

ABSTRACTS

List of Abstracts from the Sixteenth Annual Meeting of the European Chemoreception Research Organisation

Abstracts are listed according to their presentation order during the meeting

Opening address

1. Learning from the honeybee: olfactory processing in a small but intelligent brain

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Honeybees discriminate a large range of odors, and learn odors as signals for food. These abilities make bees important model-animals for the analysis of the mechanisms of olfactory coding and of memory formation. We have used optical imaging and intracellular recording techniques to examine the neural substrates of odor coding and memory formation in the primary and secondary neuropil of the honeybee olfactory system, the antennal lobe (AL) and the mushroom bodies (MBs), respectively. Each AL consists of 160 glomeruli, 40 of which can be imaged for their Ca^{2+} activity during olfactory stimulation and learning. Local interneurons and projection neurons which link the ALs to the MBs were recorded either optically or electrophysiologically, or both. Behavioral experiments were performed to relate the physiological results to odor perception and learning. Odors elicit combinatorial patterns of activity in the glomeruli of the AL. These patterns can be visualized using either bath-applied calcium-sensitive dyes or dyes backfilled via particular nerve bundles (projection neurons, Kenyon cells). The individual glomeruli can be morphologically identified according to a 3D atlas. The responses of the postsynaptic elements within the glomeruli allow the reconstruction of network properties of the AL. Some glomeruli respond with a calcium increase during odor presentation ('on' glomeruli), others with a calcium increase at stimulus offset ('off' glomeruli), and other glomeruli again with a calcium decrease during odor presentation ('inhibited' glomeruli). A comparison of odor-evoked glomerular activity patterns in bath-stained preparations and those recorded selectively in projection neurons reveal that the antennal lobe sharpens the spatial activity patterns. Two inhibitory networks can be separated pharmacologically: one globally controls overall activity in the antennal lobe, with each neuron branching off equally into many glomeruli (10–50), while neurons of the other connect single glomeruli to a subset of other glomeruli, thus performing a more glomerulus-specific odor-tuning task. Imaging of the input region of the mushroom bodies (the lip region) reveals

that odors are represented in specific patterns of microglomerular activation. These patterns are partially overlapping and highly consistent. A comparison of the dynamics of the odor responses shows that mushroom body intrinsic neurons (Kenyon cells) code odors in a sparse way with a small number of spikes per stimulus and a small number on activated Kenyon cells. Odor learning leads to an enhancement of the synaptic transmission at the input synapses to the Kenyon cells that respond to the trained odor (CS+), and a reduction of synaptic efficiency to the non trained odor (CS–). A model will be presented that captures the results of our electro- and optophysiological recordings, and assigns particular functions to the mushroom body in the bee brain. A systematic analysis of odor discrimination among many odors reveals a close relationship between discriminability and similarity of odor-induced glomerular activity patterns. Odors that are less well discriminated induce more similar glomerular activity patterns, indicating that the spatial combinatorial activity pattern in the AL is a substantial component of the neural basis of odor discrimination.

Keynote lecture 1

2. The evolution of olfaction in primates

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Olfactory receptor (OR) genes constitute the basis of the sense of smell. They are encoded by the largest mammalian gene superfamily of >1000 genes, of which >60% are pseudogenes in humans. In contrast, the mouse OR repertoire of roughly equal size contains only ~20% pseudogenes. To assess whether the high fraction of OR pseudogenes is specific to humans or a common feature of all primates, we surveyed a random subset of the OR gene repertoire of 19 primate species from all suborders (using a combination of a specific orthologous gene approach and OR degenerate primers). We found that humans have accumulated mutations that disrupt OR coding regions at least 4-fold faster than any other primate, while apes and Old World monkeys (OWMs) have a significantly higher proportion of OR pseudogenes than New World Monkeys (NWMs) or prosimian primates. Strikingly, the Howler monkey, the only NWM with full trichromatic vision, carries a similar fraction of OR pseudogene to that of the OWMs. Since all OWMs and apes also possess full trichromatic vision, these findings suggest that, in primates, the acquisition of full trichromatic color vision is coupled to the loss of OR genes.

Oral session 1: olfactory and taste receptor proteins: structure and function

3. A refined structural model for olfactory receptor proteins reveals odorant specificity characteristics

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The availability of entire olfactory receptor (OR) repertoires in several mammalian species (<http://bip.weizmann.ac.il/HORDE>) affords a unique opportunity for discerning protein structural and functional information of the individual OR proteins. We applied novel data analysis strategies to the >3000 OR genes of mouse, dog and human within HORDE. First, we identified 22 amino acid positions that are highly conserved among orthologs and variable among paralogs. This may represent the best method to date of identifying odorant complementarity determining regions (CDRs). We next used repertoire-wide sequence comparisons to generate a refined, family-specific, rhodopsin-based homology model for ORs. We employed conservation/variability signals, molecular dynamics simulations, and a comprehensive analysis of specific positions along the seven helices at which prolines generate helix-breaking kinks. This approach was applied to ORs of family 51, belonging to class I fish-like ORs, in comparison to family 6, part of the tetrapod-specific class II, and including the rodent I7 receptor. For each OR family, the model showed a different set of structural features, including idiosyncratic kink patterns, that led to significant differences in the inferred odorant-binding site structure. Intriguingly, the evolutionarily ancient class I ORs were structurally closer to rhodopsin. Combining structure and CDR information, we defined a novel concept of anchor CDRs, which are residues with specific chemical characteristics such as charge or polarity, which may be responsible shared odorant specificities of particular OR subfamilies. For example, a Lys residue in position 164 of RatI7 that is crucial for binding its cognate odorants is shared among 15 members of the OR family 6. Such analyses form a basis for a comprehensive sequence-based classification of OR proteins in terms of potential odorant-binding specificities.

4. Functional expression experiments reveal broad tuning of hTAS2TR bitter taste receptors

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Human bitter taste perception is thought to be mediated by ~30 receptors of the TAS2R family. On the other hand there are thousands of bitter-tasting substances of diverse chemical structures. To address the question how this huge number of bitter tastants can be detected by so few receptors, we aimed to deorphanize hTAS2Rs by identifying their bitter agonists. To this end, we transiently transfected hTAS2R cDNAs in HEK293T cells stably expressing chimeric G protein α -subunits. Bitter compounds were administered by bath application and cytosolic calcium concentrations recorded as a read-out for receptor activation. hTAS2R16-expressing cells responded to bitter compounds consisting of glucose

or mannose moieties, which are linked by β -glycosidic bonds to a methyl group or substituted aromatic ring systems. Two other receptors, hTAS2R43 and hTAS2R44, were activated by the two sulfonamide sweeteners saccharin and acesulfame K. Both receptors also responded to the purely bitter-tasting compound aristolochic acid, which is apparently structurally unrelated to saccharin and acesulfame K. This suggests that hTAS2R43 and hTAS2R44 mediate the known bitter aftertaste of these compounds and do not contribute to their sweet taste. This assumption is supported by the observation that chlorosaccharin, which lacks the bitter aftertaste, does not activate the two receptors. hTAS2R14 responded to chemicals with various substituted ring systems, including picrotoxinin, benzoate and α -thujone. hTAS2R38 is activated by various compounds containing an N-C=S motif. In conclusion, our data support the assumption that TAS2Rs represent cognate bitter taste receptors, although the complete repertoire is not yet characterized. Second, we suggest that bitter taste receptors are broadly tuned to detect common chemical structural motifs of diverse compounds. This may explain why humans equipped with a limited set of receptors are able to perceive thousands of bitter compounds.

5. Selective inhibition of frog olfactory receptor response by polyclonal antibodies

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Olfactory receptor-specific polyclonal antibodies were prepared against synthetic peptides from the extracellular domain of the second extracellular loop between the fourth and fifth transmembrane loops of the seven-transmembrane helical structure of selected olfactory receptor XR106. These antibodies were used to investigate whether selective inhibition of odour responses could be observed when the antibodies were applied to the olfactory mucosa. Frog olfactory bulb responses to odourant stimuli were tracked by the changes in fluorescence observed when the olfactory bulb membranes were impregnated with the dye RH414. The pre-immune response with 1,8-cineole was used as a reference, and a decrease in the 1,8-cineole response was observed when one of the polyclonal antibodies was applied to the olfactory mucosa of the frog, *Rana temporaria*. The inhibition seen with this particular odourant in the presence of the antibody seems to be partially reversible, because some of the original response is retained after washing the olfactory mucosa with the Ringer's solution. The response to isoamyl acetate is hardly affected by the antibody. The evidence indicates that this polyclonal antibody selectively prevents 1,8-cineole sensitive olfactory receptor neurons from firing, without interfering with other odour responses and would also indicate high odourant selectivity of the XR106 olfactory receptor.

6. Response profiles of olfactory receptor neurons of *Xenopus laevis* tadpoles

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It is known that in olfactory receptor neurons (ORNs) of aquatic animals amino acids are potent stimuli. Using calcium imaging in slices of the olfactory mucosa of *Xenopus laevis* tadpoles we were able to determine the response profiles of 283 ORNs to 19 amino acids, where one profile comprises the responses of one ORN to 19 amino acids.

Two hundred and four out of the 283 response profiles differed from each other. Thirty-six response spectra occurred more than once, i.e. there were 36 classes of ORNs identically responding to the 19 amino acids. The number of ORNs that formed a class ranged from 2 to 13. The shape and duration of amino acid-elicited intracellular calcium transients showed a high degree of similarity upon repeated stimulation with the same amino acid. Different amino acids, however, in some cases led to clearly distinguishable calcium responses in individual ORNs. Furthermore, ORNs clearly appeared to gain selectivity over time, i.e. ORNs of later developmental stages responded to less amino acids than ORNs of earlier stages.

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Oral session 2: olfactory receptors: functions and genes

7. Genetic analysis of odor-evoked behaviors in *Drosophila*

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Olfactory sensory systems detect structurally diverse odors, identify them chemically and measure their concentration. All three tasks are performed by a set of different types of olfactory sensory neurons (OSNs) that are sensitive to different but overlapping groups of odors. The sensitivity of an OSN is determined by the odorant receptor (OR) gene it expresses. A large number of OR genes is encoded in the genome but only one or a few OR genes is expressed per OSN. We used *Drosophila melanogaster* to study how the olfactory system performs these complex recognition tasks. Behavioral experiments were carried out with intact flies and flies in which olfactory input is manipulated with microsurgical methods. We also used genetic manipulation to modulate the types of OSNs that are active in the fly, silencing the majority of OSN types or OSNs with known ligand sensitivity. The universe of chemically diverse odors that can be detected, as well as behaviorally determined detection thresholds and measurements of the resolution of the olfactory system, will be presented. The behavioral requirement for a given population of OSNs and the consequences of blocking subsets of OSNs will be discussed. The robustness and odor-specificity of detection, concentration measurement and odor discrimination we observe in behavioral experiments is used to present a tentative model of how these three tasks are performed by a model olfactory system.

