouse *T2R* genes as probes, we further verified the presence of *T2R* genes at the *SOA* locus, and determined that *T2R* genes are indeed expressed by a subset of mouse taste receptor cells, as would be expected for bitter taste receptors. In subsequent experiments, we searched for genes encoding GPCRs in the region of the human genome that is syntenic to the mouse *Sac* locus, which controls sensitivity to certain sweet compounds. Here, we identified a gene encoding a candidate sweet taste receptor, *T1R3*. *T1R3* is related to *T1R1* and *T1R2*, two receptors that had been found in taste cells but had no known functions. Using radiation hybrid mapping panels, we found that the *T1r3* gene is located near or at the *Sac* locus. We also determined that mouse strains that differ in *Sac* phenotype have different *T1r3* alleles. Moreover, we found a perfect correlation between the different *T1r3* alleles and *Sac* phenotypes in a series of

recombinant inbred mouse strains. In contrast to T1R1 and T1R2, which are selectively expressed in the anterior and posterior papillae of the tongue, respectively, we found that T1R3 is expressed in a subset of cells in all three types of tongue papillae. Using double fluorescence *in situ* hybridization, we determined that T1R3 and T1R2 are expressed in the same cells in circumvallate and foliate papillae, raising the possibility that the two receptors function as heterodimers.

### Amygdala activation in rats exposed to urinary pheromones

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Mitral cells of the rat accessory olfactory bulb are activated by urinary pheromones via inputs from the vomeronasal system, and project to the medial amygdala, which then transmits to the hypothalamus, ultimately affecting reproductive physiology. Male rat urine contains high levels of a lipocalin (Alpha-2U), involved in conspecific chemical communication. Aspartate (ASP) and glutamate (GLU) extracellular levels in the amygdala posteromedial cortical nucleus (PMCoA) were detected by microdialysis in freely moving Wistar rats exposed to urinary pheromones. The microdialysis probe, 24 h after implantation in the PMCoA, was perfused with artificial CSF. After 90 min, samples were collected every 20 min for 3 h. Three samples were used to detect basal levels, before exposing rats to pheromones. Exposure of oestrus female rats to male urine or alpha-2U and of male rats to female urine induced an immediate behavioural activation, accompanied by a significant increase in ASP and GLU output. After 60–100 min a second burst of behavioural activation accompanied by a second peak of ASP was observed only in female rats. Exposure to water induced a lower behavioural activation, with no change in amino acids output. In conclusion, the behavioural activation induced by exposure to urinary pheromones is accompanied, in both male and female rats, by activation of the AOB to the PMCoA excitatory pathway; the second delayed burst of activity and aminoacid release, only observed in females, may be related to long-term effects of male pheromones on female reproductive physiology.

### Urinary pheromones modulate mice aggressive behaviour

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In male mouse urine, high levels of the lipocalin MUP (major urinary protein) are found. MUP can bind and release in the environment small hydrophobic molecules, and participate in chemical communication among conspecifics. Male pheromones facilitate aggression towards adult males and females anointed with such substances. A series of experiments were performed to test whether male pheromones induce a lowering of the threshold for aggressive attacks to occur, as aggression-promoter or trigger substances. If male chemical cues are meaningful signals of male presence and not only triggers, the shaping of behavioural output should be deputed to the matching of pheromonal cues with other

sensory informations. Isolated male mice were exposed to newborns, and displayed aggressive attacks, as usually seen under these conditions. If newborns were anointed with male urine, they were attacked later than controls. This did not happen if newborns were anointed with female urine. The delay in attack was also detected if pups were anointed with MUPs purified from adult male urine. This protective effect was not due to a generic avoidance effect induced by male pheromones: when pups were substituted with pieces of bread (a stimulus that could be eaten but was unknown to mice), no difference was detected in the latency to the first sniff and to the first bite between controls and bread anointed with MUPs. We conclude that the informations carried by MUPs differentially affect mice aggressive behaviours, since they induce aggression towards adult mice, delay aggression against pups and have no effect on food items.

### Olfactory function in idiopathic Parkinson's syndrome: long-term follow-up of de-novo IPS patients

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Olfactory loss is a prominent symptom in idiopathic Parkinson syndrome (IPS). Five de-novo patients received three olfactory tests at intervals of ~6 months (range = 4–10 months) spread over a period of ~1 year (mean = 13.5 months). Four of the subjects were male, one was female; age ranged between 39 and 65 years (mean age = 50 years). The Hoehn & Yahr stages at the time of diagnosis were as follows: I = 1; II = 4. At the time of diagnosis only one patient reported the presence of olfactory deficits. Psychophysical testing of olfactory function was performed bilaterally using the 'Sniffin' Sticks' test kit; it involved tests for odor threshold, discrimination and identification. One of the de-novo patients was normosmic, three were hyposmic and one was anosmic. Follow-up investigations indicated decreased olfactory function in three patients while it undulated in one. The normosmic patient retained olfactory abilities. This patient failed to respond to pharmacological treatment. In conclusion, it can be stated that patients with IPS exhibit a specific decrease of olfactory function which appears to take place during early stages of the disease. Furthermore, tests of olfactory function may also be of interest in investigations related to treatment of IPS.

### A new clinical procedure for the quantitative assessment of gustatory function

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Assessment of gustatory sensitivity in a clinical setting is the prerequisite for correct diagnosis and adequate treatment of taste dysfunction. Despite this, no taste test has been established for the routine clinical testing. The aim of the present study was to

create a protocol which is easy to administer. The presently used technique is based on strips made from filter paper which were impregnated with different taste solutions (four concentrations each for sweet, sour, salty and bitter). These strips are placed on the tongue and subjects are asked to identify the taste quality. After establishing the concentration range of the taste solutions, the test was tried in 69 subjects. Each subject received 18 taste strips (four concentrations of each taste quality plus two blanks) in a pseudo-randomized sequence. Results from this new procedure correlated significantly with the results of the well established extensive three-drop-technique ( $r_{69} = 0.67$ ). Repeated measures indicated good reproducibility of the results for the taste strips ( $r_{69} = 0.68$ ). These data suggest the usefulness of this new technique in routine clinical practice. Major advantages are long shelf-life, convenience of administration, short time needed for testing (~8 min) and the possibility to test each side of the tongue separately.

### Affinity for odorants and acidic conformational changes of a rat odorant-binding protein

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Odorant-binding proteins (OBPs) are lipocalins secreted in the nasal mucus of vertebrates, which convey odorants to their neuronal receptors. We compared the binding properties of a recombinant rat variant (OBP-1F) using a set of six different odorants and correlated affinity constants with various conformational and oligomerization states. Binding properties were examined through both fluorescent probe (1-aminoanthracene) competition and isothermal titration calorimetry, demonstrating that OBP-1F is not precisely tuned towards chemical classes of odorants. Nevertheless, affinity constants, in the micromolar range, varied by more than one order of magnitude from one odorant to another, in correlation to the odorant size. The binding stoichiometry was found to be one odorant per dimer. We also investigated the effects of pH variations on the secondary and quaternary structures of OBP-1F. It remained dimeric at pH 4.0, as observed by light scattering, but exhibited local conformational changes, as shown by circular dichroism while its affinity was significantly reduced. At pH 3.0, binding was abolished, in accordance with a severe alteration of the secondary structure and some aggregation. pH neutralization after acidification led to restore both structure and binding properties. These results are consistent with one binding site located in one of the two calices of the OBP-1F dimer and a subtle conformational change correlated with binding of one odorant molecule, which hampers uptake of a second odorant by the other hydrophobic pocket.

### Organization of glomeruli in the main olfactory bulb of *Xenopus laevis* tadpoles

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The synaptic input layer of the olfactory bulb (OB) is typically organized into glomeruli (Gl), which serve as functional units in olfactory coding. However, it is yet unclear what exactly is meant,

functionally and structurally, by the term glomerulus. Tadpoles of the clawed frog *Xenopus laevis* provide a favourable model system to study functional and morphological aspects of olfaction at both the peripheral and central levels. The general organization of their Gl is described with special focus on the cytoarchitecture and segregation of individual Gl, the cellular basis of heterogeneity and the structural basis of glomerular borders. Responses of OB cells upon application of amino acids showed that the OB is functional. Dye injections into receptor neurons, mitral and tufted cells showed that their fibers form spherical glomerular tufts (GIT), which colocalize with distinct clusters of anti-synaptophysin immunolabeling. Periglomerular cells have an extensive field of arborizations ranging over >200  $\mu\text{m}$ . Their dendrites do not show any signs of GIT, and do not contribute to form borders around Gl. GFAP and MAG labeled no structures in the Gl layer. Vimentin-positive processes of radial glia formed a dense radial meshwork throughout all layers of the OB including the Gl layer. However, they do not form any periglomerular baskets or borders. No signs of glial processes or cellular material forming Gl borders were found at the ultrastructural level. Hence, though structural organization of Gl differs among various species, ensheathment by periglomerular or glial cells is not necessary for the function of Gl.

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### Relationship of PROP status to bitterness sensitivity

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The relationship between PROP (6-*n*-propylthiouracil) taster status and sensitivity to six unrelated bitter compounds and among the seven stimuli was investigated. Thresholds of PROP, caffeine, denatonium benzoate, limonin, naringin, quinine and sucrose octaacetate (SOA) were determined for subjects (18 males and 22 females) who were classified by their PROP taster status. A significant relationship was found between PROP status and sensitivity for three of the compounds, but only for women. Female PROP non-tasters had higher thresholds for caffeine, naringin and SOA than female PROP 'supertasters'. No relationship between PROP taster status and sensitivity to denatonium, limonin and quinine was found for either gender.

### Epidemiological aspects of olfactory disorders

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The absence of population-based epidemiological data on olfactory disorders, of importance for planning health-care efforts, motivated the present population-based investigation of this prevalence and its relation to age, gender, diabetes, nasal polyps and smoking in an adult population. A random sample of 1900 inhabitants, stratified for age and gender, was drawn from the municipal population register in Skövde, Sweden. Subjects were called to clinical visits for examination with the Scandinavian Odor Identification Test and nasal endoscopy and for questions about olfaction, diabetes, and smoking habits. In total, 1387 volunteers (73% of the sample) were investigated, well representing the

general Swedish population in age distribution. Overall, 5.8% manifested anosmia and 13.3% hyposmia (15.0% experienced poor olfactory function), with a considerable age-related increase in prevalence (anosmia/hyposmia was 0.5/8.7% in age group 20–29 years and 48.2/35.2% in age group 80+ years). A multiple logistic regression analysis showed that prevalence of loss in olfactory function was related to age, nasal polyps, diabetes and gender, but not to smoking. Self-reported prevalence of ever having parosmia was 4.0% and related to age, nasal polyps and diabetes, but not to gender or smoking. These findings suggest that olfactory disorders are more prevalent than non-population-based studies previously have indicated, and emphasize the role of nasal polyps and diabetes in olfactory disorders.

### Leptin suppresses mouse behavioral responses to sweet stimuli through its peripheral functional receptors in taste cells

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Leptin is a hormone which regulates food intake, energy expenditure and body weight mainly via activation of the hypothalamic leptin receptors. Recent studies indicate that the taste organ is a new peripheral target for leptin in mice which selectively inhibits responses to sweet substances. In the present study, we further investigated the leptin effects on sweet taste by examining expression of leptin receptors in taste cells and behavioral responses to sweet stimuli by using leptin-deficient *ob/ob* and leptin-receptor-deficient *db/db* mice and their littermates. RT-PCR and *in situ* hybridization analyses indicated that the functional leptin receptor, *Ob-Rb*, is expressed in taste cells in *ob/ob* mice and normal lean littermates of *db/db* mice. The *db/db* mice have a longer RT-PCR product containing a typical *db*-insertion with a stop codon which lead to impaired shorter intracellular domain of *Ob-Rb*. Consistently, behavioral responses to sucrose and saccharin were significantly decreased at particular concentrations for each sweet substance after *i.p.* leptin injection in *ob/ob* and lean littermates, whereas no such effect of leptin was observed in *db/db* mice. From these consistent results in the behavioral and molecular studies, we concluded that leptin suppresses behavioral responses to sweet stimuli through its functional receptors in taste cells in mice.

### A procedure to measure olfactory repetition priming

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Implicit memory is often assessed in the form of repetition priming. In olfactory cognition, no standard procedure for repetition priming has been developed and validated. This presentation depicts a series of experiments investigating the primability of edibility judgements. The results indicate that (i) edibility judge-

ments are facilitated when repeated. The facilitation concerns latency rather than correctness of the judgements. (ii) Odor memory performance is typically influenced by the identifiability of the to-be-remembered odor. In the case of edibility priming, no reliable correlation between odor identifiability and primability was found across odors. (iii) Unilateral presentation of odors can serve to tap hemispheric differences. (iv) As for visual priming, edibility priming is long lasting. (v) Edibility priming is dissociated from explicit memory in terms of forgetting curves. Altogether, the results suggest that the measurement of edibility priming is a robust and valid procedure for the investigation of implicit memory in olfactory cognition.

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### Do men and women respond differently to repeated olfactory or trigeminal stimuli?

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Sex differences in olfactory sensitivity have been reported since the late 1800s. Women often outperform men on tests of odor identification, detection and discrimination. Whether women adapt differently to odorous stimuli than men is not known. Seventeen healthy volunteers participated (nine females, eight males, mean age 22 years). ERPs were recorded in response to olfactory (phenyl ethyl alcohol, PEA) and trigeminal (CO<sub>2</sub>) stimuli presented to the left or right side. Stimuli were applied at four different intervals (5, 10, 20 and 60 s). Amplitudes and latencies of ERP peaks P1, N1, P2 and P3 were measured. Using visual analogue scales, subjects also rated stimulus intensity. When compared with PEA, the slightly more intense CO<sub>2</sub> produced larger amplitudes and shorter latencies. Responses to the trigeminal and olfactory stimuli changed similarly in relation to repetitive stimulation. Both, ratings and ERP amplitudes P3 and N1P3 decreased with a shortening of the interval between stimuli. Women had larger ERP amplitudes P3, N1P2 and N1P3; however, they tended to rate intensities lower than men. No gender-related difference in relation to repeated stimulation was observed, as indicated by the missing interaction between factors 'sex' and 'interval'. These data indicate on a psychophysical and an electrophysiological level that there is no difference between young, healthy men and women in terms of short-term adaptation to suprathreshold chemosensory stimulation.

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### Orthonasal versus retronasal olfaction—a comparison of two olfactory tests

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Most tests of olfactory function are based on the presentation of odors in front of the subjects' nostrils. Recently another smell test

based on odorized candies has been introduced by B. Renner of the University of Erlangen, measuring olfactory identification through the retronasal route. This test is performed much shorter than the former and does not need pre-diagnostic decongestion as it bypasses the nasal cavity with the frequently swollen mucous membrane. In this study we compared ortho- and retronasal olfactory tests concerning practicability and outcome. For orthonasal olfactory testing 'Sniffin' Sticks' were used. Thirty-five patients with olfactory dysfunction participated in the study. It turned out that the candy test could identify anosmia well. In our sample 42.9% of the patients ( $n = 15$ ) were suffering from a severe threshold dysfunction. These patients performed well in the candy test, but their pathology could only be assessed by the 'Sniffin' Sticks' test. Therefore the candy test can only be used to diagnose complete anosmia and identification disorders whereas normosmia and all other olfactory disorders need a more detailed examination as provided by the 'Sniffin' Sticks'.

### Electrophysiological characterization of P2X-receptors in cultured rat trigeminal neurons

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Trigeminal nerve fibers innervating the facial mucous membranes (eye, nose, mouth) can perceive and discriminate between various chemical stimuli. Until now, little is known about the receptors and signal transduction pathways of the trigeminal system. m-RNA transcripts and proteins of purin receptors (P2X) have been identified in these neurons. *In situ*, trigeminal neurons express predominantly the subunits P2X<sub>2</sub> and P2X<sub>3</sub>, which can form homomeric or heteromeric receptors. P2X receptors are ligand-activated ion-channels, activated by ATP, which is an important transmitter/co-transmitter/modulator and can play a functional role in the trigeminal system. Using whole-cell voltage-clamp technique, we investigated the response of cultured trigeminal neurons dissociated from rat ganglion gasserii to application of various concentrations of ATP. The dose-response relationship showed an EC<sub>50</sub> of 30  $\mu$ M ATP with a Hill-coefficient of 0.8. Three populations of neurons differing in the kinetics of the ATP-induced currents could be identified: phasic, tonic and phasic-tonic. Specific agonists and antagonists allowed to identify the subunit composition. The tonic currents were carried by homomeric P2X<sub>2</sub> receptors, the phasic currents by homomeric P2X<sub>3</sub> receptors and the phasic-tonic currents by homomeric P2X<sub>2</sub> and P2X<sub>3</sub> receptor populations. We never got any indication for heteromeric P2X<sub>2/3</sub> receptors. In further studies we will investigate a possible role of ATP receptors in perception of chemical stimuli.