8. Odorant receptor Or83b is essential for olfaction in *Drosophila*

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Perception of olfactory stimuli is thought to rely on the activation of subpopulations of olfactory sensory neurons that express a single

odorant receptor protein. Insect odorant receptor genes, encoding seven transmembrane domain G-protein coupled receptors, have been identified in the fruit fly *Drosophila* and the malaria mosquito *Anopheles gambiae*. In *Drosophila*, most olfactory sensory neurons express two types of odorant receptors: Or83b, a broadly expressed receptor of unknown function, and one or more members of a family of 61 selectively expressed odorant receptors. While the conventional odorant receptors, which are thought to mediate odorant specificity of olfactory receptor neurons, are highly divergent, Or83b is remarkably conserved between insect species. Or83b homologues in other insects are also expressed in most olfactory receptor neurons. Here we show that Or83b is essential for normal olfactory behaviour of both larval and adult *Drosophila*. We have characterized the function of Or83b by studying the effects of knocking out this gene through homologous recombination. Or83b mutant larvae do not respond to any odors tested. Adults lacking Or83b are severely impaired in their olfactory behaviour and have no odour-evoked receptor potentials in the antenna. Dendritic targeting of the conventional odorant receptors Or22a/b and Or43b is disrupted in Or83b mutants. These data suggest that Or83b functions to promote proper subcellular localization of conventional odorant receptors, and explain the severe olfactory phenotype observed in mutants. Or83b thus represents a novel element of the olfactory pathway that may provide a potential target for behaviour-based strategies of insect control.

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9. Analyzing the olfactory genome beyond bioinformatics

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The mouse genome contains >1400 olfactory receptor (OR) genes, some 1200 of which are likely to be functional. These genes can be organized phylogenetically into families and the families typically appear as clusters of genes on the chromosomes. Although OR genes appear on almost all of the chromosomes, there are several large clusters, most notably on chromosomes 2, 7, 9 and 11. One cluster on chromosome 7 contains all of the Class I type genes (42 families). Two questions arise regarding the possible functional meaning of this genomic organization. (i) Do the families represent functionally similar receptors, i.e. receptors with similar or related ligand-binding characteristics? (ii) Does the location of clusters on the chromosomes reflect temporal and spatial patterns of OR expression, a process known to be highly regulated in individual sensory neurons?

With a custom-designed microarray containing probes for all of the mouse ORs we have surveyed the expression patterns of ORs. We find that not all ORs are likely to be expressed at the same time during an animal's life, that genes clustered on the chromosomes tend to be expressed in spatially similar regions of the OE, and that ORs are expressed at occasionally high levels in other tissues, suggesting additional functions for these receptors. Most notably all of the Class I genes clustered on chromosome 7 are expressed in the

dorsal zone of the OE, suggesting a possible level of gene regulation. A second-generation microarray is being designed to take advantage of newer technologies. This new chip will contain the complete olfactory genomes, including pseudogenes for mouse, rat, dog, human and chimpanzee. Vomeronasal receptor genes for mouse will also be included. This tool, which will be available to the community, extends genomic investigation of these large receptor families to expression analysis.

10. Pheromones: contrasts and convergence in insects and vertebrates

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The organization of the olfactory system and brain, independently evolved across many taxa, makes it almost inevitable that chemical communication will evolve as animals are selected to respond to their chemical environment (reviewed in T.D. Wyatt, 2003, *Pheromones and Animal Behaviour: Communication by Smell and Taste*. Cambridge University Press, Cambridge). It is thus no surprise that pheromones play key roles in the lives of insects and vertebrates—but there are both contrasts and similarities. As shown by the surprising sharing of a pheromone component by moths and Asian elephants, small molecules can be important in vertebrate signalling as well as in insects. Unlike insects, activity of small molecules as pheromones in terrestrial vertebrates may depend on combination with proteins which give longevity and, perhaps, individuality to signals. There are strong parallels in the use of odours in insect and vertebrate societies for recognition. Odours are the commonest cue used for recognition—and almost all recognition systems appear to involve learning of odour cues before later discrimination. Examples abound from ants, honeybees, ground squirrels, lizards and salmon, to give just a few. In social insect and social mammal species such as honeybees and naked mole rats only one female reproduces. Studies of social insects suggest signalling by the queen rather than control by her. Could these ideas apply to some social mammals? Animals, including albatross, moths and fish, show similar zigzag behaviour when finding an odour source. What is behind the behaviour?

Poster session 1: genes/transduction/genetics

11. Modelling diffusion-limited reactions of receptor, G-protein and enzyme molecules during olfactory transduction

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As part of our ongoing experimental and modelling study of olfactory receptor neurons (ORNs) we are investigating the initial reactions in olfactory transduction. They involve the sequential interaction of three types of proteins located in the ORN ciliary membrane—receptors (R), G-proteins (G) and enzymes (E) generating second messengers—whose reaction rates are limited by lateral diffusion in the membrane. We use several complementary

approaches to describe them. The ‘classical’ approach, based on the law of mass action, leads to solving a system of coupled differential equations, utilizing the tools available for the study of dynamic systems. The application of this method is restricted to systems in which reactions take place in a homogeneous, diluted and infinite 3D volume. When these conditions are not met, which is the case for the RGE system, reaction kinetics may be profoundly affected. To take into account the effect of spatial heterogeneity and diffusion, we follow two complementary approaches. The first one consists in obtaining numerical solutions of the deterministic equations describing the reaction–diffusion system. This approach is suitable for cases in which a large number of molecules is present (i.e. where the notion of concentration is meaningful). The second approach is based on Gillespie’s methods, which we use to simulate the master equations governing the microscopic processes. This approach is appropriate when fluctuations in the spatial densities of the proteins can dominate the kinetics, e.g. when the number of molecules interacting is small, a condition that may happen close to response threshold in ORNs. Using these approaches, the number and distribution in space and time of the proteins can be determined for various numbers of odorant molecules, from as low as one to sufficiently high to be expressed as a concentration. Subsequently, the RGE model will be integrated in a more global model of the ORN for comparison with experimental data.

12. Molecular cloning and expression pattern of two pheromone-binding proteins in the corn borer *Sesamia nonagrioides* (Lepidoptera: Noctuidae)

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Two different cDNAs encoding pheromone-binding proteins (PBPs) from the antenna of the noctuid *Sesamia nonagrioides* were identified and cloned by reverse transcription-polymerase chain reaction (RT-PCR) and rapid amplification of cDNA ends (RACE-PCR). The deduced proteins showed all the characteristics of PBPs: they are small secreted proteins (indicated by the presence of a leader sequence) with acidic pI (5–6) and contain six cysteines in conserved positions. Alignment with other lepidopteran PBPs showed that maximum conservation is observed with PBPs from species that share identical or related pheromone component structure with *S. nonagrioides*. Indeed, the noctuid *Mamestra brassicae* uses the same main pheromone component—Z11-16:OAc—as *S. nonagrioides* females to attract conspecific males, and their PBPs present 77% identities. The expression patterns of the two *S. nonagrioides* PBPs were established by *in situ* hybridization to RNA in both male and female antennae. The PBPs are abundantly expressed at the base of olfactory sensilla in male antennae and some labelling could also be observed in female olfactory sensilla. These results suggest that *S. nonagrioides* females could detect their own pheromone, as already proposed, from biochemical and electrophysiological studies, in other Lepidoptera such as other Noctuidae and Sphingidae. Behavioural studies are in progress to elucidate the physiological meaning of female pheromone perception.

13. Olfactory receptor genes with clustered expression pattern: genomic organization, promotor elements and interacting transcription factors

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In the nasal epithelium of mammals, olfactory sensory neurons expressing a particular odorant receptor (OR) type are either restricted to one of several broad expression zones or, like the cells expressing a member of the mOR262-subfamily, are clustered within a small central region of the epithelium. Individual cells are likely to express only a single or at most a few receptors from the large OR gene superfamily. Very little is known about the molecular parameters and processes underlying the complex transcriptional regulation of olfactory receptor gene expression. In order to gain an understanding of the underlying mechanisms, the genomic sequences upstream from the receptor-coding regions of clustered OR genes were studied in detail. 5'-RACE experiments and bioinformatic analyses revealed complex intron/exon structures and led to the identification of short DNA stretches of ~150 bp immediately upstream of the transcription start sites. Comparative studies on the rat and human gene repertoires demonstrated the existence of similar sequence motifs across species border. To test whether these sequence motifs may indeed be part of the expression control machinery, a representative 5'-sequence was employed in gel-shift assays, demonstrating specific binding only of proteins from olfactory epithelium nuclear extracts. Yeast one-hybrid experiments identified distinct transcription factors expressed in the olfactory epithelium that interact with this region. In reporter gene assays, particular factors were capable of activating transcription. Altogether, these results suggest that the DNA-motifs present in the 5'-non-coding regions of the clustered genes may indeed be important for expression control.

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14. Olfactory genes from the noctuid moth *Mamestra brassicae*

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Using the noctuid moth *Mamestra brassicae* as a model to investigate the molecular mechanisms of olfactory reception, we have isolated and identified several genes that appear specific to the olfactory process. In addition to diverse odorant-binding proteins and chemosensory proteins, we identified and characterized the expression patterns of several genes putatively implicated in ligand reception/transduction/degradation: membrane receptors from different types, G-protein subunit and odorant-degrading enzymes. In particular, we cloned one antennal specific G-protein coupled receptor (GPCR) in *M. brassicae* that exhibits unexpected sequence conservation with putative olfactory receptors (ORs) from various insect orders. This receptor, which is expressed in numerous olfactory sensilla, belongs to the OR2 moth receptor type that may have a special and important function in chemosensory neurons of insects. As all ORs known to date belong to the GPCR family,

we have identified a lepidopteran antennal G-protein subunit belonging to the Gq family that presents high homologies with the Gq 3 from *Drosophila* proposed to be implicated in visual and olfactory responses. This Gq may be involved in the olfaction transduction process through interaction with ORs, and thus could be used in receptor functional experiments. In addition, two two-transmembrane domain receptors from the sensory neuron membrane protein (SNMP) family were cloned in antennae, although their exact role in olfaction is still unknown. The first one presents all features of SNMPs, in particular a specific expression in olfactory neurons. The other one, although very similar in sequence, is not olfactory specific. Diversity and divergence of different elements implicated in odor detection, as the ones illustrated here, could have combinatory roles and thus participate to the specificity of odor recognition.

15. Heritability of taste and smell perceptions: a Finnish family study

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The present pilot study explores genetic components underlying food perceptions, preferences and choice. The study consists of phenotyping at least 100 members of 15 Finnish families (of which 53 members have been examined to date). Genome scanning of the family members has already been performed with 400 microsatellite markers. The phenotyping includes testing of chemosensory performance and hedonics, as well as evaluation of aspects of eating behavior and dietary preferences. The subjects completed a range of dietary questionnaires prior visiting the clinic where the chemosensory tests were conducted. The chemosensory testing consisted of rating PROP (filter paper method), sweet and salty solutions, and odorous stimuli after overnight fasting. Three suprathreshold concentrations of sucrose (3, 7.5 and 18.75%) and NaCl (0.2, 0.5 and 1.25%), plus plain water included in both series, were rated for intensity and pleasantness using a labeled magnitude scale (LMS). Smell tests were performed with 12 scratch-and-sniff platelets. The subjects were asked to identify the odors and rate them for intensity and pleasantness using five-point category scales. Heritability (h^2) of each trait was calculated by the Sequential Oligogenic Linkage Analysis Routines (SOLAR) program assuming a polygenic model. The preliminary results ($n = 53$) show that the heritability was highest for the third factor of the three-factor eating questionnaire, i.e. an individual's ability to cope with the sensation of hunger ($h^2 = 0.721$) and for the perceived pleasantness of 18.75% sucrose solution ($h^2 = 0.893$). The latter finding supports the hypothesis of heritable 'sweet tooth'. Further data collection and analyses will show if these trends hold true with more family members being examined.

*The first two authors contributed equally to the work.

16. Candidate pheromone receptors of the moth

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The remarkable responsiveness of male moths to pheromones released by females is based on the extremely sensitive and selective reaction of highly specialized sensory cells in the male antennae. These cells are supposed to be equipped with very distinct, male-specific receptors for pheromonal compounds; the nature of these receptors is still elusive. A combination of genomic sequence analysis and cDNA library screening of the tobacco budworm *Heliothis virescens* led to the discovery of a divergent gene family encoding putative olfactory receptors. Comparing the encoded amino acid sequences of all candidate olfactory receptors emphasized their extreme diversity; however, a small family (identity >40%) emerged, including the three receptor types HR14, HR15, HR16. Reverse transcription polymerase chain reaction analysis revealed that all three subtypes were exclusively expressed in the antennae of male moths. More detailed *in situ* hybridization studies indicated that expression of these male-specific receptor types was confined to antennal cells located beneath sensillar hair structures which contain pheromone-sensitive neurons. Moreover, two-colour double *in situ* hybridization approaches in combination with laser-scanning microscopy revealed that each cell that expressed one of these receptor types was surrounded by cells expressing pheromone-binding proteins. These findings suggest that the novel receptor types may render neurons in male antennae responsive to pheromones.

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17. Evolution of the 'OR37' subfamily of olfactory receptors: a cross-species comparison

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Olfactory receptors (ORs) of the OR37 subfamily are characterized by several special properties, including an extended third extracellular loop and a clustered expression pattern in the olfactory epithelium. The human and mouse genome projects have allowed us to characterize the complete repertoire of OR37 receptor subtypes in these species. Uniquely among olfactory receptor subfamilies, genes encoding OR37 receptors are organized in two gene clusters. Cluster-I consists of five genes, which share a very high level of sequence homology (~90%). In line with the tendency of pseudogenization for human OR genes, the human cluster-I contains four pseudogenes, the mouse cluster-I one pseudogene. Cluster-II comprises three genes in mouse and in human, surprisingly, seven genes. The sequence similarity of cluster-II genes was only ~60%. To gain some insight into the origin and evolution of this special OR subfamily, we have cloned OR37-type receptors from animals which are considered as representative for different stages of mammalian evolution. From the great anteater, an insectivore, we have identified a total of 21 OR37 genes which are related to cluster-I and cluster-II genes, respectively; thus indicating that the arrangement of two OR37 gene clusters apparently existed prior

to 130 million years ago. In the opossum, a representative of the Marsupalia, five OR37-like receptors were identified which all seem to be cluster-II genes. Assessment of a newly available genomic BAC library revealed that the five opossum OR37 genes are located in a single cluster. All efforts to clone cluster-I-like genes from opossum failed; thus, one might speculate that cluster-II may be the original cluster and cluster-I appeared somewhere between 130 and 173 million years ago. Analyzing the egg-laying monotreme platypus, we were unable to identify any OR37 genes, although a receptor type was identified that is a member of the OR262 family; interestingly, all OR37 receptors are members of the 262 family.

This work was supported by the Deutsche Forschungsgemeinschaft.

18. Peripheral antennal clock and circadian rhythmicity of olfactory gene expression in the moth *Mamestra brassicae* (Lepidoptera: Noctuidae)

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Olfaction is the dominant sensory modality for most animals. This is well illustrated in nocturnal Lepidoptera whose pheromonal communication is particularly developed. The circadian rhythm of insect olfactory behavior has been well documented via behavioral and electroantennographic studies. Although peripheral circadian oscillators were discovered in *Drosophila* antennae, where they are necessary for circadian rhythms in olfactory responses, the molecular basis of olfactory rhythm is not yet understood. Using the noctuid moth *Mamestra brassicae* as a model, we first established that Lepidopteran antennae also contain a functional clock. Indeed, an antennal homolog of the clock gene *period* was cloned whose expression pattern is associated with olfactory sensilla in antennae. In addition, the *period* transcript appears to undergo endogenous circadian oscillations in the brain and antennae, in synchrony. To decipher the molecular basis of olfactory rhythm, we used competitive polymerase chain reaction (PCR) as well as real time PCR to follow newly identified olfactory gene expression in the *M. brassicae* antennae over a 24 h period. The olfactory genes studied encode, respectively, a pheromone-binding protein, an olfactory receptor and an odorant-degrading enzyme. We found that the olfactory gene transcripts undergo circadian oscillations with a phase rhythm relationship to the period ones. Our results provide strong evidence that genes playing critical roles in olfactory transduction are under circadian control at the molecular level, and thus may participate in the olfactory rhythms observed *in vivo*. A better knowledge of the molecular mechanisms of olfaction sensitivity modulation upon circadian rhythm is fundamental to improve olfactory-based strategies used against agricultural pests as well as disease vectors that mainly use olfactory cues to seek and select their hosts.

19. Molecular trafficking and quality control of olfactory receptors

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Secreted proteins such as membrane receptors are co-translationally translocated to the endoplasmic reticulum (ER). In this

compartment, chaperones or escort proteins ensure these proteins are correctly folded to allow them to transit to the Golgi apparatus. At this stage, proteins are sorted and sent to different domains of the plasma membrane. In heterologous cell systems, olfactory receptors (ORs) are poorly directed to the plasma membrane. The reasons why ORs are sequestered in the ER are mostly unknown. Using cellular biology and biochemistry (immunofluorescence and pulse-chase), we have studied the folding and quality control of ORs. In addition human OR912–93, which is used as a model for these studies, is constitutively activated. We are investigating the molecular bases of this activity.

20. Functional expression and pharmacological characterization of olfactory receptors in *Saccharomyces cerevisiae*

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The functional expression of olfactory receptors (ORs) is a primary requirement to examine the molecular mechanisms of odorant perception and coding and to understand olfactory detection systems. Yeast offers the attractive possibility of functionally expressing quantitative amounts of recombinant GPCRs due to its easy manipulation and relatively low cost. This simple eukaryote possesses all the machinery necessary to perform adequate post-translation protein modifications. Quantitative functional expression of an olfactory receptor and adequate trafficking to the plasma membrane was obtained under optimized induction conditions, as compared to other expression systems. Moreover, we took advantage of the functional similarities between signal transduction cascades in the budding yeast *Saccharomyces cerevisiae* and mammalian cells to hijack the yeast pheromone response pathway, so that odorant ligand binding to an heterologously expressed olfactory receptor initiates signalling. Elements in the mating signal-transduction pathway were replaced by transformation with expression vectors, to improve functional coupling and activate a luciferase reporter system. Validation using the now standard rat I7 receptor response to a range of odorants provided evidence of the system's adequacy to constitute a sensitive, rapid and inexpensive test for ligand and pharmacological screening. This yeast bioluminescence reporter system was then assayed with the human OR17–40 receptor to assess further its performance.

21. A comparative analysis of primate and human T2R taste receptors

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T2R (Tas2R) genes encode a family of 20–30 G-protein-coupled-receptors that have been implicated in gustation, and many of these are likely to be involved in bitter taste perception. So far only a few ligands for these receptors have been identified. Differences between individual T2Rs are most likely responsible for differences in taste perception. On an evolutionary scale, humans closest relative in the animal kingdom are thought to be pygmy chimpanzees (*Pan paniscus*) and chimpanzees (*Pan troglodytes*). We have analysed the

T2R bitter taste receptors of pygmy chimpanzees and compared them to those of humans, and where available from the recently released draft genome, those of chimpanzees. The changes identified range from large alterations, which would have significant impact on the function of the encoded protein, to silent changes of single bases. Taken together, the analysis identifies genes for T2Rs which have remained unchanged during evolution across species barriers, as well as others that diversify between species. The numerous changes found have been analysed and will be discussed.

22. Agonists and antagonists for two human olfactory receptors from different phylogenetic classes

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Humans are able to detect and discriminate myriads of structurally diverse odorants using only several hundred olfactory receptors (ORs) classified into two major phylogenetic classes representing ORs from aquatic (class I) and terrestrial animals (class II). Olfactory perception results in a combinatorial code, in which one OR recognizes multiple odorants and different odorants are recognized by different combinations of ORs. Moreover, recent data suggest that, in addition to their agonist role, odorants could also behave as antagonists for other ORs, thus rendering the combinatorial coding more complex. Here we describe the odorant repertoires of two human ORs belonging to class I and class II, respectively. We set up a new biomimetic screening assay based on calcium imaging in which 100 airborne odorants were applied at physiological doses. We showed that the class I OR52D1 is functional, exhibiting a narrow repertoire related to that of its orthologous murine OR, demonstrating that this class I OR is not an evolutionary relic. The class II OR1G1 was revealed to be broadly tuned towards odorants whose carbon chain length was 9–10, with diverse functional groups. The current study also demonstrated the existence of antagonist odorants for this OR, structurally related to the agonists, though of shorter length. The agonist–antagonist structural similarities and dose–response data suggest that antagonists act as competitive inhibitors. Finally, we showed that these antagonists are OR specific.

23. Antibodies recognizing olfactory receptor subtypes

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Olfactory receptors are considered as multifunctional elements of olfactory sensory neurons; they are supposed to mediate the response to adequate odorants, to play an important role in target finding of the axons and may even contribute to a monoallelic expression of one receptor type per cell. Most of these views are based on studies performed on the level of the encoding nucleic acids, and very little is known about the actual receptor proteins. Efforts to explore the features of distinct receptor subtypes have always been hampered by the fact that specific receptor antibodies were not readily available. In the present study, we have tried to generate subtype-specific antibodies. In a first approach, antibodies raised against a peptide characteristic for the mouse OR37 receptors were assessed. Immunohistochemical analyses of nasal whole-mount preparations revealed staining of individual cells; their clustered

distribution matched that previously visualized by *in situ* hybridization. In tissue sections analysed by confocal microscopy, intense fluorescence was visualized in the cell body, dendrite and most notably the cilia of individual sensory neurons. In a second set of experiments, antibodies generated against mOR256–17 were assessed; this OR subtype is expressed in the medial zone, and *in situ* hybridization suggests it may also be expressed in cells within the cribriform mesenchyme during development. In fact, in distinct developmental stages, immunoreactive cells were visible in the cribriform mesenchyme; in particular the membrane of these cells was labelled. Moreover, the notion that receptor proteins may be present in the axons of olfactory neurons was confirmed; very distinct axon bundles and glomeruli were visualized by the antibodies. These results strongly suggest that the newly generated antibodies may represent useful tools for studying olfactory receptor proteins.

24. Olfactory-like receptors mRNAs are expressed in human and murine tongues

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A few years ago, the expression of olfactory-like receptors (OLRs) in rat taste buds was described, and it was suggested that OLRs may be involved in taste perception as gustatory receptors. Previously, we have shown that 9 OLR genes and 11 OLR pseudogenes are expressed in adult and/or in fetal human tongue. Genomic studies did not reveal whether OLRs expressed in tongue share some specific features. In order to understand their function in tongue, the expression profile of these genes in gustatory papillae was studied by reverse transcription polymerase chain reaction in mice. The mouse orthologs of the OLR genes and pseudogenes expressed in human tongue were identified. The expression of eight of these orthologs (S25/mJCG1, K42/mJCG2, mJCG3, P2/mJCG5, P3/mJCG6, mT09m/mJCG9, K21/mTPCR85 and mTPCR06) was studied in the different types of mouse lingual papillae as well as in the olfactory epithelium. We found that all of them are expressed in olfactory epithelium. Four of them (S25/mJCG1, K42/mJCG2, mT09m/mJCG9 and mTPCR06) are expressed in lingual papillae. The expression of the latter was also analysed in brain, lung, kidney, intestine and skeletal muscle. All were detected in brain. Additionally, K42/JCG2 was found to be expressed in skeletal muscle and mTPCR06 in all five tissues. The non-specific expression of OLRs genes in taste tissues in mice suggests that they might be involved in other mechanisms than taste perception.