### Effects of stimulus significance and stimulus probability on the olfactory event-related potential: visualization by cortical maps

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For the auditory and visual modality it has been shown that

stimulus significance and stimulus probability are independent generators of the event-related P3. The aim of the present study was to investigate whether the P3-associated perceptual processes are generally similar within the olfactory modalities. Twelve right-handed subjects (mean age = 28.4 years) voluntarily participated in the study. Linalool and phenyl ethyl alcohol (PEA) were presented birhinally via an olfactometer within a constantly flowing airstream (100 ml/s). Three experimental conditions with 60 trials each were carried out (stimulus duration = 300 ms, inter-stimulus interval = 20 s). In the first condition both odours were presented with an equal probability and the subjects had to indicate their occurrence through a motor reaction. In the second condition, PEA was presented with a probability of 25% and only PEA had to be detected. In the last condition PEA was again presented as a target stimulus but was presented on 50% of the trials. The order of conditions was counterbalanced between subjects. A multi-channel EEG was recorded from 64 electrode positions. Therefore, voltage and current density maps could be calculated. Statistical analyses reveal strong effects of stimulus probability on the positive potentials between 400 and 900 ms after stimulus onset. The voltage maps indicate that at least three positive potentials can be differentiated. The current density maps show a permanent activation above the right frontal cortex.

### The role of major urinary proteins and low molecular weight components of male mouse urine in eliciting the pregnancy block effect in female mice

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Mouse urine contains a complex mixture of chemosignals, including a variety of small volatile molecules that are bound to major urinary proteins (MUPs). The aim of this study was to investigate whether these components convey information about individuality in the context of the pregnancy block effect. This information allows recently mated female mice to distinguish the pheromones of the mating male from those of a strange male, thus preventing pregnancy failure. Urine from males of different inbred strains was separated into high molecular weight (HMW) fractions that contained MUPs and low molecular weight (LMW) fractions that contained the low molecular weight constituents. Female mice were mated with males of the CBA strain and subsequently exposed to urine fractions of either the CBA (familiar) male or a C57/B6 (strange) male. Exposure to the HMW fraction from the strange male failed to cause pregnancy block (17% block). However, this HMW fraction was effective in blocking pregnancy after it had been reloaded with strange LMW ligands by dialysis (100% block). These findings imply that MUPs alone do not elicit the pregnancy block effect. Furthermore, the familiar male HMW fraction reloaded with strange LMW ligands blocked pregnancy (83% block), whereas the strange male HMW fraction reloaded with familiar LMW ligands did not (42% block). This suggests that the LMW constituents are important for conveying information about individuality in the context of pregnancy block.

## Functional anatomy of the task of odor familiarity judgement: influence of handedness

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We have previously shown that aspects of odor processing were lateralized depending on the type of olfactory task (Royet *et al.*, 1999, *J. Cogn. Neurosci.*, 11: 94–109; J. Neurosci., 2000, 20: 7752–7759; Royet *et al.*, 2001, *NeuroImage*, 13: 506–519). In right-handers (RH), the task of odor familiarity judgement activated the right hemisphere with mainly the orbitofrontal cortex (OFC), whereas that of hedonic judgement activated the left side. However, the neural network involved in emotional response was differently lateralized depending on handedness. The left ventral insula was activated in RH and the right one was activated in left-handers (LH). Our purpose was to determine whether functional lateralization of the familiarity judgement also depended on handedness. Whole-brain fMRI (BOLD method at 1.5 T) was performed to detect local hemodynamic changes related to the odor familiarity judgement in 14 LH and 14 RH men. A block paradigm was applied with familiarity, detection and rest conditions. Activation mappings in the familiarity judgement were compared between both groups of subjects applying random effects analyses (SPM99) with the familiarity minus detection contrast. A neural network including both the amygdala–piriform region and the right hippocampus was activated in RH, while only the right OFC was activated in LH. These findings demonstrated that functional lateralization of the familiarity judgement is not dependent on handedness. The results were further discussed in terms of olfactory information processing.

## Psychophysical properties of mechanical oral irritation

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Oral irritation during food consumption is commonly associated with chemesthetic stimuli such as capsaicin. However, irritation can also be produced via mechanical stimulation. A number of commonly consumed fruits and vegetables produce oral irritation, which impacts on the acceptability of such products. In kiwi fruit, for example, oral irritation is produced by calcium oxalate in the form of needle-like raphide crystals. The psychophysics of the mechanical oral irritation caused by raphides was studied in two experiments using a model kiwi fruit system. In the first experiment, the location of irritation sensations and perceived intensity of the irritation caused by suspensions of raphides were assessed. Stinging, burning and numbing increased significantly with increasing concentrations of raphides. Stinging occurred principally on the tongue, as well as in the throat, and numbing on the tongue. Burning was strongest in the throat and to a lesser extent on the lips. The number of oral areas irritated increased with raphide concentration. Decreases in irritation were observed over a 60 min period. In a second experiment, sugars (fructose,

sucrose, glucose and inositol), acids (citric, malic and quinic) and an enzyme (actinidin) were added to the model to examine interactions between these chemical stimuli and the mechanical action of the raphides. Acids enhanced irritation in addition to increasing sourness and suppressing sweetness, but this was only evident at low raphide levels. Like some chemesthetic stimuli (capsaicin), the sweetness of the system was suppressed by irritation, while sourness was unaffected. The addition of actinidin had no significant effects on irritation or tastes.

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## PROP sensitivity and oral trigeminal responses

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Sensitivity to the bitterness of 6-*n*-propylthiouracil (PROP) is associated with sensitivity to other taste qualities—sweet, salty and sour—as well as bitterness produced by other compounds such as caffeine. Such variations are presumably mediated by differences in the density of fungiform papillae, which is highly correlated with sensitivity to PROP. The intragemmal and perigemmal spaces of taste cells are also occupied by free nerve endings of the trigeminal nerve. The trigeminal nerve contains nociceptors and fibres that respond warming and cooling, and are responsible for sensations of oral pain, burning, irritation and cooling associated with compounds such as capsaicin (from chillies), ethanol and menthol. In addition, trigeminal mechanoreceptors also occupy taste epithelium. One implication of the associations of trigeminal fibres with taste cells is that increased trigeminal innervation should be associated with higher fungiform papillae density, and therefore may also be indexed by responses to PROP. Recent research has supported such an association. PROP tasters perceive irritation associated with capsaicin, ethanol and cinnamaldehyde as more intense than do non-tasters. Tactile properties such as those responsible for the perception of fat in foods also vary with PROP taster status. PROP supertasters are more sensitive to variations in fat content in milk, even when viscosity is held constant. The viscosity of guar gum and canola oil have also been found to be correlated with both PROP bitterness and number of fungiform papillae. Such findings suggest that fungiform papillae may act as tactile sensors as much as they function to facilitate taste perception—a notion supported by findings that the gap threshold, a derivation of the two-point threshold, was negatively correlated with number of fungiform papillae.

## Pheromone binding proteins contribute to the excitation of olfactory receptor cells in moths

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Pheromone binding proteins (PBPs) occur in millimolar concentrations in the extracellular sensillum lymph surrounding the sensitive processes of olfactory receptor cells in moths. They may

solubilize and carry the odorants to the receptor cells, protect the odorants from enzymatic degradation, mediate the interaction between odorant and receptor molecule and, finally, deactivate the odorant. Biochemical studies (Bette *et al.*, 2002, *Insect Biochem. Mol. Biol.*, 32: 241–246) have shown, that the different PBPs coexpressed in the pheromone sensitive sensilla of the silkworm *Antheraea polyphemus* interact differently with various pheromone components. The X-ray structure of the PBP of *Bombyx mori* (Sandler *et al.*, 2000, *Chem. Biol.*, 7: 143–151) suggests that the pheromone binds to an inner cavity of the 15 kDa protein. According to NMR studies (Horst *et al.*, 2001, *Proc. Natl Acad. Sci. USA*, 98: 14374–14379), it may be released due to negative charges at the receptor cell membrane. Recombinant PBPs of the silkworms *A. polyphemus* and *B. mori* were expressed in *E. coli* and provided by J. Krieger (University of Stuttgart-Hohenheim). Receptor cells were stimulated *in situ* by superfusing with the pheromone–PBP complex in the various combinations of pheromones and PBPs. The responses of receptor cells depended on both the pheromone and the PBP. Pheromones artificially bound to particular PBPs elicited nerve impulses in receptor cell types which they do not activate under natural conditions. This suggests that the PBPs contribute to the activation of receptor molecules.

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### Spontaneous and odor evoked neural population activity in the antennal lobe of the honeybee

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Extracellular multi-electrode recordings performed *in vivo* on the honeybee antennal lobe will be presented. Spontaneous as well as odor evoked neural population activities will be characterized. The capability of the technique to give access to the discharge dynamics of many neurons at once will be illustrated and discussed. In particular it will be shown that correlations between spike trains generated by different neurons can easily be investigated. Finally comparison will be made (on recordings ease and quality, neural populations behavior, etc.) between honeybee and another insect species: the locust (*Schistocerca americana*).

### Expression of Na<sup>+</sup>/Ca<sup>2+</sup>/(K<sup>+</sup>) exchangers in olfactory receptor neurons (ORNs)

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Cytosolic Ca<sup>2+</sup> is a critical participant in intracellular signaling cascades in virtually all eukaryotic cells. In olfactory receptor neurons (ORNs), entry of Ca<sup>2+</sup> in response to odor reception is a critical early step in the transduction of chemical to electrical signals and plays an important role in controlling excitation and adaptation of ORNs. Ca<sup>2+</sup> amplifies its effect and increases the transduction current by activating Ca<sup>2+</sup>-activated chloride channels. In conjunction with calmodulin, Ca<sup>2+</sup> mediates desensi-

tization of the cAMP-gated channels. Furthermore, Ca<sup>2+</sup> also affects the activities of the AMP-phosphodiesterase and adenylate cyclase, as well as other events in ORNs. Thus, regulation of Ca<sup>2+</sup> flux is a critical cellular activity. Tremendous progress has been made in identifying and characterizing the molecules associated with these early events in chemoreception and Ca<sup>2+</sup> entry in ORNs of many vertebrate species. In contrast, only little attention had been paid to the downstream mechanisms that terminate the response and reduce intracellular Ca<sup>2+</sup> in order to recover the sensitivity of ORNs. This is of particular interest since the cilia of ORNs, the site where the signal transduction takes place, contain very little cytoplasm and no discernible endoplasmic reticulum that could serve as a sink for entering Ca<sup>2+</sup>. Recent reports (Reisert and Matthews, 2001) redirect attention to these late events and demonstrate the role of Na<sup>+</sup>/Ca<sup>2+</sup> exchange in terminating the transduction current. However, there is virtually no information about which exchangers are expressed in the olfactory system. Based on studies performed in heart, brain and retina, the existence of three Na<sup>+</sup>/Ca<sup>2+</sup> exchanger genes (NCX1, 2, 3) and three Na<sup>+</sup>/Ca<sup>2+</sup>/K<sup>+</sup> exchanger genes (NCKX1, 2, 3) has been shown. Transcription of some of these genes is subject to differential splicing, generating multiple mRNAs. Therefore, we analyzed the mRNA expression of these exchanger genes by RT-PCR and *in situ* hybridization in mouse olfactory tissue at different developmental stages. Our data demonstrate the presence of all six exchanger mRNAs in olfactory and vomeronasal neurons with an early embryonic onset of expression.

### Intensity based cognitive clue enables lateralization of a pure odor

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Subjects cannot indicate the side of a pure odor when an odorless blank is administered simultaneously into the opposite nostril. The question analyzed was whether this becomes possible when a cognitive clue, based upon a slight intensity difference between stimuli addressed into the right and left nostril, is being introduced. Phenyl ethyl alcohol (PEA) was used at strong and weak concentration. First, strong PEA versus blank and weak PEA versus blank were presented repetitively, the sequence of both types of stimuli and the side of odor being randomized. Lateralization was impossible. Secondly, the same was repeated with a single difference: without informing the subject, the strong stimulus was always administered to the same side (e.g. right), with the weak one to the opposite side. The subjects were asked to discover the principle enabling correct lateralization. Few subjects were able to solve the task spontaneously. However, all of them could be taught to do so. Measurement of the threshold intensity difference between both nostrils allowing lateralization indicated an increase of the ability of intensity discrimination during this phase of the experiment. Thirdly, when the subjects lateralizing according to the intensity difference were deprived of this clue, lateralization became impossible. A predictable, intensity-based cognitive clue made learning of odor lateralization possible.

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## Assessment of different methods for PROP status classification

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We are interested in investigating populations that vary in their taste and smell sensitivities. Understanding population differences helps us in the creation of improved flavors and fragrances. To gain reliable insight into such differences we need reliable methods of population classification. One of our interests at IFF is to better understand the relationship between taste responsiveness to 6-*n*-propylthiouracil (PROP) and perception of food. However, to date, there seems to be no reliable method of classifying individuals based on their PROP status. We have therefore compared three different methods for classifying individuals into supertasters (ST), tasters (T) and nontasters of PROP, and looked at the within- and between-method reliability. In the first study subjects ( $n = 194$ ) rated the perceived intensity of three different concentrations of PROP and three of sodium chloride (NaCl). In the second study a subset of the same subjects ( $n = 121$ ) rated one concentration of PROP and one of NaCl. In the third study we investigated a method in which subjects rated the intensity of one concentration of PROP solution against a reference sample of NaCl at a specified intensity. The studies were conducted in two replicates and the intensity ratings were made using the Labeled Magnitude Scale (LMS). The subjects were then classified into three groups for each method separately. The within-method reliability was ~70–80% and the between-method reliability was ~60–80%. The between-method reliability increased to >85% when subjects were classified into tasters and nontasters only. Based on these classifications, we selected the ‘most’ reliable supertasters and nontasters and conducted three pilot studies to investigate differences between the two groups based on their perception of key attributes of different types of products. Overall, the results revealed some differences between supertasters and nontasters. However, those differences seemed less impactful than those reported in the literature for simpler systems, such as taste solutions.

## Human PROP insensitivity is not accounted for by single nucleotide polymorphisms in the putative bitter receptors TAS2R3, TAS2R4 and TAS2R5.

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The best-described individual difference for the ability to detect and experience a particular taste is for the compounds phenylthiocarbamide and its chemical relative 6-*n*-propylthiouracil (PROP). In all human populations tested, some people cannot detect low concentrations and fail to experience the bitterness of high concentrations of these compounds. Family and twin studies in humans suggest this trait is partially mediated by genetic influences, and linkage studies indicate regions on chromosomes 5

and 7 contain relevant genes. These linked regions are near putative bitter taste receptors. Previous research has shown that TAS2R4 responds to PROP in a cell system, so this receptor would be considered a candidate gene for this trait. To determine whether TAS2R4 and two closely related bitter receptors on chromosome 7 contain nucleotide sequence variants that contribute to PROP insensitivity, we sequenced these genes using DNA from people at the extremes of the phenotypic distribution (TAS2R3, TAS2R4 and TAS2R5;  $n = 18$  subjects; 60.6 kb sequenced). Several variants were found within these three genes ( $p =$  allele frequency of the most frequent allele; for TAS2R3, intron -45 nt  $p = 0.46$ , P117S  $p = 0.03$ , G269G  $p = 0.53$ , L284L  $p = 0.94$ ; for TAS2R4, intron -10 nt  $p = 0.97$ , F7S  $p = 0.56$ , V96L  $p = 0.56$ , S171N  $p = 0.53$ ; for TAS2R5, intron -55 nt  $p = 0.53$ , S26S  $p = 0.53$ ). However, no sequence variant was found more frequently in PROP insensitive subjects compared with sensitive subjects. There are two possible interpretations of this observation. First, nucleotide variants within the protein coding regions of these particular putative bitter receptors may not be involved in human PROP sensitivity. Second, it is possible that one or more of these variants may contribute to the trait, but may not be sufficient to fully account for it. Genetic and anatomical evidence suggest that PROP insensitivity may have a more complex inheritance pattern than previously supposed.

## Heritability estimates for intensity ratings of sucrose and saccharin in human families

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It is generally accepted that people differ in their liking for sweet foods, so much so that the label ‘sweet tooth’ is applied to those who eat them frequently. Some health educators believe that the excessive consumption of sweet foods contributes to obesity, diabetes and a nutrient-poor diet, but little is known about why people differ in this regard. Family and twin studies suggest that macronutrient preference aggregates in families and has genetic origins. However, it is unclear if the perception of sweet taste itself is genetically mediated in humans, or how the perceived intensity contributes to the liking for sweet foods. As a step toward understanding the genetic architecture of sweet taste perception, we tested 94 members of a large human pedigree. Subjects rated the taste intensity of 0.53 M sucrose (twice, on two separate testing days) and  $7.3 \times 10^{-4}$  M saccharin (once) for intensity on the Labeled Magnitude Scale (Green *et al.*, 1996). Heritability was calculated using a polygenic model computed using the Sequential Oligogenic Linkage Analysis Routines (SOLAR) program (Almasy & Blangero, 1998). The results of this analysis suggested that genetic variability accounted for ~20–30% of the total phenotypic variance for ratings of sucrose intensity ( $h^2 = 20\%$  for the first test day, 32% for the second test day) and saccharin ( $h^2 = 33\%$ ). In comparison, ~40% of the phenotypic variability in 6-*n*-propylthiouracil bitter intensity ratings is heritable. These results suggest that DNA sequence variants in a gene or genes may partially account for the experience of sweet taste intensity in humans; dissecting the factors that contribute to individual differences in the human sweet tooth may be possible. However, other sources of variation, such as learning, individual experience and measurement error, may also make a substantial contribution.