25. External acidification of the taste bud impedes the generation of action potentials by rat taste receptor cells

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Taste buds are comprised of dozens of taste receptor cells (TRCs), some of which are able to generate action potentials by activation of a voltage-gated fast sodium inward current (I_{Na}). Patch-clamp

recordings were performed to study the impact of acidification of the basolateral membrane of rat fungiform TRCs upon I_{Na} . The lingual epithelium was peeled from the tongue, after subepithelial enzymatic treatment, and pinned, basolateral side upwards, on a Sylgard layer. Under Nomarsky illumination (upright Zeiss Axiocrop, $\times 400$) individual cells could be distinguished in the taste bud. Cell-attached recordings indicated the ability of many cells to spontaneously generate action potentials. Acidification with HCl-Tyrode (pH 3.5) blocked the spontaneous firing. In the whole-cell mode, voltage-clamp recordings distinguished cells with voltage-gated fast inward and outward currents from cells with only voltage-gated outward current. The inward current was suppressed in a sodium-free solution. The voltage range of activation and the amplitude of the current varied from one cell to another. In current-clamp mode, action potentials were observed upon electrical stimulation. Acidification rapidly blocked I_{Na} . The exact role of local action potentials in taste receptor cells is not yet known. In cells synaptically connected to gustatory nerve fibres and having voltage-gated calcium currents, the action potential could trigger or facilitate the synaptic transmission of the sensory information. Action potentials within the taste bud could also play a role in the communication between cells. During natural stimulations of the surface of the tongue with acids, protons are expected to diffuse through the apical tight-junctions and reach the basolateral side of the receptor cells. In that case, the blocking effect observed in the present study could have severe repercussions on the generation of the sensory information.

Perireceptor mechanisms

26. Study of signal transduction pathways of orexin A and orexin B in the rat olfactory epithelium

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Orexins A and B are multifunctional neuropeptides implicated in the regulation of energy metabolism (Sakurai *et al.*, 1998), wakefulness and neuroendocrine functions. Orexins activate two receptors (Ox1R and Ox2R) which belong to G-protein-coupled receptors (GPCR). Ox1R, which has 10 times more affinity for orexin A than for orexin B, is coupled to Gq protein and Ox2R (which has equal affinity for both orexins) to Gq and/or Go/i proteins in neuron-like BMI transfected cells (Zhu *et al.*, 2003). Recently, we have demonstrated the presence of both orexins and their receptors at all levels of the rat olfactory system: epithelium, bulb, piriform cortex (Caillol *et al.*, 2003). No data are presently available concerning the transduction pathways of orexins in the olfactory epithelium (OE); to study this, we have chosen three model systems: OE primary cultures; dissected epithelium and explants. Spectrofluorometric assays demonstrated that stimulation of OE primary culture with orexins (10^{-7} M) led to an increase of intracellular Ca^{2+} concentrations in $\sim 50\%$ of cases. Both orexins are thus capable of activating OE cells. Inositol phosphate formation was increased ~ 2 times after stimulation of dissected OE with orexin B, thus indicating PLC activation. As the olfactory receptors are GPCR coupled to Golf and their stimulation results in adenylyl cyclase (AC) activation and an increase in cAMP production, we examined the possibility that orexins activated the AC pathway. No change

in cAMP level was detected after orexin A or B stimulation of EO explants. In contrast, preliminary data indicated that pretreatment of explants with orexin A reduced the forskolin-induced increase in cAMP, suggesting Gi and/or Gq coupling of OxsR or a potential crosstalk between adenylyl cyclase and PLC pathways. Preliminary data demonstrated that orexins activated MAP kinase of the ERK type. These results suggest multiple orexin pathways in OE, corresponding to different possible roles that remain to be studied.

27. Odorant-degrading enzymes of noctuid moths

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Olfactory systems of terrestrial animals are specialized in the detection of odorant volatile hydrophobic molecules. Many of these compounds are cytotoxic and the olfactory systems have thus evolved mechanisms for detoxifying these substances and minimizing the signal saturation. In Lepidoptera, antennal enzymes able to transform the functional groups of the sex pheromone components were first identified by biochemical approaches. More recently, molecular approaches led to the characterization of various odorant-degrading enzymes, including cytochrome P450s, in the antennae of the sphinx moth *Manduca sexta* and the fruit fly *Drosophila melanogaster*. Looking for P450s involved in odorant metabolism in the noctuid moth *Mamestra brassicae*, we have used a polymerase chain reaction-based strategy to clone four cytochrome P450 cDNAs from antennae. One of them is specifically expressed in the olfactory sensilla, and is tuned to detection of sex pheromone and plant volatiles, suggesting a role in odorant clearance (M. Maïbèche-Coisne *et al.*, 2002, *Insect Mol. Biol.*, 11: 273–281). All these P450s present structural features of microsomal P450s and they presumably act on odorants only after their internalization. Moreover, the cDNA encoding the NADP-P450 reductase, which is the redox partner of microsomal P450s, was also isolated from the antennae. Taken together, these results strongly suggest the occurrence of a P450-dependent oxidative metabolism in odor degradation in insects, as shown in vertebrates. In addition, we have cloned two esterases expressed in the antennae of *M. brassicae* (M. Maïbèche-Coisne *et al.*, 2004, *Chem. Senses*, in press) and *Sesamia nonagrioides*. These two noctuid moths share the same main sex pheromone component, the Z11-hexadecenyl acetate, which could be inactivated by this class of enzymes, as other acetate compounds. For a better understanding of odor termination in insects, further studies are now necessary to determine precisely the functional properties of these enzymes.

28. A new odorant-binding protein XlaeOBP identified in the aerial olfactory system of *Xenopus laevis* and *Xenopus tropicalis*

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Odorant-binding proteins (OBPs), commonly associated with aerial olfaction, are currently found in mammalian olfactory mucus, but have never been identified in fish. It is not yet clear if the absence

of OBPs in the fish olfactory system is due to phylogenetic differences, or rather due to functional differences linked to adaptation of the olfactory system to the aerial environment. The *Xenopus laevis* olfactory system, because it presents in adult animals two olfactory chambers that are thought to be devoted respectively to aquatic and aerial olfaction, offers a unique opportunity to test this alternative. We have identified for the first time an OBP in *Xenopus*. XlaeOBP is a 158 amino acid protein with an isoelectric point of 8.5 and a molecular weight of 17 696.6 Da. Using reverse transcription and 3'-RACE strategy, we obtained an amplification product of ~600 bp, which was cloned and analyzed by DNA sequencing. The cloned sequence was used to characterize the expression pattern of the gene in the olfactory system of two *Xenopus* species, *X. laevis* and *X. tropicalis*. We demonstrated that in both species XlaeOBP has been found to be expressed at the level of the Bowman glands only in the aerial olfactory cavity, and at the level of the vomeronasal organ in the Jacobson glands. For *X. laevis*, expression starts early during metamorphosis (stage 57) on isolated cells, but mature Bowman XlaeOBP-positive glands appear lately (stage 61). For *X. tropicalis*, the first expression started at stage 58 and in mature Bowman's glands at stage 60.

29. High xenobiotic metabolism activities in the olfactory tissues: possible involvement in olfactory process

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In liver and other tissues, drug-metabolizing enzymes and transporters form a detoxification network. Xenobiotic hydroxylation by cytochromes P450 is usually followed by phase II biotransformation involving enzymes such as UDP-glucuronosyltransferase (UGT) and results in a major change of water solubility. Polar molecules are then excreted by transporters (phase III) and eliminated. An olfactory-specific UGT (UGT2A1) has been identified (Lazard *et al.*, 1991). This enzyme is expressed in olfactory tissues and shows preference for various odorants. Interestingly, the ability of odorants to stimulate olfactory adenylyl cyclase is abolished by the conjugation catalyzed by UGT2A1. Therefore, it has been proposed that this enzyme plays a role in odorant signal termination. We studied the quantitative expression and the cell localization of UGT2A1 mRNA. In olfactory mucosa UGT2A1 mRNA are localized mainly in sustentacular cells and Bowman glands but also in olfactory sensory neurons (Heydel *et al.*, 2001). We also showed a weaker expression in olfactory bulbs (granular cells), which emphasizes a potential role of this enzyme in brain protection. We observed important age-related changes in the glucuronidation of odorant molecules and in UGT mRNA expression in rat (Leclerc *et al.*, 2002). Mitral cell activity was recorded during exposure to different odorant molecules. Interestingly, the highest responses were obtained with molecules that were not efficiently glucuronidated by olfactory mucosa. The study of cytochrome P450 and transporters in olfactory mucosa and bulb is in progress in our laboratory. Preliminary results confirm the presence of at least 17 isoforms of P450 and demonstrate the mRNA expression of a number of transporters in these tissues. Our findings suggest a possible role of drug-metabolizing enzymes in the olfactory process which needs to be confirmed by appropriate experiments.

30. Conformational changes of rat odorant-binding protein OBP-1F upon odorant binding

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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 OBP (OBP-1F) using a set of six odorants of various chemical structures. We examined the binding properties by both fluorescent probe competition and isothermal titration calorimetry. OBP-1F affinity constants, in the micromolar range, varied by more than one order of magnitude and were roughly correlated to odorant size. The observed binding stoichiometry was found to be around one odorant per dimer. Using tyrosine differential spectroscopy, the binding of ligand was shown to induce local conformational changes. A three-dimensional structure of OBP-1F, modelled using the known structure of aphrodisin as a template, allowed us to suggest the location of the observed structural changes outside of the binding pocket. These results are consistent with one binding site located in one of the two barrels of the OBP-1F dimer and a subtle conformational change correlated with binding of an odorant molecule, which hampers uptake of a second odorant by the other hydrophobic pocket.

Peripheral processing

31. Leptin as a potential regulator of olfactory epithelium functions

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Leptin, one of the major hormones controlling energy balance, is produced by adipocytes. Leptin activates its target cells in brain feeding centers through interaction with several receptor isoforms that have different transduction capabilities. The longest isoform, Ob-Rb, supports all the signal transduction capabilities. Recent studies have emphasized the production of leptin and receptors by other tissues, and have supported a role for peripheral leptin in mediating direct metabolic effects in several key organs. Neuro-modulation of sensory perception by leptin has been proposed for taste buds, supporting the hypothesis that modulators of food intake may act on the primary steps of sensory perception. We have recently identified orexins and their receptors in the olfactory epithelium (OE), and showed that it might be a target of modulation by the nutritional status. Here, we checked whether leptin might be an additional regulator of OE functions. We examined the presence of leptin and its receptors using both reverse transcription polymerase chain reaction and immunocytochemical analysis. We showed that leptin is transcribed in OE and exhibits a patterned expression both in neuronal and in supporting cells, mainly located in the apical region, as demonstrated by ultrastructural data. Different isoforms of the receptor, the long form Ob-Rb as well as some truncated forms, are also transcribed in OE. Ob-Rb is mainly expressed in neuronal cell types, as assessed by the use of a specific

antibody, suggesting a possible auto- or paracrine regulation of neurons by leptin. We also show anatomical evidence for the co-expression of Ob-Rb and proteins involved in leptin transduction pathways in neurons. In addition, exogenous treatment with leptin is able to induce Stat3 phosphorylation in OE. Altogether, our anatomical and functional data show that leptin, its receptors and the molecules involved in its transduction pathways are present in the EO, suggesting that leptin may be a regulator of various OE functions.

32. Olfactory receptor neurons tuned to (–)-germacrene D in weevils and moths

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Germacrene D is a volatile chiral sesquiterpene produced in many plant species. The synthesis of the enantiomers can be controlled by two different synthases, and the (–)-configuration is the most common enantiomer in higher plants. From an evolutionary point of view it is interesting to compare, in closely and distantly related insect species, the specificity of receptor neurons responding to plant volatiles. However, comparison of results obtained in different species is in general difficult, due to the different protocols that have been used. In our laboratory we used gas chromatography linked to single-cell recordings and to mass spectrometry for functional characterization of olfactory receptor neurons. Essential oils, headspace samples, isolated fractions and chemical standards were used as test samples. We have identified olfactory receptor neurons tuned to the same primary odorant germacrene D in the weevil *Anthonomus rubi* and in three heliothine moths, *Heliothis virescens*, *Helicoverpa armigera* and *Helicoverpa assulta* (Røstelién *et al.*, 2000, Chem. Senses; Strandén *et al.*, 2003, J. Comp. Physiol. A), by using similar protocols in all the studies. These receptor neurons showed similar enantioselectivity, (–)-germacrene D having ~10 times stronger effect than the (+)-enantiomer as shown by dose-response curves. However, the other odorants eliciting secondary responses were different in the two insect groups. Thus, the receptor neuron type evolved for the detection of (–)-germacrene D in the weevil *A. rubi* differs from the type evolved in the three heliothine moths.

33. Effect of gambierol on voltage-gated ion currents of mouse taste cells

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Ciguatera is a type of food poisoning found world-wide caused by the consumption of fish containing toxins produced by *Gambierdiscus toxicus*, a marine dinoflagellate. The neurological features of this intoxication include sensory abnormalities, such as paraesthesia,

heightened nociperception and taste alterations (Pearn, 2001). One of the possible toxins involved in ciguatera, gambierol, has been recently synthesized (Fuwa *et al.*, 2002), making it possible to perform detailed biological studies on its mechanism of action. Here, we have evaluated the effect of gambierol on the voltage-gated ion currents in taste cells. Taste cells are excitable cells endowed with voltage-gated Na^+ , K^+ and Cl^- currents (I_{Na} , I_{K} and I_{Cl} , respectively). By applying the patch-clamp technique to single cells in isolated taste buds obtained from the mouse vallate papilla, we have recorded such currents and determined the effect of bath-applied gambierol. We found that this toxin markedly inhibited I_{K} in the submicromolar range, whereas it showed no significant effect on I_{Na} or I_{Cl} even at high concentration (1 mM). I_{K} was indeed very sensitive to the action of gambierol, as indicated by an IC_{50} of ~ 2.5 nM. This toxin acted as a partial blocker, with a maximum current reduction of $55 \pm 1.6\%$ ($n = 10$) at a saturating concentration of 0.1 mM. The block of I_{K} was irreversible even after a 50 min wash. In addition to affecting the current amplitude, we found that gambierol also slowed significantly the activation kinetics of I_{K} (>4 -fold increase in the activation time constant). In conclusion, our results indicate that voltage-gated potassium currents in taste cells represent a specific target for the gambierol action. These currents play an important role in the generation of the firing pattern during chemotransduction. Thus, gambierol may alter action potential discharge in taste cells and this could be associated to the taste alterations reported in the clinical literature.

34. Recording of mucosal potentials in response to ortho- and retronasal application of trigeminal and olfactory stimuli

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Ortho- and retronasal olfaction represent two different functional aspects of a shared sensory system, yet seem to evoke different sensations. The observed differences between ortho- and retronasal olfaction have triggered a number of studies which pointed towards a lower sensitivity of the retronasal olfactory pathway. The aim of this study was to investigate whether this effect is also present at the level of the olfactory receptor neurons. Trigeminal (60% v/v CO_2) and olfactory (8 p.p.m. H_2S) stimuli were presented ortho- and retronasally. Recordings were made at different sites of the olfactory epithelium. Responses to the trigeminal stimulant CO_2 were obtained in 10 participants (6 women, 4 men, mean age 23.4 years), responses to olfactory stimulation with H_2S were recorded in 9 subjects (5 women, 4 men, mean age 23.6 years). Typical responses were recorded after orthonasal stimulation with CO_2 and H_2S . In case it was possible to record responses after retronasal stimulation, response amplitudes were typically smaller compared to responses obtained after orthonasal stimulation. The present data indicate that differences between ortho- and retronasal olfaction may start as early as the mucosal level. The data support the idea that the intensity of physically identical stimuli is lower after retronasal stimulation.

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35. Topographical patterns of responsiveness of the olfactory mucosa to the rabbit mammary pheromone and structurally related molecules: a comparative study

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Mammalian newborns have developed reliable mechanisms to locate and attach to their mother's nipples immediately after birth. In the rabbit, a recent study demonstrated that trans-2-methyl-2-butenal (2MB2), a compound found in doe milk, is a strong signal inducing the typical searching–oral grasping response of the pups (Schaal *et al.*, 2003). 2MB2 acts specifically, and does not induce a similar behaviour in newborn rat and mouse. In rabbit pups, although less active, related molecular structures have been shown to elicit the typical behavioural response. In the present study, we explored the chemical specificity of the responses of the mucosa of the main olfactory system and tested their variability in phylogenetically different animal species. We recorded electro-olfactograms (EOGs) from different sites of the mucosa of the nasal septum and turbinates of newborn rabbits, rats and mice from postnatal days 2 to 12 as well as from animals after weaning. We tested 2MB2 and a series of structurally related odorants. All the odorants were delivered in identical dilutions based on their vapour pressures. Whatever the animal species, in all the tested preparations, most recording sites of the septal and the turbinate mucosa were responsive to 2MB2. All stimuli elicited typical EOGs with a rapid rising phase and a slower decline. Response amplitude to a given stimulus at a given recording site varied from animal to animal and was not age dependent. Animals showed one or two locations of maximum sensitivity for 2MB2. All recording sites sensitive to 2MB2 responded also to the seven other compounds. 2MB2 was the most potent stimulus in 30% of the instances tested. Surprisingly, this indicates that the olfactory mucosa of the rabbit exhibited neither regions of specific selectivity nor a greater sensitivity to 2MB2 than rat and mouse.

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36. Calcium spectrofluorimetry assays on primary cultures of olfactory epithelium cells as a screening tool for functional identification of active neuropeptides and hormones

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Olfactory receptor neurons (ORNs) and the olfactory epithelium have long been considered to be the detection site for odorants, but no or little consideration has been given to the possibilities of modulation. However, recent data strongly suggest that the olfactory epithelium is subject to various modulatory actions. Furthermore, olfactory epithelium is continuously generating new ORNs from basal cells. Little is known about the turnover of ORNs and supporting cells and the control of this mechanism, but numerous paracrine or endocrine factors are probably involved in this

control in addition to the intrinsic, genetically driven determination. Both considerations led us to set up an *in vitro* model that would allow a fast and efficient screening of potentially acting molecules. We have developed a primary culture of cells dissociated from rat olfactory epithelium. These primary cultures contained ~30% of developing neurons as early as after 2 days *in vitro* (DIV); they were maintained up to 30 DIV. We have used these cultures in a calcium spectrofluorimetric assay to screen the action of various modulating peptides. In parallel, we have tested the presence of mRNA transcripts for the peptides and their receptors in the cell culture at different stages. We demonstrate evidence of the action of many peptides linked to the physiological state of animal (leptin, orexins, arginin-vasopressin, endothelin) as well as of odorants. Both peptides and odorants proved to induce different calcium responses from 2 to 30 DIV. Furthermore, the expression of the corresponding mRNA receptors varied in parallel, demonstrating an evolution of the primary culture with time. This suggests that various peptides are involved in differential mechanisms in the olfactory epithelium. These screening methods lead to identification of new acting peptides. Moreover, used together with immunohistochemical methods, and completed by calcium imaging, it allows for the potential role of olfactory modulators to be deciphered for first time.

37. The relative contribution of somatosensory and taste perception for acids: a combined study in man and hamster

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The taste properties of HCl (pH 1.5–2.4) and weak acids (acetic, citric, lactic and malic; pH 2.1–3.9) were compared in 11 high saliva flow rate (HF) and 11 low flow subjects (LF) using the time–intensity technique. Continuous evaluation of the pH on the subjects' tongue surface with an ISFET electrode showed a better buffering effect in HF versus LF subjects, though HF perceived higher intensities than LF for acids. The CT and Vth nerves were simultaneously recorded in 39 hamsters for acids, capsaicin (100 μ M) and tastants (monosodium L-glutamate, quinine, sucrose, NaCl). Repeated application of acids, which gave responses on both nerves, resulted in a progressive decrease (41%) of CT responses to acids and to tastants ($67 \pm 48\%$; $n = 122$). No such desensitization was ever observed for repeated application of tastants at concentrations that gave very few responses on the Vth nerve. Capsaicin elicited small responses in CT and/or Vth nerves and significantly increased or decreased CT responses to tastants in 13 hamsters. Nerve sections (both nerves, $n = 17$; CT, $n = 6$; Vth, $n = 5$) affected the basal activity and the responses of each nerve in a specific way. The desensitization of CT responses by stimuli efficient on the Vth nerve, the effects of capsaicin on tastant responses, the effect of nerve sections on basal activity and on acid responses suggest functional interactions between taste and somatosensory systems. At high concentrations, acetic acid was more efficient relative to citric acid in HF than in LF subjects and acetic acid was more efficient relative to citric acid in the Vth nerve compared to CT in hamsters. It has previously been suggested that the hydrophobic acetic acid molecule has better access than the hydrophilic citric

acid to the epithelium and releases protons within taste receptor cells and nearby trigeminal nerve endings (Lyall *et al.*, 2001). Hence, a higher level of saliva production in HF subjects might result from a higher response level of the HF Vth nerve.

38. Mechanisms of intracellular Ca²⁺ level by umami stimuli

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Umami is one of the basic tastes, and is believed to act via a receptor-mediated mechanism. Monosodium glutamate (MSG) and inosine monophosphate (IMP) are umami substances and a mixture of these chemicals produces a synergistic effect. We have recorded the synergistic responses in single taste cells to glutamate agonist–IMP mixture using the whole-cell patch-clamp method and have demonstrated that G-protein was involved in the synergism mechanism of umami perception. We also observed that intracellular Ca²⁺ level of taste cells increased by umami stimuli using Ca²⁺ imaging. Two putative G-protein coupled receptors for glutamate have been identified: taste-mGluR4 and amino acid receptor T1R1/T1R3 heterodimer. However, precise transduction mechanisms are still unknown. In this study, we examined the participation of intracellular Ca²⁺ store in the umami transduction pathway. Taste receptor cells of mouse circumvallate and foliate which loaded the Ca²⁺ indicator Fluo-4 were stimulated by 10 mM MSG and a mixture of 10 mM MSG and 0.5 mM IMP by bath application. At the end of the series, the cells were stimulated by 60 mM KCl to ensure depolarization. When extracellular Ca²⁺ was present, MSG increased intracellular Ca²⁺ levels by bath application in many taste cells. Even though extracellular Ca²⁺ was not present, some MSG responsive cells increase intracellular Ca²⁺ levels. However, taste cells that respond to MSG only under Ca²⁺-free conditions were not observed. The same result was also obtained from stimulation with the mixture. These results suggest the possibility that there are two intracellular Ca²⁺ level increase pathways in umami transduction, namely release of Ca²⁺ from the intracellular store and influx of Ca²⁺ from extracellular sources in mouse taste cells.