## Transduction of acid stimuli in primary chemosensory neurons

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Tissue acidosis is a frequent condition in diseases causing chronic pain, such as inflammation, ischemia and malignant tumor growth. Pain and local pH are well correlated, and Aspirin-like drugs are effective against acid-induced pain. Nociceptive nerve endings are excited and sensitized by low pH, in skin, dura and visceral organs, thresholds range between pH 6.9 and 6.1, with maximal activation around pH 5.2. Discharge is slowly activating (over minutes) and non-inactivating at constant low pH, kinetics that are similar to those of an unspecific cation current induced by protons in DRG cells in culture. Although action potential generation in DRGs is blocked upon low pH stimulation, sensory neurons also respond with release of the pro-inflammatory neuropeptides substance P and CGRP triggered by calcium influx. This particular responsiveness is also present all along the axons of nociceptive neurons, which implies a role in neuropathic pain syndroms, such as diabetic neuropathy. Inflammatory mediators such as bradykinin, serotonin and prostaglandin E2 induce a lasting sensitization to low pH, presumably through activation of protein kinases. The capsaicin receptor antagonist capsazepine can prevent this interaction, which suggests a causal contribution of the vanilloid receptor (VR1), an unselective cation channel, that is not only operated by capsaicin but also by noxious heat and low pH. VR1 knockout mice lack neuronal proton responsiveness in skin, peripheral nerve and dura. However, proton sensitivity is retained in the colon where it can be blocked by amiloride, implying an involvement of the 'acid-sensing ion channel' ASIC3 that is expressed in DRG cells as an unselective cation channel. The phenomenology of acid responsiveness in sensory neurons still leaves room for smaller, non-inactivating mechanisms such as H<sup>+</sup>-induced block of TASK-3, a tandem pore K<sup>+</sup> channel also expressed in DRGs. The elucidation of sensory transduction of acid stimuli may provide new specific targets for pain therapy.

## Simultaneous measurement of nasal airway resistance by posterior rhinomanometry and forced oscillation

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Nasal congestion frequently follows exposure to irritants. We wished to compare nasal airway resistance measurements using two different methods—active posterior rhinomanometry (Rn) and the forced oscillation technique (RFOT)—applied simultaneously both at baseline and post-decongestant. We tested 10 volunteers—seven males and three females, aged 22–53 years—before and after administration of 0.2 ml/nosril of 0.1% xylometazoline nasal spray. Subjects breathed nasally through a full-face mask, with a flexible silicone tube held between the lips. RFOT involved superimposing oscillations at 5.0 Hz over spontaneous nasal breathing. Rn and RFOT were both measured at

+75 Pa; measurements were averaged for inspiration and expiration over 2–3 respiratory cycles. The efficacy of the decongestant was assessed by the percent change of Rn and RFOT to their respective baseline values (% Rn and % RFOT). The mean  $\pm$  SD values of Rn and RFOT before decongestant were  $1.98 \pm 0.97$  and  $2.26 \pm 0.87$  cmH<sub>2</sub>O·s/l, respectively. The two indices were highly correlated (RFOT =  $0.57 + 0.86 \cdot \text{Rn}$ ;  $r = 0.92$ ) and the mean coefficients of variation were not significantly different ( $10.3 \pm 6.4\%$  for Rn and  $10.3 \pm 5.5\%$  for RFOT). After the administration of the decongestant, both resistance indices decreased significantly to  $1.11 \pm 0.25$  and  $1.55 \pm 0.30$  cmH<sub>2</sub>O·s/L respectively ( $P < 0.05$ ). Rn and RFOT were highly correlated, and both responded to decongestant treatment. The two techniques appear to be equivalent with regard to both ease of measurement and coefficient of variation.

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## Diversity of the mammalian vomeronasal receptor gene repertoire

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Seven-transmembrane-domain proteins encoded by the vomeronasal receptor *V1r* and *V2r* gene superfamilies, and expressed by vomeronasal sensory neurons, are believed to represent pheromone receptors in mammals. Taking advantage of the recent major advances in the sequencing of the mouse genome, we obtained a first global draft of the mouse *V1r* repertoire, including 104 novel *V1r* genes. The repertoire comprises eight novel and extremely isolated families. Phylogenetic analyses suggest an ancient original radiation, followed by the isolation, divergence and expansion of families by extensive gene duplications and frequent gene loss. The isolated nature of these gene families probably reflects a specialization of different receptor classes in the detection of specific types of chemicals.

## Olfactory priming through nasal inhalation in humans?

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Studies on chemosensory event-related potentials (CSERPs) often use the velopharyngeal closure breathing technique (VC) to maintain a constant airflow within the nasal cavity. However, it has been shown that the structure of CSERPs can be altered by the breathing technique. This study has been designed to evaluate whether olfactory processing varies with the phase of the breathing cycle, and whether this effect might be modulated by the breathing technique. Three female and five male right-handed non-smokers voluntarily participated in the study. Isobutylaldehyde and phenyl-ethylalcohol were presented 80 times each per session via a constant flow-olfactometer. Each stimulus lasted for 300 ms and was triggered by inhalation or exhalation, respectively. In each of two sessions, subjects had to perform either VC or natural nasal

breathing (NNB). The order of sessions was counterbalanced across subjects. EEG was collected from 32 scalp sites at 500 Hz. The results show that the N1 amplitude is more pronounced in VC compared with NNB. Additionally, the N1 seems to be enlarged during inhalation compared with exhalation, independent of the breathing technique. The late information processing (P3-1 and P3-2) shows combined effects of respiratory cycle and breathing technique: in NNB the amplitudes were larger during inhalation than during exhalation, while no differences between the respiratory cycle phases were found in VC. The results indicate that inhalation activates priming processes for olfactory perception not only in NNB but in VC as well.

## Psychophysiological correlates of affects in olfaction

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Several studies have suggested that in olfaction emotional categorization is the most important dimension for odor grouping, is rapid and involuntary, and involves specific neural structures. Many authors contend that affective categorization comprises only the single dimension of pleasure, and fail to consider other dimensions such as arousal. We present four experiments that examine this issue. The first aim of this series of studies was to examine the relationships between the dimensions of pleasantness and arousal in the olfactory modality. In the visual and the auditory modalities many studies using measures such as facial electromyographic (EMG) activity and skin conductance have found those parameters to vary as a function of either arousal or pleasantness; for example, facial corrugator EMG activity covaries with the pleasure dimension, while skin conductance increases linearly with arousal. The second aim was to study the covariation between peripheral measures and pleasantness and arousal in olfaction. We found, for olfactory stimuli, a strong increase in arousal as a function of unpleasantness (experiment 1). Results also showed that, as in other modalities, facial corrugator EMG activity decreases as a function of pleasure and that skin conductance level increases with arousal (experiments 2 and 3). Finally, we observed that affective reactions change according to the nostril stimulated (experiment 4). In conclusion, results of the present studies are in line with certain authors who postulated the existence of two systems (defensive and appetitive) in the brain, each of which can vary in terms of metabolic arousal. Arousal is not viewed here as having a separate substrate, but rather as representing intensity of activation of either the appetitive or the defensive system, or both.

## Influence of the cue significance acquired following an olfactory discrimination learning task on Fos expression in the rat piriform cortex

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The piriform cortex (PCx), the main area of the primary olfactory cortex, receives dense inputs from the olfactory bulb. Several lines of evidence suggest that the PCx, together with other brain areas,

could play an important role in olfactory memory. The expression of the immediate early genes such as the proto-oncogene *c-fos* is known to characterize learning-related changes in neuronal activity. By using immunocytochemistry of the Fos protein following a two-odor discrimination learning test, we examined the PCx activation according to the biological significance i.e. positive (association with a reward) or negative (a lack of reward) of an olfactory cue. Olfactory discrimination training was performed in a four-arm radial maze. Four groups of rats were used: trained (T), pseudo-trained (P), odor-only (O) and naive (N), i.e. home-cage control animals. T and P rats were water-deprived. The T rats learned to discriminate between two odors (geraniol and limonene). In experiment 1, geraniol was rewarded by water delivery. In experiment 2, geraniol was the non-rewarded odor of the pair. Discrimination learning task was considered as acquired when the animals chose the rewarded odor in 80% of trials. Then, the rats were stimulated by geraniol and sacrificed 1 h later to process Fos detection. The P rats were randomly rewarded with water. The O rats had no behavioural training and they were stimulated by geraniol to determine the effect of meaningless odorless stimulation upon Fos expression. Countings of immunoreactive cells were performed at eight rostro-caudal PCx levels. In the anterior part of PCx, the Fos labeling was significantly lower in the T rats from experiment 1 in comparison with all the other groups. Such data sustain the assumption of a differential information processing in the PCx.

## Functional anatomy of the emotional response to odors: influence of hedonic valence, hedonic judgement, handedness and gender

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We previously showed that the neural network involved in emotional responses to pleasant (P) and unpleasant (U) odors was lateralized to the left hemisphere of right-handed (RH) men (Royet *et al.*, 2000, *J. Neurosci.*, 20: 7752–7759). This study examined activation in these networks as a function of handedness (RH versus LH), gender and hedonic valence (P versus U) during both explicit hedonic judgements (HJ) and passive (PAS) stimulation. Whole-brain fMRI (BOLD method at 1.5 T) was used to detect changes related to hedonic judgement in 28 subjects (RH and LH men; RH and LH women). Two functional runs (HJ, PAS) were performed. For each run, a block paradigm was used with both hedonic (P and U) and rest conditions. Activation from emotional response (PAS) and explicit judgements (HJ) was then compared between subject groups using random effects analyses (SPM99). The piriform-amygdala area and ventral insula were more activated by U than P odors; that is, by more hedonically intense odors. U odors activated the left ventral insula in RH and the right ventral insula in LH. P and U odors activated left orbitofrontal cortex (OFC) during HJ, but not during PAS. Left OFC was also more activated in women than in men. The results suggest that hedonic intensity and explicit hedonic judgements activate different areas of an emotional neural network, and that activation patterns change as a function of handedness and gender.

## Cross-cultural study on umami taste; perception, behavioral response and brain activity

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We reported the perceptual difference among different countries, influenced by their own culture. In this study we investigated cross-cultural difference on taste perception of umami, which is very popular in Japan but not in Europe. We also examined the behavioral response and brain activity of the primary gustatory area, which we found at the transition between parietal operculum and insula (area G) using magnetoencephalography (MEG). The subjects, 20 Japanese and 20 European, estimated the intensity, taste quality and hedonic quality for 2.2, 6.7, 20, 60 and 180 mM disodium 5'-inosinate (IMP), 300 mM NaCl and 30 mM Na-saccharin. The threshold of IMP and reaction time for 60 and 180 mM IMP, 300 mM NaCl and 30 mM Na-saccharin were measured, and gustatory evoked magnetic fields were measured by a 64-channel whole-head SQUID MEG system for 180 mM IMP and 300 mM NaCl. The results showed no significant differences in threshold, intensity and RT between the two groups. However, in the hedonic evaluation, a difference was found for the lowest concentration of IMP (2.2 mM). In the taste quality description, the Japanese often used a special word for umami like 'dashi' or 'umami', while the Europeans named the food as fish, boiled pork-meat or mushroom. Activity of area G was observed for IMP and NaCl in both groups. These results suggest that the cognitive and hedonic processes of umami taste were influenced by cultural difference, but the sensitivity process was not.

## Behavioral and neural mechanisms involved in odor-taste association in rats and humans

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Human participants rate intensity of a taste stimulus (sucrose, Na-saccharin, etc.) sweeter when it is presented with a vanilla flavor than when presented alone. This phenomenon is called 'taste enhancement by odor'. Although there have been some studies of this phenomenon, the underlying mechanisms remain unclear. In this presentation, we first introduce some human psychophysical studies on 'taste enhancement by odor', which suggest that associations between odor and taste are related to this phenomenon. To investigate brain mechanisms involved in this kind of association, some studies with rats and humans have been done. In the behavioral neuroscientific study with rats, it was shown that rats could acquire the association between 'salty taste' and an odor. It was also found that rats with lesions in some brain regions, such as insular cortex or prefrontal cortex, did not show acquisition of this kind of taste-odor association. According to these results, it is suggested that humans and animals develop some 'taste images' for odor based on an association between odor and taste, and use these images in perception and cognition of chemical stimuli. We are investigating the brain areas activated in correlation with a 'taste

enhancement by an odor' using non-invasive measurement of human brain activity with magnetoencephalography.

## Exploring the brain areas activated during evaluating pleasant-unpleasant of odors with fMRI

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To clarify brain mechanisms involved in processing pleasantness-unpleasantness in humans, we investigated human brain activities during smelling odors with the functional magnetic resonance imaging (fMRI). In this experiment, 13 participants were asked to smell three kinds of odor (triethyl amine, rose P and citral) and to evaluate pleasantness-unpleasantness of each odor while an echo-planar image of the brain was acquired. The pleasantness-unpleasantness of stimuli was rated on the scale from -3 (extremely unpleasant) to +3 (extremely pleasant). According to the participants' ratings, 39 stimuli were divided into one of the following conditions: negative condition (rated pleasantness  $\leq -1$ ), neutral condition ( $-1 < \text{rated pleasantness} < 1$ ) and positive condition (rated pleasantness  $\geq 1$ ). The cerebellum, the insular cortex and the prefrontal cortex (Brodmann area 10) showed significant change of signals correlated with the olfactory stimulation in all three conditions. Correlated activity was found bilaterally in the prefrontal cortex (Brodmann areas 45, 46) in the negative condition, whereas the amygdala showed correlated activity in the positive condition. These results are discussed with those of preceding studies.

## Smelling of odorous sex hormone-like compounds causes sex-differentiated hypothalamic activations

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The anatomical pathways for processing of odorous stimuli include the olfactory nerve projection to the olfactory bulb, the trigeminal nerve projection to somatosensory and insular cortex, and the projection from the accessory olfactory bulb to the hypothalamus. In the majority of tetrapods, the sex-specific effects of pheromones on reproductive behavior is mediated via the hypothalamic projection. However, the existence of this projection in humans has been regarded as improbable because humans lack a discernable accessory olfactory bulb. Here, we show that in women smelling an androgen-like compound, the hypothalamus is activated, with the center of gravity in the preoptic and ventromedial nuclei. In contrast, in men, the hypothalamus (center of gravity in paraventricular and dorsomedial nuclei) is activated when smelling an estrogen-like substance. This sex-dissociated hypothalamic activation suggests a potential physiological substrate for a sex-differentiated behavioral response in humans.

## Developmental pattern of peptide-immunoreactivity in the metamorphosing antennal lobe of the sphinx moth *Manduca sexta*

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The antennal lobe (AL) of *Manduca sexta*, which contains many peptidergic neurons, is newly built during metamorphosis. All neurons comprising the AL are born 2 days after pupal ecdysis (P2; Oland and Tolbert, 1996, *J. Neurobiol.*, 30: 92). To learn about the time course and regulation of peptide expression during AL development, we started to study the developmental pattern of FMRamide-, allatostatin- and allatotropin-like immunoreactivity (ir). AL-neurons labeled with the three peptide antisera belong to the local interneuron type. Typical for all three antisera was a strong increase in labeled cell numbers 7–8 days after pupal ecdysis (P7/8). The number of FMRamide-like immunoreactive cells showed an additional strong increase at stage P11/12. The rapid increase of peptide immunoreactive neurons at stage P7/8 parallels the increasing 20-hydroxyecdysone (20E) hemolymph titer (Warren and Gilbert, 1986, *Insect Biochem.*, 16: 65). To investigate the relationship between the 20E titer and the number of peptide immunoreactive neurons, we mimicked the increase of the hormone early in development by injecting an equivalent amount of 20E into the hemolymph of stage P1 pupa. The experiments resulted in high numbers of peptide immunoreactive cells, normally not seen before P7/8, 3 days (P4) after the injection. This result strongly indicates that peptide expression in the developing AL is regulated via 20E.

## Associative olfactory learning in individually assayed *Drosophila* larvae

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We have established a larval olfactory conditioning paradigm using gustatory reinforcement on individual *Drosophila* larvae. Employing chemosensory stimuli would facilitate future analyses of the circuitry underlying this form of olfactory learning, because extensive research is actually pursued concerning the functional anatomy of chemosensory systems. Testing individuals would be a prerequisite for future physiological analyses. We developed a discriminative conditioning assay in which single larvae were trained successively with either of two trial types: odorant A paired with fructose and odorant B paired with quinine. In a second experimental group, A was paired with quinine and B with fructose. Initially, we used the attractants octanol and amylacetate as olfactory cues. After such training, larvae of both groups were tested in an olfactory choice assay. A difference of odor choice between the two groups can be considered an associative learning effect. Indeed, such a learning effect was reproducibly observed. To further increase the learning effect, we plan to introduce an inter-trial interval. To test the general applicability of our procedure, we want to use NaCl instead of quinine. Finally, we will test the three gustatory reinforcers independently of each other in an absolute conditioning situation.

## Processing of food cues in patients with eating disorder

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In humans, food selection depends on physiological demands, sensory cues and cognitions about food and body weight. This study was designed to investigate the influence of attitudes towards eating on the central nervous processing of olfactory and visual food stimuli. Thirty-two women (age = 19–31 years; body mass index = 18.5–40.5) were categorized as restrained eaters (REs; eaters who cognitively restrict their eating behaviour), unrestrained eaters (UEs) or patients with eating disorders (Ps), using standardized questionnaires and a clinical interview. Palatable and unpalatable food odours (caramel, onion) were presented via a constant-flow olfactometer in randomized order. Afterwards, pictures of delicious or rotten foods were shown on a screen. Phenyl ethyl alcohol and pictures of landscapes served as a neutral non-food control condition. EEG was recorded from 64 scalp positions and peaks were detected in the latency ranges of the olfactory and visual components N1, P2, P3 and pSW. Analyses of the chemosensory event-related potentials revealed a reduced P3 amplitude to caramel odour in REs and Ps compared with UEs ( $P = 0.025$ ). Similarly, in the visual event-related potentials, the pSW amplitude to pictures of desserts was smaller in REs than in UEs ( $P = 0.04$ ). However, Ps did not differ from either of the compared groups. In both modalities, groups differed more in the late components, whereas early components seemed to be less affected by individual attitudes towards food associated stimuli.

## Repair and regeneration in the peripheral olfactory system

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The peripheral olfactory system is able to recover after injury—the olfactory epithelium reconstitutes, the olfactory nerve regenerates and the olfactory bulb is reinnervated—with a facility that is unique within the mammalian nervous system. Cell renewal in the epithelium is directed to replace neurons when they die in normal animals or at an accelerated pace following damage to the olfactory nerve. Neurogenesis persists because neuron-competent progenitor cells, including transit amplifying and immediate neuronal precursors, are maintained within the population of globose basal cells. Notwithstanding events in the neuron-depleted epithelium, the death of both non-neuronal cells and neurons directs multipotent globose basal cell progenitors to give rise individually to sustentacular cells and horizontal basal cells, as well as neurons. Multiple growth factors, including TGF- $\alpha$ , FGF2, BMPs and TGF- $\beta$ s, are likely to be central in regulating choice points in epitheliopoiesis. Signalling via the Notch/HES/MASH-1 cascade is likely to play a critical role in regulating the choice between multipotency and neuropotency. Reinnervation of the bulb is rapid and robust following peripheral injury. When the nerve is left undisturbed, i.e. by lesioning the epithelium directly, the projection of the reconstituted epithelium onto the bulb is restored to near-normal with respect to rhinotopy and in the

targeting of odorant receptor-defined neuronal classes to small clusters of glomeruli in the bulb. However, at its ultimate level, i.e. the convergence of axons expressing the same odorant receptor onto one or a few glomeruli, specificity is not restored unless a substantial number of fibers of the same type are spared. Rather, odorant receptor-defined subclasses of neurons innervate an excessive number of glomeruli in the rough vicinity of their original glomerular targets. The limits and capacities on repair and regeneration in an experimental setting inform our understanding of clinical olfactory disease and give direction toward our attempts to remediate sensory dysfunction.

### Effect of olfactory mucosal application of lectins on the odorant response activity of the frog olfactory bulb as measured using the voltage sensitive dye RH414

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Studies of the electro-olfactogram (EOG) in frogs and rats report partial inhibition of responses to fatty acid odours in the presence of the lectin Concanavalin A (Con A) (Wood *et al.*, 1983, *Biochem. Soc. Trans.*, 11: 781–782; Shirley *et al.*, 1987, *J. Biochem.*, 245: 175–184; Shirley *et al.*, 1987, *J. Biochem.*, 245: 185–189). This suggests that the response to C4–C6 alkanolic, alicyclic and carboxylic acids, and possibly the response to aliphatic thiols, is preferentially inhibited by Con A. The original results were interpreted as evidence for specific fatty acid odorant receptors that are specifically blocked by Con A. However, the counter-argument is that application of Con A to the olfactory mucosa may cause nonspecific effects on the EOG that are not receptor mediated—the EOG being the integrated generator potential over a relatively large area of the olfactory mucosa. Because of the convergent nature of sensory connections to glomeruli in the olfactory bulb, we deduced that if localized areas of the olfactory bulb were monitored, then the activity of selected receptor types that may be geographically distributed across the olfactory mucosa could be monitored simultaneously. If a fatty acid receptor was selectively blocked, then we should see a corresponding decrease in neural activity in certain glomerular areas in the olfactory bulb. The olfactory bulb (OB) from the frog *Rana temporaria* was exposed and stained using a voltage-sensitive dye (RH-414). The resulting fluorescence from the stained tissue was imaged with a charge-coupled device camera, attached to an epifluorescent microscope, synchronized with a frame grabber, to follow neural events in real time over the course of an odour stimulus. Spatial and temporal features of the response profiles were detected using digital filters implemented within specific software. The results show that application of Con A to the olfactory mucosa of the frog brings about a pattern of selective inhibition of olfactory bulb responses of the odorants *n*-butyric acid, dimethyl disulphide and propylmercaptan. The data support the existence of at least two Con A sensitive receptors. The two receptors may exist in the form of one being sensitive to a fatty acid (*n*-butyric acid) and the other being sensitive to both dimethyl disulphide and propylmercaptan.

### Behavioral and molecular analyses in the *dpa* (D-phenylalanine sensitivity) congenic strain

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It is known that there are two loci influencing responses to sweet substances on mouse chromosome 4, *dpa* and *Sac* (Saccharin preference). Recently, the *Sac* locus is found to be a candidate gene encoding a G-protein-coupled sweet receptor, TIR3. Although the site of action of the *dpa* locus has been proposed at the peripheral taste system, details remain unknown. In this study, we produced the *dpa* congenic strain having a BALB background except a segment including the *dpa* locus derived from D-phe responsible C57 strain. We examined whether the *dpa* segments from C57 contain *TIR* loci. The cDNA subtraction was also carried out to identify components involved in the D-phe response. Behavioral analysis demonstrated that sweet-tasters of two generations (fifteenth and sixteenth) were differentiated into two sub-groups according to their partial differences in responses to sucrose and saccharin. *TIR2* and *TIR3* loci of the congenic strain were found to be derived from BALB, but not C57. The typing of the *TIR1* locus could not be determined because of none of polymorphisms between C57 and BALB. In the subtraction analysis, we obtained 20 cDNA clones including four unknown genes. One of them was highly expressed in fungiform papillae in RT-PCR. These results suggest that the gene segment derived from C57 does not encode the *TIR2* and *TIR3* but may contain some genes other than the *dpa* which may relate to sensitivities to D-phe and other sweeteners in congenic strain.

### Blockade of chemical communication by an aromatic environmental pollutant

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Many classes of environmental pollutants are found at significant levels in the environment. Concerning the reproductive field, environmental pollutants, which disrupt the endocrine system, have been shown to be endowed with hormone/antihormone activity. Gonadal functions of various animals are regulated by pheromones whose production is modulated by hormones. The purpose of this study was to explore effects of 3-methylcholanthrene (3-MC), which are widely distributed in a variety of environmental products, on excretion of pheromones from male mice. On day 1 after the i.p. dosage of 3-MC, the density of Fos-immunoreactive cells, which is correlated with cellular activity, in the accessory olfactory bulb of female mice after exposure to urine excreted from treated males was lower than that after exposure to urine from non-treated males. SDS analyses were performed on the urine on day 1 after the 3-MC treatment. The level of proteins, including major urinary proteins (MUPs), in urine from treated males was similar to that from non-treated males, suggesting that 3-MC lowers pheromonal activity in urine without significant changes in urinary MUPs on day 1. It is likely that environmental

pollutants interfere with chemical communications between males and females.

### Mechanistic studies of nasal trigeminal reflexes using chlorine gas as a model irritant

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Nasal trigeminal reflexes include rhinorrhea, congestion, sneezing and alterations in breathing pattern. Primary trigeminal irritation, as well as these secondary reflexes, are among the symptoms most often reported in air pollution situations, including so-called 'problem buildings'. Our objective was to create a model system for nasal trigeminal irritation to both quantitate reflex responses and to explore their pathophysiologic mechanism. To do so, we chose the water-soluble irritant gas, chlorine (Cl<sub>2</sub>), administered by nasal mask at its occupational short-term exposure limit (1.0 p.p.m. for 15 min), and selected subjects with and without nasal allergies, but with no prior history of asthma. Repeated testing confirms that this system is capable of triggering nasal reflexes (specifically congestion, as documented by posterior rhinomanometry) without lower respiratory tract symptoms or decrements in pulmonary peak flow. Nasal congestion is achieved with very modest symptom ratings. In three different single-blinded cross-over studies utilizing clean air as a control, we confirmed the augmented reactivity to nasal irritants of seasonal allergic rhinitic versus non-rhinitic subjects, implying that allergy somehow plays a part in the response. However, repetition of the exposure protocol with nasal lavage tryptase levels as the outcome of interest fails to demonstrate evidence of mast cell degranulation (i.e. the nasal congestive response to Cl<sub>2</sub> is not a true 'allergic' reaction, but may be up-regulated by allergic inflammation). Other potential acute response mechanisms are neurogenic in nature, and include both central (autonomic) and local (tachykinin-mediated) reflexes. As a first step in exploring neurogenic mechanisms, we pre-treated subjects' noses with the cholinergic blocker, ipratropium bromide, and found that it does not alter the nasal congestive response to Cl<sub>2</sub> provocation. Thus, the role of parasympathetic autonomic reflexes has been downgraded in our estimation. By implication, a major candidate response mechanism would be the tachykinin-mediated local (axon) reflex. This mechanism will provide a major focus for future studies.

### Olfactory space, receptors and nervous representation of the chemical stimuli

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In several animal species, it has been shown that the olfactory experience is likely to modify the responses of the olfactory system to the smelling stimuli tested. These changes are recorded on several levels: on the one hand, they concern the neurophysiological activity throughout the olfactory tract, from the receptor organ and the olfactory bulb to more central structures. On the other hand, they are also manifest on the behavioural responses guided by olfaction. By way of assumptions, the modifications of

nervous activity are interpreted like modifications the nervous representation of the olfactory stimulus, and could be correlated with the perceptive variations which we deduce from the observation of the behavioural responses. However, in the light of the results of molecular biology and the neurophysiological data, what idea can we have of the nervous representation of the olfactory stimulus?

### Suppression of taste by oral irritants

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Irritant chemicals are commonly found in many foods and evoke a burning or pricking sensation by activating nociceptive nerve endings in the oral mucosa. In combination with sensations of taste and olfaction, oral irritation contributes to the overall flavor profile elicited by a given food or beverage. Few previous studies have investigated whether and how the presence of oral irritants modify perceptions of taste, with the majority reporting capsaicin suppression of the intensity of certain taste qualities, particularly sweetness. We employed a multidisciplinary approach to investigate the effect of pretreating the oral cavity with two irritant compounds—capsaicin (the pungent principle in chili peppers) and nicotine—on taste sensation and neural processing. Human psychophysical experiments employed a two-alternative forced-choice paradigm coupled with intensity rating, in which capsaicin was applied to one side of the tongue followed by bilateral application of one of five tastants (NaCl, citric acid, sucrose, quinine, glutamate). Subjects consistently chose the capsaicin-treated side as tasting less intense (and assigned lower intensity ratings to that side) for sucrose-induced sweetness, quinine-induced bitterness and glutamate-induced umami. In electrophysiological experiments using pentobarbital-anesthetized rats, responses of gustatory neurons in the nucleus tractus solitarius (NTS) to tastant stimuli were recorded before and following lingual application of capsaicin or nicotine. Units were characterized as responding best to one of the five tastants used in the psychophysical study. Immediately after capsaicin, responses to each tastant were in nearly all cases depressed (mean 57% of control), followed by recovery within ~12 min in many but not all units. These data support a physiological mechanism as underlying the capsaicin-induced reduction of taste intensity as perceived in humans. To differentiate between peripheral and central mechanisms of taste suppression, tastant-evoked responses were recorded prior to and following lingual capsaicin application in rats subjected to bilateral trigeminal ganglionectomy. Taste-evoked responses following lingual capsaicin were suppressed in ganglionectomized rats to the same degree as that seen in intact animals. Furthermore, capsaicin applied to the tongue in a manner identical to that used in the electrophysiology experiments evoked plasma extravasation with a resulting accumulation of dye in the fungiform papillae, indicating that capsaicin acts peripherally to suppress taste transmission. Lingual application of nicotine also suppressed tastant-evoked NTS unit activity in a concentration-dependent manner. Interestingly, following bilateral ganglionectomy, nicotine no longer suppressed tastant-evoked NTS unit responses, suggesting a central mechanism of suppression dependent upon activation of trigeminal pathways. In addition, topical application of nicotine

did not evoke plasma extravasation in the tongue, further differentiating the mechanisms by which capsaicin and nicotine might alter gustatory processing.

### Dynamic processing of tastants in gustatory cortex of awake rats

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The gustatory system has evolved to detect and discriminate among foods, to select nutritious diets and to initiate, sustain and terminate ingestion. These processes evolve over several seconds and involve the integration of multiple sources of information. Historically, and even at present, most neural responses to gustatory stimuli have been analyzed by simply counting the number of spikes in a given interval (usually 3–5 s) to obtain an average activity. We show that by analyzing recordings from small ensembles of neurons from the gustatory cortex (GC) of awake rats that were evoked by tastants delivered by intraoral cannulae, that the static analysis, whether applied to a labeled line or across firing pattern paradigm, does not account for either the dynamic and interactive aspects of gustatory neural processing. Analyses of the GC responses revealed that they contain somatosensory information during the first 200 ms, chemosensory information during the next second and somatosensory responses related to the hedonic valence of the tastants at latter times (Katz *et al.*, 2002, *J. Neurosci.*, 22: 1850–1857). Thus, various neural networks contribute differentially to the GC spike trains at different times. We have also tested whether between-neuron interactions may contribute to the shaping of single-neuron responses (Katz *et al.*, 2001, *J. Neurosci.*, 21: 4478–4489). Ensemble recordings from GC neurons revealed tastant-specific patterns of long (300 ms) cross-correlations between pairs of neurons that provide additional information to the gustatory system to discriminate among tastants.

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### Plasticity in odor-elicited behavior in the moth *Spodoptera littoralis*

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We have studied different aspects of plasticity in the behavioral response to odor-cues in the moth *Spodoptera littoralis*. First, we have investigated the effect of experience on the male's response to female sex pheromone. In olfactometer and windtunnel tests we evaluated the males' response rate with and without pre-exposure to the complete sex pheromone, which consists of several components, and the main component of the pheromone. We found that males with a single exposure to the pheromone showed a 10–100 times higher response rate than naïve males. The effect was still prominent after 27 h post-exposure. Pre-exposure to the complete pheromone elicited a higher increase in response rate than did exposure to the main component by itself. Thus, the effect

was quality dependent. Secondly, we have investigated plasticity in host plant choice behavior in larvae of *S. littoralis*. The larvae were reared on different plants up to third or fourth instar. They were then exposed to the odor of the plants and to clean air in a two-choice olfactometer, where their orientation behavior was monitored. Some plants attracted the larvae after feeding experience, while others did not. This might reflect the plants' varying suitability as a host for the larvae. Current and future studies investigate plasticity in host plant choice in adults, as well as the effect of experience on the neurophysiological responses by means of several different methods.

### Connections between the olfactory epithelium and the brain can support olfaction in the absence of the olfactory bulbs

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After removal of one olfactory bulb in P2 rats, the homolateral forebrain grows forward to fill the resulting cavity, and axons from maturing olfactory sensory neurons extend through the cribriform plate and terminate in the overlying cortical tissue. We assessed olfaction in such cases by training on odor detection and discrimination tasks at P70 and then retesting animals after removing the remaining olfactory bulb. Connections between the epithelium and forebrain were documented using anterograde transport of horseradish peroxidase (HRP) applied to the epithelium. In nine experimental cases, both olfactory bulbs were completely absent, and the forebrain ipsilateral to the neonatal bulb removal contained HRP reaction product characteristic of axon bundles and clusters of axons that formed glomerular-like structures in frontal pole neocortex or in the olfactory peduncle. In most cases with rich input to olfactory peduncle, rats were able to detect a variety of odors and discriminate between acetic acid and propionic acid, and between the enantiomers of carvone. Performance in some cases was at or near that of controls (those with one intact olfactory bulb). Rats with input to only neocortex were anosmic, as were those with extensive damage to the olfactory peduncle in the neonatal operated hemisphere.

### Assessment of acute sensory irritation from isopropyl alcohol exposure in humans: a psychotoxicological approach

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Two studies were conducted to evaluate the sensory irritancy from isopropyl alcohol (IPA) in occupationally exposed workers and controls. Central to the design of these studies were the assessment of contribution of odor and response bias to reports of sensory irritation. The first characteristic of our 'psychotoxicological' approach is the inclusion of both subjective (self-report) and objective markers of irritation (e.g. ocular redness, respiratory frequency, nasal secretion and congestive changes) from a whole-body exposure of IPA at the TLV/MAC value of 400 p.p.m. (study 2). In addition, nasal irritation thresholds for IPA were determined independently from odor thresholds using the

lateralization method (study 1). The second characteristic consists of the inclusion of two control exposure conditions—to phenyl ethyl alcohol (PEA) and to clean air (CA)—to determine the relative contributions to irritation reports of perceived odor (PEA) or of response bias (CA) (study 2). Results (study 1) show that lateralization thresholds (3361 versus 6083 p.p.m. for controls and workers, respectively) were significantly higher than the odor thresholds (11 versus 39 p.p.m.), and that current TLV of 400 p.p.m. is below the fifth percentile of the lateralization threshold distribution. It was concluded that the current TLV is adequately protective of nasal sensory irritation. In study 2, subjects reported significantly higher intensity ratings for irritation, annoyance and odor in the IPA condition than in the PEA and CA conditions. The only objective endpoint that showed a change exclusively in the IPA condition was respiration frequency: relative to baseline, respiration frequency increased in response to IPA in both groups. This change was interpreted as mediated by the perception of an unpleasant odor. Overall, the psychotoxicological approach enables the evaluation of relative contributions of cognitive and physiological effects of VOC exposures.