39. Odour response spectrum of a population of genetically identified olfactory sensory neurons: characterizing the input to a single glomerulus in *Drosophila melanogaster*

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The olfactory system provides an animal with information about odours in its environment. The first processing center in this system, the olfactory bulb (OB, vertebrates) or the antennal lobe (AL, insects), is remarkably similar across species. A common feature is the spherical subunits called glomeruli. A single glomerulus consists of three cellular elements: the input, represented by the olfactory sensory neurons (OSN), the output (mitral and tufted cells in vertebrates, projection neurons in insects) and neurons of a local

network. The majority of OSNs converging in a particular glomerulus is believed to express the same receptor gene(s). Forty-three glomeruli have been described for the fruitfly *Drosophila melanogaster*. Knowledge about the input to a system provides the basis of understanding how information is processed within it. It is our long-term goal to know which odours are processed in each glomerulus at the input level. In order to achieve this goal we are currently characterizing the odour response spectrum of an exemplary OSN population in great detail. We use the GAL4-UAS system to express the calcium sensor *cameleon* under the control of the promoter of the olfactory receptor gene *OR22a* expressed in the OSNs converging into glomerulus DM2. This allows us to monitor odour-evoked activity *in vivo* by employing optical imaging, both at the level of the primary dendrites and somata on the antenna and in the presynaptic endings of the glomerulus. We have tested 100 odours from a variety of different chemical groups. We found graded responses to more than a third of the odours tested, indicating that *OR22a* has a broad response profile. The best ligands were ethyl and methyl hexanoate. Comparing the odour response spectrum measured on the antenna to that measured in the glomerulus will give us a first indication of whether the sensory input to the olfactory system is already altered before its first relay station.

40. Calcium plays a central role in insect olfactory transduction

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The role of Ca^{2+} in insect olfactory transduction was studied *in vivo* in *Spodoptera littoralis* (Lepidoptera, Noctuidae). The sensillar potential (SP) and the action potential discharge of olfactory receptor neurons (ORNs) were recorded in response to 200 ms stimulations with the main pheromone component (*Z9,E11-14:Ac*) using the extracellular tip recording technique. The $[\text{Ca}^{2+}]$ of the sensillum lymph was modified using electrolytes within the recording electrode containing 6.10⁻³, 10⁻³ or 2.10⁻⁸ M of Ca^{2+} . (i) With all three external $[\text{Ca}^{2+}]$ similar SP amplitudes were recorded. Thus Ca^{2+} influx is not required to generate a response. (ii) SPs were significantly prolonged at low extracellular $[\text{Ca}^{2+}]$, demonstrating the important role of Ca^{2+} in ORN repolarization. (iii) W7, a calmodulin antagonist, strongly inhibited responses recorded at both low and high external $[\text{Ca}^{2+}]$. Thus calcium—calmodulin complexes are necessary to initiate the response. Since at low (2.10⁻⁸ M) external $[\text{Ca}^{2+}]$ the required intracellular calcium elevation cannot be due to a calcium entry, it must originate from intracellular stores. (iv) The effects of an intracellular increase in $[\text{Ca}^{2+}]$ were tested inducing either a Ca^{2+} influx with a Ca^{2+} ionophore (A23187 tested in high external $[\text{Ca}^{2+}]$) or depletion of internal stores with thapsigargin (tested in low external $[\text{Ca}^{2+}]$). Both drugs did not modify the spontaneous firing of action potentials, thus Ca^{2+} alone cannot activate a response. (v) Following pheromone stimulation, thapsigargin did not modify SP amplitude while A23187 increased it. Considering that A23187 most likely induces a stronger intracellular $[\text{Ca}^{2+}]$ increase than thapsigargin, these data indicate that a Ca^{2+} rise amplifies the pheromone response. We propose that Ca^{2+} activates a protein kinase C in synergy with the pheromone-induced lipid messenger, diacylglycerol, and thus activates PKC-dependent ion channels.

41. Experience-dependent plasticity in the antennal lobe of *Drosophila melanogaster*

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The ability of the brain to adapt structurally and functionally in response to sensory stimuli is a striking property across animal phyla. Several studies have reported that continuous exposure to odors leads to global morphological effects in the first olfactory neuropil, the olfactory bulb of vertebrates or the insect antennal lobe. To investigate the specific effect of an odorant on the neuronal network it activates, we studied the plasticity in identified olfactory neurons with known odor response profiles in *Drosophila melanogaster*. Fruit flies are highly sensitive to CO_2 , which activates a population of ~25 olfactory sensory neurons that project to a single glomerulus in the antennal lobe (De Bruyne *et al.*, 2003, XXV AChemS meeting, no. 379). Using the GAL4/UAS system to visualize the different olfactory neurons innervating this specific glomerulus, anatomical changes on separate processing levels due to long-term CO_2 exposure were investigated. The results showed an enlargement of the CO_2 -glomerulus in a concentration-dependent manner. This effect was stimulus- and glomerulus-specific. To determine whether these stimulus-evoked changes in olfactory circuitry produced behavioral consequences, we tested olfactory-evoked locomotor responses to CO_2 and other stimuli. Flies showed a reduced sensitivity to CO_2 after CO_2 pre-exposure, but showed normal responses to all other odors tested. The behavioral changes were stimulus-specific and concentration-dependent. Importantly, both anatomical and behavioral effects of CO_2 exposure were reversible. We are in the process of identifying the cellular and molecular basis of these experience-dependent changes.

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42. Analysis of cellular mechanisms leading olfactory sensory neurons to simultaneously integrate several chemical signals in a mixture: patch-clamp electrophysiological study on slices of rat olfactory mucosa

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Odours present in the natural environment of animals and humans are mostly complex mixtures of several tens or even hundreds of compounds that the olfactory system recognizes and discriminates over a remarkably wide range of qualities and intensities. Single olfactory sensory neurons (OSNs) have been shown to simultaneously integrate two chemical signals of a binary mixture. Indeed, their electrical responses reflected the existence of complex interactions occur between compounds (Duchamp-Viret *et al.*, 2003, Eur. J. Physiol., 18: 2690–2696). Odour molecules interactions are thought to happen during the first steps of peripheral events, namely receptor binding and transduction. We plan to decipher some odour mixture interaction mechanisms by investigating the intracellular transduction currents initiated in OSNs. An

analysis and comparison of transduction current kinetics generated by single and binary molecular stimuli may provide insights in these interaction mechanisms. Furthermore, contribution of different transduction pathways and cross-talks between these pathways will be assessed by pharmacological tests. To date, transduction currents in mammals have only been studied in OSNs whose spike activity was suppressed by dissociation injuries. In order to optimize experimental conditions by preserving the physiological environment of the OSNs, we set up slices of main olfactory mucosa, a biological preparation rarely used in patch-clamp recording studies. Odour stimulations were performed through a multibarrel pipette from which single odours or binary mixtures were delivered by pressure ejection close to the cilia. OSN activity was recorded in both attached- and whole-cell configurations. In slices, we observed that OSN spike spontaneous activity is preserved. Our preliminary results have already led us to conclude that *in vitro* OSN spike responses to binary mixtures reflect types of interactions similar to those described *in vivo*.

43. Roles of taste nerves in the perception of bitterness

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There are many bitter tastants, ranging from toxins and medicines to food. These divergent bitter substances have their own intensity of bitterness. We examined whether each intensity of bitterness was discriminated in taste nerves. Nerve responses to various bitter substances were recorded from chorda tympani (CT) and glossopharyngeal (GL) nerve in C57BL/6J strain mice. Quinine hydrochloride evoked strong responses in both CT and GL nerves. On the other hand, denatonium benzoate, cycloheximide, isohumulones and caffeine evoked only small responses, although some of these compounds are remarkably bitter. Thus, both taste nerve responses did not correspond to the intensities of bitterness. These results suggested that perception of bitterness involved other mechanisms (e.g. somatic sensation, olfaction). In order to examine the relationships of taste nerves in signal transduction of bitter substances, the bilateral CT and GL of mice were transected (CTx+GLx). In two-bottle choice tests, the sensitivities of quinine hydrochloride and caffeine in CTx+GLx were reduced, indicating that the CT and GL nerves were important for their perceptions. On the other hand, the recognition of denatonium benzoate or isohumulones differed little between CTx+GLx and sham operation mice, indicating that other mechanisms were involved in their perception. These data suggested that not only taste nerves but also other nerves or factors played an important role in bitter perception.

44. Odorant responses of different subpopulations of olfactory receptor neurons in *Xenopus laevis* tadpoles

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It is known that in olfactory receptor neurons (ORNs) of aquatic animals, amino acids and nucleotides are potent stimuli. Using calcium imaging in slices of the olfactory mucosa we were able to show that ORNs of *Xenopus laevis* tadpoles also respond to amino acids

and nucleotides. Interestingly, ORNs that responded to amino acids did not respond to nucleotides and vice versa, i.e. two different subpopulations of ORNs are involved in the detection of these two groups of molecules. The application of forskolin, a known pharmacological agent activating the adenylate cyclase, evoked responses in a third subpopulation of ORNs. This shows that tadpoles of *X. laevis* have at least three subpopulations of ORNs and that both amino acids as well as nucleotides are not transduced by the well-described cAMP-dependent transduction pathway. To show that the three subpopulations of ORNs are connected to the olfactory bulb we imaged olfactory bulb neurons upon application of the three different stimuli to the olfactory mucosa using a nose-olfactory bulb preparation. Any of the stimuli evoked responses in the olfactory bulb. Furthermore, the nucleotide-responsive ORNs did not respond to well-known P2X and P2Y receptor agonists. This in turn shows that the reaction to nucleotides must be driven by different mechanisms, possibly by olfactory receptors specific for nucleotides.

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45. Electrophysiological correlates of behavioural responses to the mammary pheromone in rabbits: development from fetal to preweaning stages

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Newborn rabbits rely on odour cues to find the nipples and initiate sucking. One such cue, the mammary pheromone (MP), is emitted into milk and causes oral grasping movements in newborns. Here, we studied the development of that MP-induced behaviour and the electro-olfactogram (EOG) from prenatal to preweaning ages. The releasing effect of the MP was assessed in four groups of pups: (i) pups delivered preterm (E28) and never directly exposed to the MP ($n = 8$); term-born pups: (ii) on P0 ($n = 9$) after minimal exposure to the MP, (iii) on P4 ($n = 6$) before onset of hearing and sight; and (iv) on P28 ($n = 6$), 3 days before weaning at a stage when they consume more non-milk fodder than milk. Over 80% of the pups responded by oral grasping to the MP between E28 and P4, but this response vanished by age P28. The electrophysiological responses of the olfactory mucosa of rabbit pups were recorded at E28, P0, P4 and P28. The chemical stimuli, isoamyl acetate (control odorant) and MP, were obtained by gaseous licking of the pure compounds or their dilution (10–2 v/v) in mineral oil. They were delivered onto the olfactory mucosa of post-mortem animals. Three locations were systematically studied, one on the posterior part of the nasal septum, the other two on the medial aspect of the turbinates. At any age, the MP and the control odour elicited typical EOG responses. These data reveal that the behavioural-sensory mechanisms of the rabbit pups' oral reactions are predisposed. The response amplitudes to MP can reach >4.5 mV, and were location-dependent. The inter-individual variability of the amplitudes of the responses for both odours appeared to be high. As the EOG responses remained relatively high at P28, the decrease of the behavioural response at this age cannot be ascribed to a decreased sensitivity of the olfactory mucosa to the mammary pheromone.