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## European odour concentration measurement by dilution olfactometry

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The almost complete acceptance of the CEN/TC264/WG2 draft standard for odour concentration measurement by dilution olfactometry by the odour laboratories throughout Europe and in some laboratories the USA means that odour concentrations can be directly compared and that the measurements can be relied upon to be repeatable. The draft standard prEN13725 is a performance-based standard that demands that measuring laboratories test and record their compliance to the quality criteria continuously. Factors are prescribed in the prEN that ensure the overall performance quality. These factors are the materials of construction of the instruments and sampling equipment, the instrumental accuracy, selection of the sensors (in this case human assessors), behaviour of the assessors, the conditions of the measurement and treatment of the data when calculating the results. The reference material prescribed is *n*-butanol and the assessors collectively shall have a mean detection threshold of 123 mg/m<sup>3</sup> (40 p.p.b.). Laboratories must be able to demonstrate they can achieve this criterion. The criterion for precision, expressed as repeatability, is  $r < 0.477$  (in non-logarithmic terms it is  $10 r < 3$ , which implies that the ratio between two measurements on the same material will not be  $>3$  in 95% of cases) and the criterion for accuracy (bias and repeatability)  $A < 0.217$ . The prEN also requires the laboratory precision to be the same for real environmental samples. Measurements at SRI have shown that compliance with the criteria for the reference material ensures the results with environmental (nuisance) odours well exceed the repeatability requirement. The prEN has been developed in such a way as to reduce the subjective assessment of odours to as close to an

objective measure as possible. The same selected panel has been successfully used to calibrate electronic nose systems for detection of contaminants in grain.

## Age-related trends in food preference and intake

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Data from our laboratory (Snyder *et al.*, 2001) show that high-fat and bitter preferences increase with age, while that for sweets declines with age for women but remains stable for men. Diet has a pronounced effect on disease risk; food preferences are believed to predict intake. To assess this, we compared preference data for 26 foods ( $n = 2330$ ) with 24 h food diaries from NHANES III and the USDA Continuing Survey of Food Intakes, as well as NHANES III food frequency data. Intake measures for each food were averaged by age (decade); foods were studied individually and by food group (sweet, salty, bitter, fat). Bitter foods showed the most stable agreement between preference and intake, which increased with age. Although fat preference increased with age, intake declined, perhaps due to concerns about health risk. For similar reasons, salt intake declined over time, although preference was unchanged. Generally, sweet foods were consumed more with age, perhaps as sensory compensation for diets containing less fat and/or salt. Analysis of individual foods revealed strong agreement among bitter foods, but marked variability among fats. In sum, food preferences loosely predict intake, and agreement is maximized when foods are studied individually. Since individual foods in the same nutritional group differ broadly in terms of sensation, hedonics and perceived healthfulness, nutritionally based food groups may be too blunt to capture the subtleties of specific food choices; thus, they may be impractical for studies of food preference and intake.

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## Taste and trigeminal inputs interact to produce phantom sensations: clinical implications for supertasters of PROP

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Several reports from our laboratory show that taste loss from the chorda tympani (VII), either from pathological damage or anesthesia, can lead to more intense pain and taste sensations in other parts of the mouth. Phantoms (sensations without stimulation) can also result. These findings suggest that inputs mediating oral sensation inhibit one another centrally, such that damage to one increases sensation from others; in particular, central interactions between inputs of VII and the glossopharyngeal and trigeminal nerves are demonstrated. Clinical disorders implicated by this 'release of inhibition' model include burning mouth syndrome (BMS), an oral pain phantom, and atypical odontalgia (AO), a tooth pain phantom. Patients with BMS and AO show a loss of bitter (and other) taste perception compared with controls, particularly on the anterior tongue.

Individuals with BMS and AO tend to be supertasters of 6-*n*-propylthiouracil (PROP); BMS pain intensity is correlated with the density of fungiform papillae. Based on the similar profiles of sensory change between research subjects and patients, both BMS and AO appear to be oral pain phantoms created centrally by the absence of robust taste input. Clinically, oral phantoms (including BMS) are reduced by the GABA agonist clonazepam, which may restore inhibitory control; we are exploring clinical options for patients with AO. In sum, taste loss produces phantoms, patients with phantoms exhibit taste loss, and supertasters appear to be most susceptible to both.

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### Parkinson's disease as possible cause of 'idiopathic' olfactory dysfunction

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Olfactory loss is an early sign of Parkinson's disease (PD). It is unclear whether and how far olfactory dysfunction precedes first clinical signs related to the motoric system. The present study aimed to investigate whether 'idiopathic' olfactory dysfunction might relate to the presence of Parkinson's disease. A total of 18 patients diagnosed with 'idiopathic' olfactory dysfunction participated in this investigation (12 men, 6 women; mean age = 59 years, age range = 39–73 years). Using transcranial sonography, nine patients (50%) exhibited signs of nigral degeneration. When submitting seven of these patients to SPECT imaging, reduced density of dopaminergic neurons was observed in three. These data indicate that, in many patients, 'idiopathic' olfactory dysfunction may be an early sign of PD. Consequently, in clinical practice, cases with 'idiopathic' olfactory loss should be seen by an experienced neurologist. Further, while more research is needed, the combined investigation of olfactory function and nigral degeneration might become a valuable tool in the early diagnosis of Parkinson's disease.

### 3-Phosphoinositides modulate cyclic nucleotide signaling in olfactory receptor neurons

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Phosphatidylinositol 3-kinase (PI3K)-dependent phosphoinositide signaling has been implicated in diverse cellular systems coupled to receptors for many different ligands, but the extent to which it functions in sensory transduction is yet to be determined. We now report that blocking PI3K activity increases odorant-evoked, cyclic nucleotide-dependent elevation of  $[Ca^{2+}]_i$  in acutely dissociated rat olfactory receptor neurons, and does so in an odorant-specific manner. These findings imply that 3-phosphoinositide signaling acts in vertebrate olfactory transduction to inhibit cyclic nucleo-

tide-dependent excitation of the cells, and that the interaction of the two signaling pathways is important in odorant coding, indicating that 3-phosphoinositide signaling can play a major role in sensory transduction.

### Spatio-temporal dynamics of odor representations in the mammalian olfactory bulb

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We explored the spatio-temporal dynamics of odor-evoked activity in the rat and mouse main olfactory bulb (MOB) using voltage-sensitive dye imaging (VSDI) with a new probe. The high temporal resolution of VSDI revealed odor-specific sequences of glomerular activation. Increasing odor concentrations reduced response latencies, increased response amplitudes and recruited new glomerular units. However, the sequence of glomerular activation was maintained. Furthermore, we found distributed MOB activity locked to the nasal respiration cycle. The spatial distribution of its amplitude and phase was heterogeneous and changed by sensory input in an odor-specific manner. Our data show that in the mammalian olfactory bulb, odor identity and concentration are represented by spatio-temporal patterns, rather than spatial patterns alone.

### Multisensory mimicry in the dead horse arum

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Some plants rely on deception for pollination. These plants typically mimic a favorable insect resource. Attracted insects are subsequently exploited as pollinators without being rewarded. Here we describe the multisensory targeting mimicry of the dead horse arum, *Helicodiceros muscivorus*, that enables it to efficiently dupe insects into performing unrewarded pollination. Linked gas chromatography–electroantennodetection recordings show that the involuntary pollinators, primarily female blowflies, by relying on odor cues, cannot separate the plant from a true resource. Odor collections from the plant and from rotten meat contain the same active oligosulphide components, eliciting identical olfactory responses from the blowfly olfactory organ, the antenna. The identified oligosulphides are very attractive to blowflies. Adding odor to odorless plants, which are not attractive to blowflies, restored their attractivity. In addition, oligosulphide baited traps also caught large numbers of blowflies, indicating that the odors are attractive by themselves. The arum is thermogenic; it is capable of generating a temperature of  $>10^{\circ}C$  above ambient. This temperature production in the arum is similar to that of a true carcass, which suggests that the thermal adaptations enforce the carrion mimicry. Furthermore, several visual and tactile adaptations also contribute to the mimicry. The dead horse arum is a

striking example of how during evolution a plant can acquire the ability to mimic multiple aspects of complex objects.

### Identification of novel natural odor ligands in *Drosophila*

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The fruitfly *Drosophila melanogaster* has become a very important model organism in olfactory research. Despite all the research invested, few natural odor ligands have been identified. By using a combined gas chromatographic—single receptor neuron recording technique we set out to identify active odorants in head space-collected volatiles from preferred food sources. In all, we performed 101 GC–SC experiments on 87 contacted sensilla. Using GC–mass spectrometry, we identified 29 active compounds. Synthetic samples of these compounds were used to establish dose response curves for several of the receptor neuron types encountered. In total, we identified eight distinct sensillum types based on response profiles of 12 olfactory receptor neuron types. In most recordings a single GC peak would produce a strong response, whereas a few other, often chemically related, compounds would produce weaker responses. Dose–response investigations also revealed a very low response threshold to the tested compounds in several of the receptor neuron types described. The *Drosophila* food odor detection system seems to be built of selective receptor units and work according to a labeled line principle. In addition, we have also examined the behavioral effect of some of the identified ligands. All except one compound elicited attractive behavior, as we expected, as most of the odorants tested are typical microbial volatiles, thus indicative of the favored food sources of *Drosophila*.

### Hyperpolarization-activated channels mediate responses to sour stimuli in a subset of taste cells in the rat vallate papilla

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The responses of taste cells in vallate papilla to sour stimuli applied at the taste pore were examined. Sour stimuli elicited inward current and enhanced an inward current which activated during hyperpolarizing steps ( $I_h$ ). Over 92% of cells exhibiting  $I_h$  responded to sour stimuli, while 22% of all taste cells responded. There was agreement between the effects of sour stimuli on  $I_h$  and the activation of inward current. In cells in which a large inward current was elicited, there was a reduction in the amount of  $I_h$  activated by hyperpolarization. This is consistent with an activation of  $I_h$  channels by the sour stimulus, resulting in fewer  $I_h$  channels available to open during hyperpolarizing pulses. Blockage of  $I_h$  by 1–2 mM Cs<sup>+</sup> resulted in a 52% reduction in the inward current elicited by sour stimuli.  $I_h$  was activated in a voltage- and time-dependent manner, and fit the Boltzmann equation ( $V_{1/2}$  near –100 mV). The rate of activation was voltage-dependent, with

faster activation at more negative membrane potentials. The estimated reversal potential of  $I_h$  was consistent with a mixed cationic current. In all aspects,  $I_h$  was similar to the  $I_h$  reported in neurons and cardio-myocytes. During sour stimuli, the  $I_h$  activation rate was accelerated and activation shifted to more depolarized membrane potentials, mimicking the effects of hyperpolarization. Thus protons may directly interact with the activation mechanism of  $I_h$ . The results indicate that protons can gate  $I_h$  and that  $I_h$  can function as a sour receptor in taste cells.

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### Characteristics of parosmic patients

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Disorders of the chemical senses are frequent. They can severely affect our lives. Appropriate testing to determine the nature of the patient's complaints is nowadays possible by standardized psychophysiological and electrophysiological testing. These methods allow the assessment of threshold, discrimination and identification, whereas parosmia is a condition without clinical signs. Forty-one patients suffering from parosmia from the Department of ENT, University of Vienna, Austria, were administered a psychophysiological test for odor identification, discrimination and threshold ('Sniffin' Sticks'), and a medical history questionnaire. Sixteen were retested after individual test–retest periods ranging from 1 to 12 months. Females are more often affected by parosmia than men (f = 23, m = 18; mean age = 55 years). Ten patients (24.4%) had anosmia, 19 (46.3%) had hyposmia and 34 (82.9%) had normosmia. The aetiology of the parosmia were mostly viral, followed by idiopathic and post-traumatic causes, but all causes (toxic, surgical) were identified. The presence of parosmia did not correlate with the aetiology nor with the degree of the olfactory disorder. The results of the retest showed an improvement of the parosmia regardless of the therapy and aetiology. Occasionally the values of the olfactory function were stable, but parosmia improved. Patients complaining of parosmia associated with olfactory dysfunction have a good chance of a decrease of their striking symptom in the time of recovery. A proper method to measure parosmia would be favourable to evaluate these patients.

### The larval olfactory system of *Drosophila*: a simple model system?

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There is emerging evidence that vertebrate and insect olfactory systems may be organized according to common principles, though differing in cell numbers. This is why *Drosophila*, with its 1300 odorant receptor neurons (ORNs), has become a focus in chemosensory research. Surprisingly, larvae include no more than 21 ORNs, suggesting an even simpler olfactory model system. But is this drastic reduction of ORNs accompanied by simplicity at

the central level? Studying the expression pattern of selected P[GAL4] lines at the confocal level suggests that the larval antennal lobe (LAL) is composed of subunits. Similar to adult glomeruli, these subunits seem to be recognized by the arborizations of afferent fibers and projection neurons. Hence, the central structures may be no less complex than in the adult. Also, larval and adult systems resemble each other regarding the distribution of neurotransmitters. In contrast, other aspects of the larval system differ significantly from the adult system. First, its peripheral organization is very distinct. Secondly, there are striking links between olfaction and gustation, as shown, for example, by an association of certain smell and taste sensilla. Remarkably, one of the LAL subunits seems to be a target of gustatory sensilla. Accordingly, larvae in which synaptic transmission in the corresponding afferents is blocked by shibirets transgene expression show defects in gustatory rather than olfactory behavior. Hence, this subunit is indeed part of the gustatory system. We hypothesize that for larvae, living on their food supply, distinction between smell and taste may not be very crucial.

### Olfaction in *Drosophila*: peripheral perception and central processing

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Insects sense much information about their environment by olfactory cues. Tremendous progress in the understanding of underlying molecular processes came from the identification of >60 putative olfactory receptors. We have characterized one olfactory receptor, the olfactory receptor Or43a, and confirmed that it indeed has an olfactory function. We misexpressed Or43a in almost all olfactory receptor neurons on the third antennal segment and identified benzaldehyde, cyclohexanol, cyclohexanone and benzyl alcohol as ligands for this receptor. In subsequent studies we want to identify the sensillae that express Or43a and the corresponding glomerulus in the antennal lobe that forms a connection with Or43a-expressing olfactory receptor neurons. There are three morphological different types of sensillae situated on the third antennal segment that bear 2–4 olfactory receptor neurons. We used the Gal4 enhancer trap technique to express the marker green fluorescence protein together with Or43a in olfactory receptor neurons and identified several sensillae at the distal end of the third antennal segment. This subset of sensillae expresses Or43a in the dendritic portion of receptor neuron in the sensillum shaft. The olfactory signals from the antenna are processed in the antennal lobe. The antennal lobes in invertebrates are divided into so-called glomeruli. All olfactory receptor neurons that express one identical type of receptor protein form a synaptic connection with one glomerulus. We were interested in the connectivity of olfactory receptor neurons expressing Or43a in the antennal lobe. Several studies demonstrated that the connection of olfactory receptor neurons with the first olfactory processing centers follows a determined pattern and seems to be part of the olfactory coding mechanism. We have now identified a single glomerulus that forms synaptic connections with all Or43a-expressing receptor neurons. We now have a system in our hands that allows us to manipulate olfactory processing in *Drosophila*.

### Correlation between subjective disturbance and olfactory disorders in olfactory impaired patients

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Complaints about olfactory dysfunction exhibit large inter-individual variation. So far little is known about the correlation between these complaints and the results of psychometric testing. The aim of the present study was to examine this phenomenon in a relatively large population of patients with olfactory loss (56 female, 47 male, mean age  $49 \pm 15$  years). Most frequent causes of the disorder were trauma (47%) and viral infections (18%); ~1/5 had idiopathic olfactory dysfunction (18%). The mean duration of disease was  $19.3 \pm 30.8$  months. Using a visual analogue scale ('complaint scale'), patients were asked to rate how much olfactory dysfunction affected their lives. Psychometric testing was performed using the 'Sniffin' Sticks' test battery. The degree of complaints differed significantly in relation to the degree of olfactory loss (anosmia versus hyposmia,  $P = 0.002$ ; anosmia versus normosmia,  $P = 0.005$ ). Moreover, a weak but significant correlation was observed between duration of disease and subjective impairment ( $r = -0.21$ ). There was no correlation between the cause of disease and the different tests of the 'Sniffin' Sticks' test battery. In conclusion, the present results indicate a correlation between complaints and the degree of olfactory dysfunction. Nevertheless, it appears that patients learn to cope with the loss of smell over time.

### Intrinsic organization of the mushroom bodies of *Drosophila melanogaster*

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Classical light and electron microscopical methods have been used to reveal a level of cellular complexity and precision in the *Drosophila* mushroom bodies that is comparable to organization in the mushroom bodies of honeybees and cockroaches. At least seven types of Kenyon cells have been identified, the dendrites of which in the calyces designate zones that are equivalent to the lip/collar and basal ring regions of the honeybee calyx. Axons from these calycal regions segregate into discrete laminae and tracts within the pedunculus. These peduncular subdivisions further sort out to form discrete divisions ('lobes') that together comprise the compound structures called the medial and vertical lobes. Previous studies describe three divisions medially and two vertically. The present studies reveal five divisions medially and four vertically, each of which is defined by its immunoaffinity to antisera raised against taurine, aspartate and glutamate. Glutamatergic axons represent the last Kenyon cells to be generated before eclosion. These comprise a core lobe posteriorly in the mushroom bodies, here termed the j and k lobes. Studies on mushroom body development by Kurusu *et al.* (2001) have shown that this lobe exists at each instar. It is therefore homologous to the glutamatergic downgrowth lobe in cockroaches and the basal ring division

of the lobes in the honeybee, all of which are generated last at each developmental stage and which show various degrees of transdifferentiation to aspartate immunoreactivity. Homologies have also been established between the g lobe of *Drosophila* and the g lobes of the honeybee and cockroach. Electron microscopical observations of the lobes shows that in *Drosophila*, as in the cockroach and honeybee, Kenyon cells are postsynaptic to afferents arriving at the lobes. Kenyon cell axons are also pre- and postsynaptic to each other and to efferent neurons. These findings suggest that systems of Kenyon cell axons comprise local circuits between afferents and efferents, thus reflecting the phylogenetic default organization of mushroom bodies in taxa that originally lacked antennal lobes and calyces. The present study demonstrates that the *Drosophila* mushroom bodies have elaborate internal structures comparable to those of larger species.

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### Brain targeting and glomerulus formation of olfactory sensory neurons

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Olfactory sensory neurons located in the nasal neuroepithelium send their axons directly into the olfactory bulb, where they contact the dendrites of second-order neurons in specialized structures called glomeruli; neurons expressing the same odorant receptor send their axons to a small number of common glomeruli. The glomeruli of neurons expressing a subtype from the highly homologous mOR37 receptor subfamily are grouped together within a small focal area of the bulb; nevertheless, each glomerulus receives input precisely from only one mOR37 population. Employing a transgenic approach, the targeting of mOR37 axons to their specific glomeruli during development was examined. The onset of expression for the different subtypes occurred simultaneously between embryonic day E11.25 and E11.5. From E13 onwards, axons of all mOR37 populations were detectable within the mesenchyme between the olfactory epithelium and the telencephalic vesicle. At E15, axons from different mOR37 populations entered the ventral region of the developing bulb, where they were diffusely arranged and intermingled to a high extent. Two days later, condensations of axons formed, at still largely overlapping positions. Even at the day of birth, the axons of different mOR37 populations were only marginally separated into distinct glomerular structures. The segregation process was largely completed at postnatal day 3 (pn3). From this time, projection of mOR37 neurons to distinct glomeruli was observed, with only individual fibers or fiber bundles targeting an 'inappropriate' glomerulus. These results demonstrate that targeting of neurons expressing the highly related mOR37 receptors is largely unfocused during development and the projection into distinct glomeruli is mainly achieved within a short postnatal period until pn3.

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### Impact of intensity and hedonic tone of environmental odours on degree of annoyance: results from field studies

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Regulation of environmental odours from industrial and agricultural activities in Germany is largely based on established associations between frequency of odour events (exposure) and annoyance responses in the population. We investigated if, in addition to well-known modifying factors, perceived odour strength (intensity) and hedonic tone (pleasantness/unpleasantness) must be considered in order to increase the validity of exposure–annoyance associations. Since 1998 a pertinent field study was conducted in the vicinity of six odour emitting sources preclassified in terms of hedonic tone of their immissions. A standardized sensory method was developed in order to quantify odour intensity and hedonic tone within the assessment of the degree of odour exposure by systematic field inspection using calibrated human observers as well as within the assessment of annoyance responses by means of direct interview. Altogether, data from 1409 subjects were used. Systematic odour–response relations were found. The degree of annoyance in residential areas with 'pleasant' odour immissions is much lower than in the vicinity of 'unpleasant' or 'neutral' odour sources. Inclusion of odour intensity does not increase the precision of annoyance prediction.

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### Is olfactory mental image sensory-like?

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Similarity judgements were made for 17 everyday odors on the basis of perceived odors, imaged odors and meaning of odor source names. Twenty-five students were instructed to smell the members of each possible pair of odors and to make similarity judgements on a 11-point scale. Another 20 students were asked to image odors from odor source pictures and to make similarity judgements. It seemed to be difficult to image odors properly, so they were then trained to form associations between perceived odors and their names. After the associative learning, they made similarity judgements of odor images again. A further 20 students made similarity judgements of 17 odor source names in terms of general word meanings. Non-metric MDS analysis was applied to each of the similarity data, and a three-dimensional solution was accepted. All spaces had a common dimension, which was labelled as 'edibility'. In the sensory space, three substantial groups, 'dimethyl-sulfide', 'fats oxide' and 'pyrazine', were found. Seventeen elements in the other spaces were superimposed onto the sensory space using a procrustes rotation. Then distances between the elements in the sensory space and the corresponding elements in different spaces were calculated. It was found that the average

distance between the sensory and the mental (either before or after learning) was smaller than that between the sensory and the semantic [ $F(2,32) = 11.25, P < 0.01$ ]. Olfactory mental space seemed to be similar to the sensory space. It was suggested that olfactory mental representation was sensory-like, especially after the associative learning between perceived odors and their names.

### Taste responsiveness to 6-*n*-propylthiouracil as a marker for fat intake and obesity: implications for chronic disease risk

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Individuals who are insensitive to the bitterness of 6-*n*-propylthiouracil (PROP) (i.e. non-tasters) are also less responsive to other bitter tastes, sweet taste, the oral burn of capsaicin and the mouthfeel of some fats. Earlier findings of an association between taste blindness to PROP and a higher preference for and intake of fats has prompted this laboratory and others to investigate possible interrelationships among PROP status, body weight and chronic disease risk. Our work has consistently shown that non-tasters maintain the highest body mass indices (kg/m<sup>2</sup>) as compared with taster groups. This difference begins to emerge in childhood and becomes progressively larger in middle adulthood. This talk will discuss the implications of these findings and other from the literature for understanding dietary-induced weight gain and susceptibility to chronic disease. It will also suggest future research directions that might help to elucidate the role of this phenotype in health and disease.

### ETOC: a short olfactory test culturally validated across Europe

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The aim of this study was to develop a reliable and quick olfactory test to evaluate efficiently the olfactory abilities of the European population. The ETOC (European Test of Olfactory Capabilities) was designed in order to be a cross-cultural tool. It is based on two indices relevant to the olfactory performance, provided by a supra-threshold detection task and an identification task. More than 1000 healthy subjects in five European countries performed the test. The results indicated that scores to the ETOC (i) are not culture-dependent; (ii) reflect well the decrease in olfactory abilities with increasing age; and (iii) are higher for women than for men. A test-retest study showed that the ETOC is also highly reliable. As first results of application of the ETOC, we present data on the relation between olfactory losses and food habits.

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### Nasal function and chemoreceptive symptoms during controlled exposure to 2-ethylhexanol

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Occupational solvent exposures can cause sensations of strong odors and sensory irritations. For the process of chemical exposure regulation, sensory irritations are critical endpoints. The present study aims at such effects during controlled 2-ethylhexanol exposures. In an exposure laboratory (28 m<sup>3</sup>) 24 male volunteers were exposed to 1.5, 10 and 20 p.p.m. of 2-ethylhexanol (German TLV: 50 p.p.m.) for 4 h. Half of the subjects were selected because of heightened chemical odor sensitivity. Rhinomanometry was applied before and after the exposures. Symptoms aiming on odor intensity, nasal and eye irritations were rated on 6-point rating scales before, during and after the exposures. When compared with the pre-exposure values, exposure to 1.5 p.p.m. reduced the nasal airflow by ~2.5%, 10 p.p.m. ~7.5% and 20 p.p.m. ~12.2%. Only the decrease after exposure to 20 p.p.m. was significant ( $F = 9.72; P < 0.01$ ). Simultaneously, the symptom scores showed a significant dose-dependent increase. Symptoms of odor intensity rose by ~33% at 1.5 p.p.m., 87% at 10 p.p.m. and 107% at 20 p.p.m. The respective values for aforementioned concentrations were 22, 49 and 78% for nasal irritations, and 31, 62 and 89% for eye irritations. Both symptoms and flow-values were not affected by chemical sensitivity. However, a correlation between decrease of flow and increase of nasal irritations was only observable among sensitive subjects. The results strongly suggest that below the German TLV 2-ethylhexanol causes sensory irritations.

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### Sensitivity of the trigeminal sensory system to chemical stimulation with CO<sub>2</sub>

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CO<sub>2</sub> is frequently used in experimental sensory models because of its specific trigeminal stimulation properties. Peripheral trigeminal activation can be determined by recording an electrical summated response, the negative mucosal potential (NMP), from the nasal mucosa. The aims of the current study were (i) to investigate the sensitivity of the NMP to small increments of CO<sub>2</sub> concentrations (3% v/v CO<sub>2</sub>) and (ii) to characterize the sensory input of CO<sub>2</sub> by determining NMP, detection and pain thresholds. In the first experimental sessions we investigated NMP responses to stimuli of 62, 65 and 68% v/v CO<sub>2</sub> (stimulus duration = 1000 ms,  $n = 10$ ). A dose-dependent increase of the NMP amplitudes and areas under the curve (AUCs) was observed. In the second experimental sessions we determined the thresholds for NMP, detection and pain (stimulus duration = 1000 ms). MANOVA analysis revealed significantly different thresholds for detection, NMP and subjective pain judgements (detection =  $20.6 \pm 9.6$ , NMP =  $42.6 \pm 12.5$ , pain

= 50.4 ± 12.0% v/v CO<sub>2</sub>). We could demonstrate the existence of a prepain range with activation of trigeminal nociceptive sensors resulting in the generation of NMPs. The detection threshold of 20.6% v/v CO<sub>2</sub> was surprisingly low, i.e. 22% v/v CO<sub>2</sub> below the NMP threshold. Our recording in rats revealed that stimulation with 20% v/v CO<sub>2</sub> elicits single A-delta-fiber responses in most but not all trigeminal primary afferents. The low detection threshold of CO<sub>2</sub> in man could be explained by the high sensitivity of trigeminal primary afferents with or without involvement of newly discovered  $\alpha$ -gustducin positive trigeminal chemosensory cells.

### Responses of oral mucosal nociceptors to bitter solutions in rats

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It has been suggested that intraoral nociceptors are involved in production of bitter sensation in addition to gustatory receptors. However, there are no reports about the effects of bitter solutions on the activities of oral mucosal nociceptors. The present study was undertaken to investigate the bitter responses of polymodal nociceptors in rats. The experiments were conducted in an *in vitro* jaw-nerve preparation. Single unit responses of 21 Adelta and 28 C polymodal nociceptors to bitter stimuli were recorded from the lingual nerve innervating the gingival mucosa. For identifying the subtypes of the nociceptor, von Frey, heat or bradykinin stimulations were used. After identifying nociceptor subtype, bitter solutions were applied to the surface of the mucosa. Mucosal polymodal nociceptors were well excited by caffeine (10<sup>-2</sup> M) and/or quinine (10<sup>-6</sup> M). The Adelta polymodal nociceptors were more sensitive to bitter solutions than the C polymodal ones. In Adelta polymodal units, about half of the units responded to both caffeine and quinine. By contrast, in C polymodal units, both-sensitive units were ~30% of the observed units. In conclusions, the present results show that bitter stimuli can excite polymodal nociceptors, suggesting that these nociceptors play a significant role in producing bitter sensation. Furthermore, oral mucosal nociceptors may participate in the rejection of various noxious foods containing bitter components.

### Hypothyreosis: influence on sense of smell before and after therapy

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Smelling disorders are reported to occur in various endocrine diseases (e.g. diabetes, hyperparathyroidism). We investigate 50 patients (40 females, aged 17–86 years, mean 47 years) with certified (T3: ng/ml, T4: ng/ml, TSH: 16 µg/ml) hypothyroidism. Patients are tested before and after beginning (16–56 weeks, mean 40 weeks) with levothyroxin therapy. Besides general data, subjective judgement of the patients sense of smell is also reported. The psychophysiological testing (threshold, discrimination, identi-

fication) is performed using 'Sniffin' Sticks'. Only 5 of 50 patients (10%) reported subjective complaints with olfaction. The initial psychophysiological testing showed just 15 of the 50 patients (30%) normosmic, 33 (66%) show hyposmia and two patients (4%) are anosmic. The results psychophysiological testing gave a significant correlation ( $P < 0.05$ ) with the findings of laboratory markers (T3, T4). TSH did not correlate significantly with SDI scores. Only discrimination and identification are causative for these results; it seems that there is no connection between T3 values and threshold. To date, 12 patients have been retested after beginning levothyroxin therapy, and no significant changes in ability of olfaction are detectable. The results of this study show that the sense of smell is affected by hypothyroidism. In the recent literature it has been shown that thyroid hormones are needed for proliferation and expansion of olfactory epithelium in rats as well as for maturation of olfactory receptor neurons (ORNs). In the human nose the life-span of ORNs is just a few weeks, and the lack of hormone could explain the impairment of olfaction. On the other hand, a decrease of discrimination and identification shows an influence on central nervous parts of the sense of smell. After 40 weeks of substituting thyroid hormones no significant changes in olfaction are detectable. These results are in contrast to the findings of McConell.

### Discrete depolarizations in hamster vomeronasal neurons in response to aphrodisin

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Cell-attached patch-clamp recordings were performed from well-differentiated neurons isolated from male hamster vomeronasal sensory epithelium. The analysis of the current flowing through the channels in the patched membrane, with a pipette filled with an intracellular-like solution, indicated a stable resting potential around -60 mV. Most neurons were silent or presented a very low spontaneous impulse activity. Spontaneous action potentials were systematically associated with discrete depolarizations, usually lasting <2 s and separated by variable long quiescent periods. In some neurons, particularly those without spontaneous activity, spiking responses were evidenced during stimulation with diluted hamster vaginal secretions or recombinant aphrodisin. Spiking bursts appeared on the top of transient depolarizations. Whole-cell recordings confirmed that neurons had a resting membrane potential more negative than -60 mV. In spontaneously active cells, spiking activity corresponded to transient short-lasting depolarizations to the firing threshold around -40 mV. These depolarizations resulted from transient inward currents of a few picoamperes amplified by the high cell input resistance. Injection of long-lasting depolarizing currents through the recording electrode revealed the inability of these neurons to fire action potentials repetitively for more than a few seconds. The results suggest that continuous signalling of sustained presence of pheromones in the vomeronasal lumen is made by transient and repetitive depolarizations rather than long lasting voltage changes.

## Olfactory identification and detection tests in the prediction of odor and flavor intensity ratings in the elderly and young

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Two recently developed olfactory tests, SOIT (Nordin *et al.*, 1998, *Acta Otolaryngol.*, 118: 226) and TOE (Thomas-Danguin *et al.*, 2001, *Chem. Senses*, 26: 1062), were examined for their ability to predict individual odor and flavor intensity ratings of vanilla (0–0.31% w/v) in aqueous solutions and in a liquid food (whey drink) among the elderly ( $n = 28$ , age range 66–83 years) and young ( $n = 33$ , age range 21–36 years). Both tests consist of 16 common odors to be detected (TOE) and identified (TOE, SOIT) using four-alternative stimulus sets (detection) or verbal labels (identification). For the elderly, the mean scores (range) were as follows: SOIT, 11.0 (5–16); TOE detection, 14.6 (7–16); and TOE identification, 10.6 (2–15). For the young, these scores were: SOIT, 14.1 (11–16); TOE detection, 15.8 (14–16); and TOE identification, 14.1 (11–16). Low odor and flavor intensity ratings of vanilla in aqueous solutions and in whey drink were predicted by low (<11) SOIT scores, whereas higher SOIT scores were unrelated to intensity ratings. Also, low odor and flavor intensity ratings were predicted by imperfect (<16) TOE detection scores. TOE identification scores were less predictive of flavor ratings than the other two scores, although all three measures were well correlated. Overall, SOIT and TOE predicted perceived intensities when the olfactory performance was markedly impaired. In this study, such performance was observed in the elderly subjects.

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## Interpapillar functional interactions: a single unit study in the hamster

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Fungiform papillae are innervated by branched chorda tympani fibers (Beidler, 1969). Miller (1971) found a modulation of chorda tympani single fiber responses when stimulating a single papilla with or without the neighbouring ones. Simultaneous recording and stimulation of single fungiform papillae were performed in order to further investigate these complex interactions. Unitary nerve fibers were recorded in the taste pore, using glass micro-electrodes as described earlier (Faurion and Courchay, 1989). A new technique allowed iontophoretic stimulation of two nearby papillae using 20–30  $\mu\text{m}$  glass micropipettes filled with NaCl, acesulfam K or monosodium glutamate. A constant current generator delivered positive or negative shocks in order to apply either the cation or the anion to the taste pore. Sixty-three papillae were recorded, while 149 were stimulated. Eighteen out of 111 stimulations with  $\text{Na}^+$ , 2/34 with  $\text{K}^+$  and 4/72 with L-glutamate showed an increase of the frequency of unitary firing during the shock, eventually lasting after the shock. Eight out of 72 stimulations with L-glutamate showed inhibition. In 6 out of 30 tests, the simultaneous stimulation of both papillae (double shock) showed a modulation of the response obtained with the stimulation of only

one papilla (single shock). Further work is in progress to check whether interpapillar interactions involve peripheral circuits or central convergence and whether trigeminal fibers in the taste bud could be involved.