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46. Physiological responses of olfactory receptor neurons in *Drosophila*: relating different measures

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The fly *Drosophila melanogaster* has become an important model species for studying olfaction due to the availability of genetic tools and the accessibility of its olfactory organs for physiological measurements. The entire olfactory input to the brain is represented by a relatively low number of sensory neurons on antennae and palps, which can be classified into ~40 coding units. Activity from olfactory receptor neurons (ORNs) can be recorded in three ways: extracellularly from single sensilla, from whole organs with glass electrodes and with optophysiological recordings. Action potentials (APs) of a small set of ORNs housed in a single sensillum, and sensillum potentials (SPs) can be registered by inserting an electrode at its base. Global activity in antennae and palps, i.e. electroantennogram (EAG) and electropalpogram (EPG), can be measured as voltage deflections, similar to vertebrate electroolfactograms, from an electrode on the cuticle. Finally, optical imaging of genetically expressed fluorescent indicators can report changes in intracellular calcium concentration. We have investigated how these different measures relate to each other and how well they reflect response parameters relevant for odor coding. We deduce the contribution of each ORN in a sensillum to the SP and relate it to AP frequencies. SP amplitudes correlate with AP firing rates in a log-linear way but absolute values and time course do not. We see a good correspondence between calcium increase and AP firing, though this is not directly reflected in the time course of the signal. Calcium signals at the level of the antenna (soma + dendrite) and glomerulus (axon terminals) are similar and reflect the dynamic range of dose–response relationships. We also compared EAG/EPG amplitude and time course for certain odorants to the expected summation of SPs from single sensilla. Discrepancies in EAG/EPG measurements and action potential firing rates of single ORNs in wildtype flies and mutants are discussed.

47. Olfactory receptor neurons tuned to linalool in moths and weevils: structure–activity relationships

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The monoterpene linalool is a common plant odorant synthesized by the terpene pathway in a variety of plant species. Its attractive effects on many herbivorous insects are well documented. Of particular interest in terms of olfaction is the chirality of this compound; the two enantiomers being synthesized by two different enzymes. In our laboratory we have used gas chromatography linked to single-cell recordings (GC-SCR) and mass spectrometry (GC-MS) to identify

functional types of receptor neurons. As test odours we used head-space samples and essential oils of a variety of plant species and chemical standards. Mixtures of volatiles were used as reference samples in studies of several insect species. Thus, we are able to compare across species the specificity of the receptor neuron types. We here present receptor neurons tuned to linalool, which were identified in three species of moths (*Mamestra brassica*, *Heliothis virescens*, *Helicoverpa armigera*) and one species of weevil (*Anthonomus rubi*). The receptor neurons showed enantioselectivity, responding best to one of the enantiomers. The presence of one or both of the (+)- and (–)-linalool receptor neuron types differed in the species of moths and weevil. In common for all the receptor neurons was a weaker response to another chiral compound. In one moth species (*M. brassica*) three other compounds eliciting weaker responses were also identified. So far, comparisons across species indicate similar specificity of the two enantioselective linalool receptor neuron types.

48. Inhibitory effects of glutamate receptor agonists on chorda tympani basal activity and responses to tastants

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The hamster whole chorda tympani nerve and fungiform papillae single units (Vandenbeuch *et al.*, 2004) were recorded during the application of various agonists and antagonists of brain glutamate receptors either on fungiform papillae taste pores or injected via the lingual artery. Both responses and long-term modulation of the basal activity were observed in chorda tympani and single unit recordings. Applying an electrode filled with glutamate receptor agonists or antagonists on taste pores elicited inhibitions (22/328) and activations (24/328) of the spontaneous activity of single fibers. Moreover, the iontophoretic application of the same agents induced 2 responses by activation and 13 responses by inhibition of single units recorded in a nearby papilla, out of 328 trials. Comparatively, 7 activations and 14 inhibitions out of 326 were obtained with monosodium glutamate (MSG) in the same conditions. Systemic stimulation with glutamate receptor agonists or antagonists induced an activation (7/95) or an inhibition (31/95) of the recorded unitary activity. Furthermore, whole chorda tympani responses to whole tongue application of NaCl or MSG were possibly reduced by systemic injection of these agents. The double apical and basal location of glutamate receptors is discussed, with different roles envisaged for glutamate acting both as a tastant and as a neuromodulation agent of chorda tympani responses to tastants.

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Central processing

49. Spatio-temporal activity evoked by the mammary pheromone in the main olfactory bulb of rabbit pups

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Rabbit newborns find the maternal nipples through the expression of a searching–oral grasping behaviour that is mainly released by

odour cues. A representative of these cues is the mammary pheromone (MP; 2-methylbut-2-enal) identified in rabbit milk. Since different results reveal that the accessory olfactory system seems not to be implicated in this odour-guided behaviour, we explored the spatio-temporal activity of the main olfactory bulb (OB) evoked by the MP, and by structurally related odorants that induce the neonatal behaviour (in lower proportion compared to the MP) or not. Experiments were performed on anaesthetized rabbit pups from d2 to d10 (d0 = birth). OB activity was recorded using 16 channel Michigan probes. From a gross broadband signal, both local field potentials (LFPs) and spiking discharges were extracted. Both time–frequency analysis of LFP oscillatory activities and mitral cell spiking discharges were performed with regard to the phase of the respiratory cycle. Odours were delivered with different concentrations of the saturated vapour pressure. Preliminary results show that (i) between d2 and d4, mitral cell spontaneous activity is almost absent; it appears around d5, along with odour-evoked responses; (ii) oscillatory LFP activity progressively develops from d2 to maximal amplitudes around d5; (iii) both odour-evoked LFP waves and mitral cell discharges are related to breathing; moreover, mitral discharges are most often phase-locked with LFP oscillations; (iv) oscillatory LFP activity greatly depends on odour concentration: long-lasting ~20 Hz oscillatory activity appears with concentration $>10^{-3}$; (v) spatio-temporal patterns of oscillatory LFP vary with odour quality; (vi) no specific pattern was observed in response to the MP, neither in LFP pattern nor in spiking activities. All these results suggest that the MP signal is not selectively processed in the pup OB.

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50. Electrophysiological changes in the vomeronasal system associated with mate recognition in mice

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The ability of female mice to recognize individuality chemosignals in their mate's urine is critical for their reproductive success by preventing the pregnancy blocking effect elicited by exposure to unfamiliar males. The chemosignals that enable this discrimination are dependent on MHC type and represent a mechanistic link between the representation of individuality at both immunological and behavioural levels. Learning about the strain identity of the mating male's urinary chemosignals occurs during a sensitive period of a few hours following mating and appears to involve changes in the inhibitory control of mitral cell projection neurons in the accessory olfactory bulb (AOB). The local field potential (LFP) recorded from the AOB of behaving mice was found to be dominated by theta oscillations, which increased in frequency and amplitude when male urine was presented. Lasting changes in the electrophysiology of the AOB were observed following mating, including a differential response to urine from the mating male compared to urine from an unfamiliar male. These changes were also reflected in the activity of neurons recorded in the medial amygdala, which fired at higher rates in response to unfamiliar male urine compared to mating male urine. These findings are consistent with the selective enhancement of inhibition of the mate's pheromonal signal at the level of the

AOB. This is proposed to underlie recognition of the mating male by selectively disrupting the central transmission of his pregnancy blocking signal, via the corticomедial amygdala.

51. Temporal sharpening of plant odour representation in populations of moth output neurons

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Plant-associated odours are represented as unique combinations of activated glomeruli in the moth antennal lobe, as observed by calcium imaging. The recordings have, however, been non-selective with respect to the neural elements involved. To overcome this limitation we have selectively stained a large population of moth antennal lobe output neurons, projection neurons, by retrograde filling from the inner antenno-cerebral tract with the calcium-sensitive dye FURA-dextran. We found that different odours evoked unique and reproducible patterns of activated glomeruli in a similar manner as seen in non-selective glomerular staining. Principally activated glomeruli had the same relative positions across individuals, suggesting a hard-wired organization also at the output level. Temporal resolution in the recordings was high enough to investigate the evolution of slow temporal patterns. Activity patterns were dynamic so that temporally late patterns were different from initial ones. All glomeruli responded with a fast transient increase in calcium concentration whereafter the decrease of activity differed between glomeruli and stimuli. Within the period of odour exposure, correlation of patterns evoked by different odours decreased as a function of time. Thus, there was a sharpening of odour representations over time. Our results suggest that olfactory information is contained in a code with both spatial and temporal components and that the discrimination ability may improve over time.

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52. Dynamic activation of neuron assemblies as a mechanism for odor encoding in the mammalian brain: an electrophysiological investigation in the rat olfactory bulb using LFPs and extracellular activity recordings

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The aim of our work was to study the encoding strategy used by the mammalian brain structures dedicated to olfactory perception to represent odorants. The works of Laurent's team has demonstrated that, in the insect, odor is encoded by spatiotemporal activity patterns across dynamic assemblies of neurons, synchronized in regard to an oscillatory global activity. We wondered if such a coding scheme could be found in mammals where different oscillatory regimes exist, including respiration-related modulation, and where anatomical organization is more complex. Our work was conducted on anaesthetized rats. Bulbar activity was recorded as a broadband

signal (0–3000 Hz) using multichannel recording probes positioned in the mitral cell layer. From this signal we extracted both action potentials from different single cells and local field potentials (LFPs). The odorants used were simple aliphatic compounds, varying in carbon chain length and chemical function. Data processing was conducted in two phases: LFP oscillatory activity was analyzed using morlet wavelet transformation and time–frequency representations to determine if the network activation pattern is odor-specific; then cellular synchronization in regard to the oscillatory regime of LFPs was quantified during odor response. Preliminary results indicate that LFPs present marked oscillations in the β (10–30 Hz) and γ (35–80 Hz) range. Plotting those oscillations on the respiratory cycle phases showed that different odorants elicited different LFP activity patterns.

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53. Modulation of evoked responses in the amygdala by odor emotional intensity in patients with temporal lobe epilepsy

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The odor hedonic judgment task was assessed in 34 control subjects and 43 patients with unilateral temporal lobe epilepsy. Stereotactic electro-encephalography (SEEG) recordings were taken in patients prior to surgical treatment. Twelve odorants including six pleasant and six unpleasant stimuli were successively presented 60 s apart in a pseudo-random order identical for all subjects. Each odor was administered monorhinally in the nostril ipsilateral to the epileptogenic focus, while the patients held the contralateral nostril closed with one of their fingers. A low-pressure airflow sensor was used to record breathing and monitor the inspiration phase. Subjects were asked to judge pleasantness using rating scales. Behavioral results showed that patients found odors slightly less emotional than control subjects. The amygdala SEEG activity associated with odorant stimulation was recorded in 17 patients. Nine patients were excluded from the study because of epileptic discharges during the test or frequent interictal spikes in the amygdala, and unclear respiratory signals precluding a reliable chemosensory evoked potential (CSEP) average. Electrophysiological recordings collected from the amygdala of eight patients demonstrated that CSEPs associated with unpleasant odors had mean peak amplitudes slightly higher than those obtained in response to pleasant odorants. No significant differences were observed for latencies between pleasant and unpleasant odorants. For each one of eight patients, relations between peak amplitudes and rating scale values obtained for 12 odorants were examined. Significant correlations were found for two subjects, showing that the more odors were found unpleasant by subjects, the higher the CSEP amplitudes. These data are discussed in relation to recent findings in cerebral imaging indicating that the amygdala participates in emotional intensity processing of odors, and is thus involved not only in intensity, but also in hedonic valence of odors.