## The sensitivity to puberty modulating chemosignals changes during postnatal ontogeny in Campbell's hamsters, *Phodopus campbelli*

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The role of the secretions (SS) released by the supplementary succuli located at the opening of the cheek pouches in adult Campbell's hamsters (*Phodopus campbelli*) was studied. These secretions are suspected to be involved in the regulation of the sexual maturation. In experiment 1 pups were treated with male secretions (M-SS) from postnatal day D11 to D31. The results indicate that M-SS accelerate the development of young females and suppresses the maturation of males. In experiment 2 female secretions (F-SS) were applied from postnatal day D11 to D25 (group I), from D11 to D31 (group II) and from D25 to D45 (group III). The results reveal that F-SS suppress the sexual maturation of females; the effect was most pronounced in group II. The effect of F-SS on males differed between three groups: sexual maturation was delayed in group I; there was no effect in group II; sexual maturation was accelerated in group III. Our data indicate that the sacculi secretions of adult hamsters influence the sexual maturation of juveniles; the character of such an influence may depend on the duration of the treatment and on the age of the developing animal.

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## Encoding by neurons in the primate orbitofrontal cortex of taste, texture and temperature

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The primate orbitofrontal cortex contains the secondary and tertiary taste and olfactory cortices, and also receives somatosensory inputs (Rolls, 1997, *Crit. Rev. Neurobiol.*, 11: 263–287; Rolls *et al.*, 1999, *J. Neurosci.*, 19: 1532–1540). In recordings in awake rhesus macaques we now report that some single neurons in the OFC have firing rates that depend on the viscosity of aqueous methyl cellulose in the range 100–104 cPoise. Of these neurons, 64% were tuned to have non-linear response functions within this viscosity range, while 21% had increasing and 14% had decreasing response functions. Of these oral texture-sensitive neurons, 67% responded to taste stimuli. Of taste-responsive neurons, 24% also responded to viscosity. A population of OFC neurons also responds differentially to the temperature of oral stimuli (water at 0, 25, 37 and 42°C), with 50% of neurons having linearly increasing or decreasing response functions. Of the taste neurons, 44% also responded to temperature. Of the temperature responsive neurons,

69% also responded to taste and 38% to viscosity. Thus neurons that show convergence between taste, texture and temperature are found in the orbitofrontal cortex, yet both are also represented independently, both by single neurons and as shown by a multidimensional scaling analysis of the stimulus space encoded by this neuronal population. Such representations may be important in coding for all the sensory properties of a food in the mouth that influence its flavor (Rolls *et al.*, 1999).

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## Robot olfaction

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Olfactory processing is closely linked to the active exploration of the chemical environment. Hence the behaviors that depend on the results of olfactory processing in turn affect the sampling of the environment. We explore the relationship between neuronal sensory processing, decision making and behavior using a synthetic approach where neuronal models are interfaced to real-world devices, i.e. robots. In this presentation the basic motivations behind this approach will be presented. As a particular example, our work on the construction of an artificial moth will be discussed.

## Testing olfactory performances in four European countries: influence of culture

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In the context of a large European study designed to evaluate the effect of culture on scores to olfactory tests ('Sniffin' Sticks', SOIT, OCT) we previously reported (Thomas-Danguin *et al.*, 2001) differences in global scores between countries when an identification task was considered and no differences on thresholds or global discrimination scores. The data presented here focus on cultural differences related to identification and discrimination efficiency for each odour used in these tests. The three tests above quoted were thus administered to 121 subjects (55–79 years old) in four European countries. The results indicated that some odours used in the identification tasks are more transcultural than others. As far as discrimination is concerned, we observed an influence of the country on the discrimination of some couples of odours. Thus, the discrimination task, although it is a non-verbal task, appears to be dependent on previous experience. In that sense, the role of verbal encoding is discussed.

## Induced olfactory sensitivity: central mechanisms are involved

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Unlike many other sensory systems, nasal chemoreception appears to be dynamic throughout the lifetime of an organism. Individual experiences with odors often interact with genetic propensities to yield a changing phenotype. In humans, there is a well-documented specific anosmia to androstenone (AND). Although there is a genetic basis for the perception of androstenone, this phenotype is dynamic. Inbred strains of mice that provide a model for specific anosmia to AND are available in the forms of NZB/B1NJ (NZB; insensitive to AND) and CBA/J (CBA; sensitive to AND). We estimated behavioral sensitivity of NZB and CBA mice to AND. NZB mice could detect 0.1% (w/v) AND in mineral oil, but not 0.05% AND. CBA mice could detect AND at a concentration of 2000-fold. After 2 weeks of exposure (16 h/day) all mice increased sensitivity to AND regardless of their initial level of sensitivity. After 1 week more, sensitivity of NZB mice was 64- to 128-fold lower than original estimates and those for CBA mice were 200- to 400-fold lower. We found significant and systematic increases in sensitivity to AND in animals exposed to the compound for 1, 2 or 3 weeks: mice with longer exposure became more sensitive. At the receptor level, additional exposures to AND after 1 week did not cause any significant changes, indicating possible involvement of central mechanisms in the sensitization process. We recorded fos-immunoreactivity in main olfactory bulb (MOB) and accessory olfactory bulb (AOB) in response to stimulation with AND in CBA mice with different level of AND sensitization. Induced AND sensitivity is correlated with elevated fos-immunoreactivity distributed differentially in MOB and AOB. Our results indicate CNS involvement in induction of sensitivity to AND.

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## Representation and processing of odorant information at the level of receptor neuron input to the olfactory bulb

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In the olfactory bulb, information about an olfactory stimulus is represented by spatially organized patterns of activity across olfactory glomeruli. To visualize how odorants are represented at the level of input to glomeruli, we have loaded mouse olfactory receptor neurons with calcium-sensitive dye and imaged odorant-evoked signals from their axon terminals *in vivo*. This approach has allowed us to monitor activation of receptor neuron populations converging onto individual glomeruli, and reveals a high level of complexity in odorant representations at this initial stage. First, we find that representations of even simple, monomolecular odorants tend to involve input to many, widely distributed glomeruli. Secondly, we find that individual glomeruli, some of which can be identified across animals, show stereotyped but complex response specificities. Finally, we observe complex temporal dynamics of receptor neuron input, with receptor neuron

activation showing glomerulus- and odorant-specific kinetics. Using this same imaging approach in an olfactory bulb slice preparation, we have also shown that calcium influx into receptor axon terminals can be suppressed via a feedback pathway involving olfactory bulb interneurons. This suppression is mediated by GABAB and D2 dopamine receptors, and serves as a means to presynaptically regulate receptor neuron input to the olfactory bulb. Thus, the complex odorant representations carried by olfactory receptor neurons are subject to inhibitory processing prior to their input to the CNS.

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## Relation of PROP tasting to depression and anxiety

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The present study investigated the hypothesis that people with depression or generalized anxiety disorder (GAD) or whose parents have either disorder are more likely to be supertasters of 6-*n*-propylthiouracil (PROP) than the general population. Two hundred college students were interviewed using the Structured Clinical Interview for DSM-IV (SCID) for major depression, generalized anxiety disorder (GAD), alcohol abuse and substance abuse. To assess familial symptomatology, participants were given the Family History-Research Diagnostic Criteria Interview (FH-RDC) for depression, anxiety, alcoholism and substance abuse in their mother and father. All participants took a PROP taste test, using filter paper saturated with PROP and indicating their judgement on the Labeled Magnitude Scale (LMS). They were classified as nontasters, medium tasters and supertasters based on their ratings on the LMS. In a group of participants with no diagnoses in themselves or their parents (control,  $n = 20$ ), the distribution of tasting was 25% nontasters, 50% medium tasters and 25% supertasters, matching the expected population distribution. The distributions of tasting in participants with depression ( $n = 118$ ), GAD ( $n = 63$ ), or mixed depression and GAD ( $n = 48$ ) were significantly different from controls: each had significantly more supertasters. Additional analyses that divided participants according to parental history showed that subjects with parents who were depressed ( $n = 97$ ), anxious ( $n = 47$ ) or both ( $n = 23$ ) were also significantly more likely to be supertasters. In contrast to previous results from our laboratory, results for participants with alcoholism in themselves or their parents were inconclusive. The results support other findings indicating a genetic link between depression and GAD, and suggest that the gene for PROP tasting may be located near a gene for depression and GAD.

## Different effects of phenyl ethyl alcohol (PEA), H<sub>2</sub>S and CO<sub>2</sub> on face encoding and recognition

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Twenty healthy young participants had to classify visually presented faces with regard to whether they liked or disliked them, while some of the faces were simultaneously associated with either phenyl ethyl alcohol (PEA), H<sub>2</sub>S or CO<sub>2</sub>. During this decision task magnetic field recordings were made using a 148-channel whole-cortex magnetoencephalograph (MEG). Olfactory stimulation was

administered with a Kobal olfactometer. Subsequently, all previous faces were shown again together with the same number of new faces, and the task this time was to discriminate between the repeated and the new faces. Face recognition performance has been found to vary as a function of the simultaneously associated stimulus during prior face encoding. Compared with the control condition, PEA led to an increased recognition performance whereas CO<sub>2</sub> led to a decreased recognition performance. H<sub>2</sub>S also led to an increased recognition performance but was not significantly different from the control condition. Neurophysiological activity related to face encoding and associated stimulus processing was also found to differ in relation to the associated stimulus. Two temporally distinctive activity components are described as to represent neurophysiological correlates of associated stimulus influences on face encoding manipulating face recognition performance.

## Sensory specific satiety caused by high and low fat, sweet and savoury snacks in obese and normal weight women

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Sensory specific satiety was measured in obese (mean BMI 33.1) and normal weight women (mean BMI 22.8), who were matched for restrained eating behaviour, physical activity, age and smoking behaviour. Four different types of snacks were served for lunch, differing in fat content and sweet versus savoury character: low fat sweet (pears in syrup), high fat sweet (chocolate candy bar), low fat savoury (chicken fillet pieces) and high fat savoury (cheese biscuits). Before and after the lunch subjects were requested to score satiety-related feelings, such as appetite for meals, appetite for snacks and hunger, and to score the liking for each of the four different snacks. Sensory specific satiety was defined as the difference between the decrease in liking for the product eaten in comparison with the decrease in liking for the three products that were not eaten. Sensory specific satiety was observed for all products, in both subject groups. Taste (sweet, savoury) had a stronger sensory specific satiety effect than fat level. No differences were observed between the obese and non-obese subjects, in either sensory specific satiety or food intake, for any of the products or product categories tested. Appetite for a meal or snack after lunch was significantly higher in obese than in non-obese subjects. In contrast to an earlier study by ourselves on sandwiches, sensory specific satiety for high fat snacks was not higher than for low fat snacks, suggesting that factors other than fat content predominate in determining sensory specific satiety.

## Centrifugal projections to the olfactory bulb in adult ferrets

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The olfactory bulb (OB) processes afferent information from the olfactory epithelium but is modulated itself by higher brain

centers. In the ferret (*Mustela putorius*), which depends mainly on olfactory cues, food imprinting phenomena take place in the olfactory bulb. Therefore, we were interested in the origin of centrifugal projections to the bulb. To reveal primary afferent central projections we injected the retrograde tracer horseradish peroxidase (HRP) into the main olfactory bulb of one side. Labeled neurons were found bilaterally in the following structures: nucleus olfactorius anterior (all parts), cortex praepiriformis, cortex frontalis, tuberculum olfactorium, nucleus amygdalae anterior, nucleus tractus olfactorius lateralis and nucleus coeruleus. Only ipsilateral labeled structures were: gyrus praesylvius, nucleus horizontalis lemnisci diagonalis, area periamygdaloidea, nucleus amygdalae pars centralis and corticalis, cortex piriformis, both diencephalic parts (thalamus and hypothalamus) and brainstem regions (nucleus raphae pontis and dorsalis, and close to decussatio brachii conjunctivi). Additionally, on the contralateral side, labeled cells appeared in the OB. Many brain regions that project primary centrifugal to the olfactory bulb also receive a primary centripetal projection from the bulb. They belong to neuronal networks functionally involved in respiration, food intake, the hormonal status of social and sexual behavior, the emotional status and memory. The contralateral labeled cells in the OB suggest a bulbar crosstalk, so far not described in other species.

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### Effect of local anesthesia on chemosensory event-related potentials

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Olfactory and trigeminal inputs both contribute to our perception of odorous substances. Our aim was to examine the effect of local anesthesia in different parts of the nose on chemosensory event-related potentials (CSERPs) and to study the interaction of the two systems. Twenty healthy volunteers with a normal olfactory function were anaesthetized in three test sessions on one side of the nose; Tetracain 1%, 2 ml was applied with a swab either to the middle or the upper part of the nose, or both parts. Moreover, in 10 volunteers, Xylocain 4% was applied directly with a syringe into the olfactory cleft in a head down position. CSERPs were elicited by stimulation with phenyl ethyl alcohol (PEA), H<sub>2</sub>S and CO<sub>2</sub> stimuli before and after anesthesia with Tetracain. Potentials elicited from the anaesthetized and not anaesthetized side were compared statistically (general linear model). After anesthesia with a swab all volunteers still were able to smell and CSERPs could be recorded, in contrast to complete anosmia after Xylocain in the olfactory cleft. Comparing the anaesthetized and non-anaesthetized sides, no statistically significant effect was seen in the amplitudes; in cases of anesthesia in the middle part of the nose, most of the latencies showed significant prolongation (Pz, latency P2 PEA:  $P = 0.008$ , H<sub>2</sub>S:  $P = 0.016$ , CO<sub>2</sub>:  $P = 0.045$ ), while anesthesia in the upper part as well as in both parts did not show significant effects. Anesthesia of olfactory fibres is difficult to achieve and the routinely used anesthetic only reduces sensibility, not olfactory perception.

### Arachidonic acid plays a role in rat vomeronasal signal transduction

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Sensory neurons of the vomeronasal organ (VNO) detect volatile chemicals that are released by conspecific animals and convey information for social and reproductive behavior. The signal transduction pathway in the vomeronasal receptor neurons (VRNs) is not known, but is distinct from that of the sensory neurons of the main olfactory system. Using Ca<sup>2+</sup> imaging and electrophysiological recordings, we investigated the signal transduction pathway of urine perception in freshly dissociated rat VNO neurons. We found that application of urine induced a transient increase in intracellular Ca<sup>2+</sup> that was dependent on the activity of phospholipase C and diacylglycerol lipase. The Ca<sup>2+</sup> transient was not dependent on depletion of intracellular Ca<sup>2+</sup> stores but on the presence of extracellular Ca<sup>2+</sup>. The urine response was not sensitive to modulators of adenylyl cyclase and inhibitors of inositol 1,4,5-trisphosphate receptors. Application of polyunsaturated fatty acids (linolenic acid and arachidonic acid, synthesized in living cells from diacylglycerol) induced Ca<sup>2+</sup> transients in FURA2 measurements and inward currents in whole-cell voltage-clamp recordings. Pharmacological inhibition of lipoxygenase and cyclooxygenase induced a transient increase in intracellular Ca<sup>2+</sup>, possibly by increasing the endogenous level of polyunsaturated fatty acids, leading to activation of transduction channels. These data provide some new insight into the signal transduction pathway of rat VRNs.

### Learned associations with smells influence taste naming speed

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Do implicit associations between odorants and tastants that have been formed through experience cause olfactory input to ready the cognitive system to expect a particular type of taste? Previous experiments (White and Prescott, 2001) with a chemosensory Stroop-like reaction time task indicated that the larger the conflict between odor/taste pairs, the slower one is to name the tastant. The present experiment attempted to implicitly form associations between smells and tastes, then assess whether those associations would facilitate or inhibit reaction times to name tastes in the presence of conflicting or matching odors. Subjects first rated the strength, sourness and sweetness of two odorants, then participated in a mock triangle test to provide the subject with extensive experience of a particular odor-taste combination. Subjects repeated the same odorant ratings, and then judged the identity of a tastant presented in the presence of odors that were either conflicted or associated. A third set of ratings followed the reaction time task. Shifts in ratings following the triangle test showed that implicit odor-taste associations were formed. Reaction time measures indicated an effect of these learned associations on the speed of naming tastes, with congruent odors facilitating taste identification. The results of these experiments emphasize

olfaction's significant contribution to the human perception of flavor, and provide support for an exceptionally strong interaction between cognitive processes associated with smell and taste.

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### Expression of neurotrophins and their receptors in lingual taste bud fields of hamster after sensory denervation

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Unlike taste buds in most mammals, fungiform buds on anterior tongue of hamster survive sensory denervation. Neurotrophins and their tyrosine kinase (Trk) receptors may play a role in the survival of denervated buds. In unilaterally denervated mature hamsters, we compared the degree of gemmal cell immunoreactivity (IR) (i.e. number of immunoreactive cells/bud profile) and density of nerve fiber-IR for neurotrophins BDNF and NT-3, and their respective receptors, TrkB and TrkC, in the fungiform bud field after chorda tympani/lingual nerve resection, to that in nerve-dependent, posterior tongue foliate and circumvallate bud fields after glossopharyngeal nerve resection. Four weeks post-lesion, the number of denervated fungiform buds matched that on the unoperated side, whereas denervated foliate and circumvallate bud counts respectively decreased by 72 and 38%. In taste buds that survived on the posterior tongue, the degree of foliate bud cell BDNF-, NT-3- and TrkB-IR, and circumvallate bud cell BDNF- and NT-3-IR, significantly decreased as compared with the unoperated side. In contrast, for anterior tongue fungiform bud cells, the degree of neurotrophin and receptor expression was relatively less affected: NT-3- and TrkB-IR were unchanged; BDNF-IR, though significantly decreased, was also maintained. Moreover, TrkB fiber-IR was eliminated within and surrounding fungiform buds. Hence, expression of NT-3, BDNF and TrkB in fungiform gemmal cells may reflect an autocrine capacity promoting survival. Since TrkC-IR in bud cells is absent (i.e. immunonegative), and sparse in fibers intragemmally and perigemally, NT-3 may also bind to bud cell TrkB so as to sustain fungiform gemmal cell viability, postdenervation.

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### Identification and characterization of arrestins in taste cells: possible role in taste signal transduction

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Taste sensations are elicited by a variety of structurally diverse compounds triggering rapid and transient changes in the levels of second messengers (cNMPs, IP<sub>3</sub>). According to other signaling cascades, the turn-off reaction for taste transduction is supposed to be mediated by feedback reactions, leading to a phosphorylation of the receptor and a subsequent binding of a 'capping protein', an arrestin. As a first step to evaluate whether arrestin may in fact be involved in terminating the gustatory signaling process,

RACE-PCRs were performed to identify which arrestins may be expressed in taste cells. From rat papillae a fragment encoding a protein corresponding to the C-terminus of  $\beta$ -arrestin2 was amplified. In Northern blot analysis the length of the hybridizing RNA was ~1.6 kb, which differs from the mRNA of  $\beta$ -arrestin2 (2.4 kb). Furthermore, in Western blot analyses using antibodies generated against the C-terminus of  $\beta$ -arrestin2 in taste fractions a major protein band was recognized that migrates with a molecular weight of 43 kDa and therefore deviates from the expected size of  $\beta$ -arrestin2 (47 kDa). Immunohistochemistry revealed that the immunoreactive proteins are localized in the cytosol and in the apical taste pore; furthermore, colocalization with the effector enzyme PLC $\beta$ 2 has been demonstrated in taste cells. These results indicate that a novel arrestin-like protein is expressed in taste cells which may participate in regulating gustatory transduction processes.

### The effect of time after kidney transplantation on olfactory and gustatory function

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Very few data are available about the effect of a kidney transplantation on odour perception. Since odour perception has a known influence on nutrition (lack of appetite, reduced eating), we examined the dependence of the period of time after kidney transplantation for the improvement of the olfactory capacity. In this pilot study we have subdivided the patients into two groups: group A (the period of time after kidney transplantation was <3 months;  $n = 8$ ) and group B (the period of time after kidney transplantation was >3 months;  $n = 8$ ). Psychophysical testing was performed by means of the 'Sniffin' Sticks'. Odour thresholds for *n*-butanol, odour discrimination and odour identification were assessed. In addition, taste detection and recognition thresholds were obtained using the 'three drops' method. Statistical analysis was carried out with Sigmaplot 5.0. The patients of both groups had olfactory loss (TDI score < 31). Odour thresholds and odour discrimination showed no significant difference between the two groups. However, odour identification and the TDI score, which is derived from the sum of the results obtained for threshold, discrimination and identification measures, improved significantly after kidney transplantation ( $P = 0.028$ ). In total, 87.5% of the patients in group A but only 37.5% of those in group B had a TDI score below the tenth percentile of the normal distribution. The gustatory function also correlated positively to the duration after kidney transplantation. All taste qualities were recognized better (salty,  $P = 0.001$ ; sweet,  $P = 0.0005$ ; sour,  $P = 0.006$ ; bitter,  $P = 0.0001$ ). A correlation between the chemosensory test results and the blood parameter levels could not be determined since the blood parameter levels were not significantly different. Kidney transplantation has a positive influence on both olfactory and gustatory function. Due to the still low number of patients, we could not give an exact time course. This should, however, be possible with future data.

## Effects of flavorants on oral texture sensations

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Oral texture sensations elicited during the consumption of foods are typically defined as a combination of physical food properties and perceived by the senses of touch, sight and hearing. Effects of flavorants remain unexplored, although certain texture sensations such as perceived creaminess seem to be composed of several other sensations, including flavor-related ones. Effects of flavorants on texture sensations were explored for vanilla custard desserts in a series of studies. Texture sensations for these desserts were primarily related to either fat content or viscosity. The first study, where diacetyl (fatty flavor), caffeine (bitter chemical), benzaldehyde (almond flavor) and vanillin were added to custard desserts, demonstrated that the first three flavorants affected fat-related sensations (rough, dry-mealy, fatty and creamy) but not viscosity-related ones (e.g. no effect on thickness or melting). These flavorants also elicited increased prickling sensations, which suggested trigeminal activation. Added vanillin did not affect any texture sensation. The second study demonstrated that the flavorant effect disappeared when nose-clips were applied, i.e. the effect seemed to be primarily mediated by nasal olfaction and/or irritation. The third study demonstrated significant concentration effects of added diacetyl and vanillin on creamy and prickling sensations. Melting sensations were also affected in this study. Finally, physico-chemical measurements indicated that added flavorants may affect the structure of the desserts, either directly via physico-chemical interactions with starch or indirectly via a change in viscosity of saliva. These effects, however, are unlikely to fully explain the sensory flavor–texture interactions.

## Influence of flavors on the perception of video clips

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There is considerable interest in the influence of odors on cognitive and emotional processes in everyday life. In a pilot study there was evidence that 3-methyl-2-hexenoic acid (3 MHA) modified well-being and cognition when presented in consecutive sessions. Our aim for this study was to investigate this effect including a pleasant odor, phenyl ethyl alcohol, a pleasant perfume composition, described as jasmine-like, and indole, a potentially unpleasant odor, on well-being and perception of video clips in a pleasant experimental environment. Fifty healthy volunteers with normal olfactory acuity (tested with Sniffin'Sticks<sup>®</sup>, TDI score) participated in two experimental sessions. In each session we presented 10 video clips of 3 min duration in a randomized order (five clips with animal wildlife scenes and five clips with action scenes). At the same time we presented the four different odors and a placebo (no-odor) in a pseudo-randomized order so that each clip was equally often combined with each odor across subjects. Following the presentation of a clip, subjects estimated their well-being, their interest in the clip, the attractiveness of the main actor and their willingness to see more of the respective movie.

Estimates were performed using visual analogue scales. Before and after each session well-being was additionally assessed with the BF-S' questionnaire. Also, they rated the pleasantness of the 16 odors of the identification subtest of the Sniffin' Sticks and the four test odors. Data were analyzed using analyses of variance. During presentation of jasmine, subjects felt significantly better than in most other conditions, finding the video clips most interesting (animal scenes) and the main actor most attractive (action scenes). When smelling indole, the main actors were rated most attractive; however, subjects did not want to see more of the movie. After the sessions volunteers felt more relaxed and odors of the Sniffin' Sticks had a tendency to be more pleasant than before. Interestingly, 3 MHA was far more pleasant than anticipated. As hypothesized, 3 MHA significantly increased well-being in the second session. This was also achieved by the presentation of indole, but only to a minor extent. In summary, flavors had various effects on the perception of video clips, with 3 MHA and indole being the most effective independent of the type of movie and when presented repeatedly.

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## Temporal integration in nasal localization of carbon dioxide

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The purpose of this investigation was to examine time–concentration trading in nasal irritation at threshold. A Kobal-type olfactometer presented a dilution of CO<sub>2</sub> to one nostril and clean air to the other. Subjects (two male, one female, aged 24–50 years) sought to determine which nostril received CO<sub>2</sub>. Within blocks, concentration of CO<sub>2</sub> was fixed at various levels (8.8–65%). The duration of stimulation varied in a staircase (one-up, two-down). In short, the method determined the threshold stimulus-duration needed for localization at 8–9 concentrations of CO<sub>2</sub>. The minimum stimulus-duration needed to localize CO<sub>2</sub> decreased according to a power function of concentration (linear fits to plots of log threshold pulse-duration versus log concentration had *r*<sup>2</sup> values ranging from 0.98 to 1.00, and slopes ranging from –1.52 to –2.02). Below a certain concentration (ranging from 8.8 to 12.5%), subjects failed to localize even very long pulses. Pulse-durations for minimum detectable concentrations agreed fairly well between subjects (~2–2.5 s). The following conclusions were reached: (i) the nose can integrate CO<sub>2</sub> over time at threshold. (ii) Integration fell short of perfection over the entire range of concentrations examined (more than a two fold increase in pulse-duration was required to compensate for a two-fold decrease in concentration). (iii) Integration at threshold may fail beyond ~2.5 s.

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## Time-intensity ratings of nasal irritation from carbon dioxide

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The objectives of the study were to begin to determine: (i) the rate

of desensitization with sustained presentation of CO<sub>2</sub>; (ii) the degree of individual differences in rate of desensitization; and (iii) the speed of recovery when presentation is interrupted. A Kobal-type olfactometer delivered CO<sub>2</sub> to both nostrils. In study 1, trials lasted 13 s (7 s of CO<sub>2</sub> at 0, 30, 45 or 60%, with 3 s of air before and after). In study 2, trials lasted ~16 s: 10 s of CO<sub>2</sub> (30%) with an interruption in the middle (0–500 ms). In both studies, subjects continuously tracked intensity of nasal sensation. Study 1: (i) time–intensity ratings showed clear concentration dependence. (ii) Subjects desensitized rapidly on average. (iii) Substantial individual differences occurred: some subjects desensitized completely within a few seconds, whereas others showed little or no desensitization. (iv) Individual differences remained stable across sessions. Study 2: time–intensity curves for trials with interruptions in stimulation of 300 ms or longer showed second peaks after the gap (evidence of recovery). For the longest (500 ms) gap, the second peak reached 80% of the height of the first peak. In conclusion, on average, subjects rapidly lose sensitivity with continuous nasal presentation of CO<sub>2</sub>, and rapidly regain some sensitivity when stimulation stops. However, the rate of desensitization varies between subjects. Future research might determine whether people differ in nasal biochemistry (e.g. in carbonic anhydrase), in neural properties (e.g. central integration) or in other factors.

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### Immunohistochemistry of the human vomeronasal epithelium

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The vomeronasal epithelium (VNE) in humans has long been regarded as absent or functionally irrelevant. For example, the neural connection between the VNE and the accessory olfactory bulb degenerates during the second half of pregnancy. Further, reports on the organ's occurrence in adult humans have been contradictory. The aim of this study was to collect immunohistochemical data on the neurogenic or epithelial character of the VNE (e.g. with antibodies against PGP 9.5, OMP,  $\beta$ -tubulin and cytokeratin), its proliferative capacity (e.g. PCNA), as well as the differentiation activity and the interaction with extracellular matrix components (e.g. hyaluronan receptor CD44, galectins, caveolin). To this end, we studied the vomeronasal organs of 22 human cadavers, three adult biopsies, one embryo (week 8) and one fetus (week 13) by means of immunohistochemistry. The histology of the VNE appeared extremely heterogeneous. There were sections of stratified, respiratory and typical 'pseudo-stratified' vomeronasal epithelia consisting of slender bipolar cells. Immunohistochemically, mostly negative results for OMP indicated that the human VNE does not function like the mature olfactory epithelium. In addition, the investigations did not reveal neural connections between the VNE and central brain structures. On the other hand, the presence of some bipolar cells positive for both PGP 9.5 and soybean lectin pointed to a neuron-like activity of a small subset of VNE cells. Positive reactions for CD44 demonstrated a high activity of VNE cells in terms of differentiation and migration. Some bipolar cells showed immunoreactivity for

caveolin, indicating its possible role in signal transduction and differentiation. In summary, the reaction pattern of most antibodies in the adult human VNE is different from that obtained in the olfactory epithelium and the VNO of the rat. However, it shows a specific pattern of activity unique to the mucosa of the nasal cavity. Considering the histologically fully developed epithelial structure and its steady maintenance, the VNE appears to be a highly differentiated structure, the function of which remains unclear.

### Recovery of salt-responsive neural systems after crush of the chorda tympani nerve in mice

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Recovery of salt responses and its inhibition by amiloride (Ami) after crush (AC) of mouse chorda tympani (CT) was studied by examining taste responses of single fibers of the regenerated CT nerve and PGP9.5 immunoreactivity (PGP-IR) of taste buds. At ~2 weeks AC-CT, no significant responses of the nerve to taste stimuli were observed. At ~3 weeks, responses to salts started recovering. At this period, almost all single fibers responding to NaCl were also sensitive to KCl, but were insensitive to Ami (E-type). At ~4 weeks, some single fibers best responding to NaCl and showing Ami inhibition (N-type) reappeared. After 5 weeks or more, the number of N-type recovered to the control level almost equal to that of E-type. During the recovery period from 3 to 5 weeks, impulse frequencies to NaCl both in the N- and E-types increased ~4-fold. Concomitantly, the number of taste bud showing PGP-IR and number of PGP-IR cells in each bud gradually increased. These results suggest that during the recovery period N- and E-type axons may selectively innervate their corresponding Ami-sensitive and -insensitive receptor cells, respectively. While maintaining selectivity, individual axons in each type increase branches and synaptic reformations with their matched type of taste progenitor cells. Regeneration of E-type fibers slightly precedes that of N-type fibers.

### NOS-I is expressed in the vomeronasal organ during prenatal development

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The developing vomeronasal organ of mouse embryos (E13–E20) was investigated to assess the presence and distribution of nitric oxide synthase type I and a panel of regulatory neuropeptides. Immunohistochemistry showed that nitric oxide synthase type I is first expressed in the vomeronasal nerve at embryonal day 15 and in putative extrinsic nerve fibers in the vomeronasal pump anlage at embryonal day 16. Expression of the enzyme was confirmed by northern blotting of pooled embryonal vomeronasal organs. Immunoreactivity for vasoactive intestinal peptide appeared in putative nerve terminals around developing vessels of the VNO during embryonic day 18. On the contrary, immunoreactivity for

atrial natriuretic peptide, substance P and calcitonin gene-related peptide was absent in the embryonal vomeronasal organ. The present results show that early production of nitric oxide takes place during the development of peripheral and central neural vomeronasal structures of the mouse, and indicate that nitric oxide synthesis is a very early step in the neurochemical maturation of the vomeronasal organ.

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### Trigeminal nociception in the dura—role of ATP and CGRP release

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Migraine attacks are believed to culminate in a vicious circle involving meningeal nociceptor discharge driven by inflammatory mediators, ATP and protons, and leading to co-release of pro-inflammatory neuropeptides such as substance P and CGRP. An isolated preparation of the rat dura was stimulated by various concentrations of ATP, UTP, H<sup>+</sup>, bradykinin, histamine, serotonin and PGE<sub>2</sub>, measuring iCGRP and iPGE<sub>2</sub> by ELISA. Suramin, reactive blue and PPADS were used as purinoceptor antagonists, active and inactive enantiomers of flurbiprofen as cyclooxygenase inhibitors. Naratriptan and CP93,129 were employed as 5-HT<sub>1B/D</sub> agonists reported to block meningeal CGRP release in clinical trials. ATP was unable to stimulate iCGRP but augmented iPGE<sub>2</sub> release. Acid buffers (pH 5.9 and 5.4) increased iCGRP; ATP < UTP < PGE<sub>2</sub> facilitated this response. S-flurbiprofen suppressed iPGE<sub>2</sub> and prevented the ATP facilitation of the proton response in iCGRP release. This drug effect was consistent with the antagonist profile and the UTP effect, indicating that P<sub>2Y</sub> receptors mediate the facilitation through prostaglandin release. S-flurbiprofen also suppressed the iCGRP release stimulated by a combined inflammatory mediators (BK, HA and 5-HT), whereas the triptans had no effect on either iCGRP or iPGE<sub>2</sub> release. Inflammatory mediators as well as

ATP stimulate meningeal release of PGE<sub>2</sub>, which is a necessary cofactor for the inflammatory mediators and a facilitatory factor for protons to induce nociceptor activation and CGRP release. However, since cyclooxygenase inhibitors and triptans are similarly effective in migraine treatment, inhibition of CGRP release cannot be a common action principle of the drugs and thus not essential for the antimigraine effect.

### VNO pheromone transduction analyzed in genetically altered mice

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We have developed a comprehensive approach by which the mechanisms underlying pheromone transduction in sensory neurons of the mammalian vomeronasal organ (VNO) can be dissected using wildtype and gene-targeted mice. As a first step in this analysis, pheromonal ligands acting on distinct subsets of vomeronasal neurons (VNs) are identified using a combination of electrophysiological and high resolution Ca<sup>2+</sup> imaging methods in intact VNO sensory epithelium. The availability of ligands then allows analysis of the sensory responses in VNs. We find that Gai2-positive VNs can detect single ligands with exquisite sensitivity and specificity, indicating that these neurons exhibit unusually narrow tuning profiles for stimulus discrimination. In a second step, we analyze the properties of pheromone-induced cellular responses using mice with targeted deletions in essential signaling components. We find that the cation channel TRP2, which is expressed almost exclusively in the VNO, plays a central role in mediating pheromone responsivity. As a third step, these gene-targeted mice are used to examine the role of the VNO in the generation of innate sexual and social behaviors. The results suggest that TRP2 is required in the VNO to detect male-specific pheromones that elicit aggressive behaviors and dictate the choice of sexual partners.

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