54. Postnatal development of mitral cell properties in the olfactory bulb of young rabbits

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Within the first postnatal days, rabbits display a highly stereotyped search behaviour before they attach to a nipple. It has recently been shown that this behaviour is under the control of 2-methylbut-2-enal emitted in milk, and this compound has therefore named mammary pheromone (MP) (Schaal *et al.*, Nature, 2003). To understand the role of olfactory structures underlying the processing of the MP, we examined the functional development of mitral cells (Mc) over the first postnatal week (3–9 postnatal days), the pheromonal response being independent of the accessory olfactory pathways (Saucier *et al.*, ECRO, 2004). Anatomically identified Mc ($n = 26$) were investigated by performing whole-cell patch-clamp recordings from olfactory bulb (OB) slices. In newborn rabbits, small developmental changes were observed in action potential patterns during the first week. All Mc were able to generate trains of sodium spikes at birth and displayed membrane potential subthreshold oscillations in the 15–55 Hz frequency range (as a function of the membrane potential). We further observed that stimulation of the olfactory nerve evoked a prolonged glutamate receptor-mediated response in Mc as seen in Mc of adult rabbits. In contrast, GABA receptor-mediated inhibition generated in Mc developed slowly after birth. During the first week, stimulation of the lateral olfactory tract generated a glutamate receptor-mediated response that was not accompanied by a GABA_A receptor-mediated activation. Electrical stimulation of the OB inhibitory interneurons also failed to recruit a substantial degree of inhibition. Similarly, spontaneous GABA_A synaptic events were generated in Mc at a very low frequency during this period. Since GABA_A receptor-mediated inhibition plays a major role in sculpting neuronal response to odorant stimuli in adult rabbit OB (Yokoi *et al.*, Proc. Natl. Acad. Sci. USA, 1995), our results suggest that neonatal processing of the MP is supported by different mechanisms in the OB.

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55. Odour-induced c-fos expression in the rat olfactory bulb: influence of nutritional status

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Relations between nutritional status and olfactory perception were suggested from results of electrophysiological studies on rat olfactory bulb: in satiated rats, the multiunit olfactory bulb activity in response to food odour or to isoamyl acetate was similar, whereas it was enhanced in fasted rats only after food odour exposure (Royet and Pager, 1981). On the other hand, fasting induced an increase of mitral cell single-unit reactivity for both food and non-food odour (Apelbaum and Chaput, 2003). However, these observations were carried out in anesthetized animals. Therefore we have chosen to study responses to food and non-food odours in awake animals using c-fos expression as marker of neuronal activity. We have compared c-fos expression in olfactory bulb in response to food odour and to isoamyl acetate in male rats fasted for 4 or 48 h before odorant stimulation. Fos immunocytochemistry was carried out using

a polyclonal rabbit antibody (AB5, Oncogene Research Products) diluted 1/30 000. The Fos-immunoreactive neurons of olfactory bulb were quantified with a computer-assisted image analysis system (Biocom). The immunoreactive nuclei were counted, and the olfactory bulb area was measured to obtain a cellular density (Fos-IR cells/mm²). The Fos-IR cell density in response to food odour was significantly increased in rats deprived of food for 48 h (102 ± 2 versus 60 ± 10). This increase was observed in all layers of the olfactory bulb (periglomerular cells, tuft cells, mitral cells and granule cells). However, responses to isoamyl acetate were not different after 48 or 4 h of fasting (112 ± 11 versus 124 ± 8 , respectively). These observations are in agreement with the results of Royet and Pager (1981): fasting selectively modulates olfactory bulb responses to food odour.

56. Olfactory fear conditioning induces synaptic changes in the amygdala and piriform cortex in the rat

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Current theories of the neurobiology of memory suggest that memorization of information involves a distributed neural network including limbic as well as sensory areas, and occurs via durable changes of synaptic efficacy at specific nodes of this network. We investigated whether synaptic changes could be detected at different levels of the olfactory pathways, following an olfactory fear conditioning learning. On 17 rats, a bipolar stimulating electrode was implanted in the left olfactory bulb (OB) and four recording electrodes respectively in the ipsilateral anterior piriform cortex (aPC), posterior piriform cortex (pPC), anterior cortical nucleus of the amygdala (ACO) and basolateral nucleus of the amygdala (BLA). Nine rats were then trained with six pairings of an odor (isoamylacetate, CS) with a mild foot-shock (US). Eight rats served as control animals, receiving the odor with no association with a foot-shock. On the day after training, the animals were tested for their retention of the CS, assessed by the amount of freezing exhibited in presence of the learned odor. In parallel, evoked potentials (EPs) induced in the four recording sites in response to electrical stimulation of the OB were collected before training (baseline) and during the retention test. The amplitudes of the EP signals were measured and compared. The data show that learning was accompanied by a lasting increase (present before and during presentation of the CS) in EP amplitude in ACO. In addition, a transient increase was observed in pPC and BLA during presentation of the CS. These data suggest that in this task ACO, BLA and pPC could be differentially involved in recognition of the learned odor.

57. Kenyon cells in the moth, *Spodoptera littoralis*: morphology and physiology

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The mushroom bodies (MBs) are paired neuropils in the insect brain that receive input from several sensory modalities and are involved in the formation and retrieval of odor memory, but their di-

rect function is largely unknown. The MBs are constituted by a large number of tightly packed neurons, called Kenyon cells (KCs), that extend from the main input area, the calyx, via a stalk, the pedunculus, to the two main output domains, the vertical and medial lobe. In the current study, we apply a number of methods to study the function and morphology of KCs in a moth. We used whole-cell patch-clamping on an *in vivo* preparation of the intact moth brain in order to record and stain KCs. Cells were recorded under both voltage-clamp and current-clamp conditions. Both electrical stimulation via the recording electrode and olfactory stimuli delivered to the antennae were applied. All the recorded cells expressed voltage-activated currents upon depolarization. Most of them could sustain action potentials elicited by depolarizing current pulses in current clamp configuration. A minority of the recorded KCs responded to olfactory stimuli delivered to the antennae with depolarization and spikelets. The responding neurons had a wide response spectrum and responded to both blends and single compounds. Recorded cells were stained and later scanned in a confocal microscope. We also used transmission electron microscopy, immunohistochemistry and backfills from the antennal lobe (AL) and from the calyx of the MBs to study the morphology of and connectivity between AL projection neurons (PNs) and KCs. These experiments revealed a diverging connectivity pattern from PNs to KCs. In addition, we found a region of the calyces not innervated by olfactory PNs and discovered that KCs in the moth are arranged in two separate tracts. We hope to use the above techniques to further study and elucidate the role and function of the MBs in the moth.

58. Peripheral plasticity in the human olfactory system

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The aim of this study was to determine the location of changes in the olfactory system that occur during olfactory sensitization to androstenone. Of those people who are anosmic to androstenone, a proportion can acquire sensitivity to it by repeated exposure, and even those who are able to smell it can lower their threshold with this treatment. Olfactory thresholds were determined by a three-repetition, two-alternative forced-choice test of an ascending dilution series of androstenone and a control odour, amyl acetate. Subjects were then required to sniff androstenone daily (3 min, 3 times) for three weeks. A control group sniffed amyl acetate. Their thresholds for the odorants were determined at weekly intervals. At the same time their electrophysiological responses to androstenone and amyl acetate stimulation were measured. The electro-olfactograms (EOGs) were recorded using an intranasal electrode and the olfactory event-related potentials (OERPs) was recorded using EEG electrodes. The EOG measures peripheral events in the olfactory system and the OERP measures central plus peripheral events. The subjects' detection threshold to androstenone decreased following repetitive exposure to androstenone but not to amyl acetate. The androstenone threshold was proportional to the amplitude of the EOG recorded inside the nose and the EOG amplitude was correlated with the amplitude of the OERP. As the androstenone threshold decreased with repetitive exposure the olfactory evoked potential (EOG) and event-related potential (OERP) increased. No changes in either the EOG or the OERP were observed in response to amyl acetate. These observations support the

existence of odorant-specific plasticity in the peripheral olfactory system.

Symposium 1: Developing chemosensation and behavioural development

59. Human infants' memory for an odour acquired during breast-feeding: positive retention tests at 7 and 21 months

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This longitudinal study examines whether an odour associated with breast-feeding can be retained for a year or more. Previous studies had shown that odour exposure in the early feeding context could induce a shift in preference for that odour lasting for several weeks or months. Advantage was taken here of the local use of a camomile (Ca)-scented pomade prescribed for nipple protection. Two groups of breast-fed infants were compared for differential responses to Ca at 7 and 21 months, the Ca-exposed group (Ca+; exposure duration: 45 ± 39 days) and the Ca-naïve group (Ca-) which never experienced Ca in association with the maternal breast. At 7 months, both groups were videotaped while being presented in counterbalanced order two scented toys bearing either Ca or a scent of violet (V). The quantitative decoding of the videotapes revealed that Ca+ infants mouthed the Ca-toy longer than the V-toy, whereas Ca- infants explored orally the V-toy longer than the Ca-toy, and introduced both toys into their mouth for equal times. At 21 months, infants were tested for their differential choice and sucking of two bottles of water which were externally odorized with either Ca or V. It emerged that Ca+ infants chose the Ca-bottle more often than the V-bottle. In contrast, the Ca- group did not display any preferential choice in these conditions. These results confirm that infants are aware of odours during breast-feeding and further reveal that this context has a strong potency to reinforce the acquisition of odours, as long-term retention can be evidenced >1.5 years after the discontinuation of odor exposure. In sum, this experimental study emphasizes again the influence of such neonatal olfactory experience on the development of stable odour preferences in toddlers.

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60. Unique neurobiology of neonatal olfactory learning

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In a variety of neonatal altricial species, attachment is expressed as approaching the caregiver and tolerating considerable abuse to maintain contact with the caregiver. Due to the importance of attachment to survival, a brief period of facilitated learning generally underlies an infant's attachment formation to their caregiver. With some structures normally associated with learning nonfunctional (hippocampus, frontal cortex), the neonatal central nervous system most relies on a unique, specialized learning circuit. To explore the neural basis of attachment learning, we used

the infant rat mammalian model of imprinting, where pups exhibit an increased ability to acquire odor preferences and a decreased ability to acquire odor aversions. Thus far, our work has shown that the increased ability to acquire odor preferences is dependent upon the locus coeruleus (LC) releasing copious amounts of NE into the olfactory bulb. Termination of the rapid odor preference conditioning appears to be controlled by the emergence of LC alpha2 autoreceptors around postnatal day 10 that inhibit the LC and greatly reduces NE release. On the other hand, the decreased ability to acquire odor aversions appears to be due to lack of amygdala participation in at least some aversive learning situations (odor-shock 0.5 mA conditioning results in an odor preference). Amygdala participation in odor-shock conditioning appears to emerge in conjunction with pups' ability to easily learn an aversion from odor-shock conditioning. Together, these results indicate that the infant brain is designed to maximize attachment to the caregiver regardless of the quality of maternal care.

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61. Early odor preference learning and the cAMP/PKA/CREB cascade: new insights

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We propose a rat pup odor preference learning model in which the critical events underlying learning occur in mitral cells of the main olfactory bulb. Serotonin (5HT) depletion in the olfactory bulb prevents learning, but increased activation of beta-adrenergic receptors restores learning. We hypothesize that serotonergic and noradrenergic inputs act synergistically to elevate cAMP in mitral cells and that this mediates the unconditioned stimulus (US). Beta-1 and 5HT2a/c receptor co-localization on mitral cells reinforces our hypothesis. We find learning effective pairings of odor (CS) and US, but not ineffective pairings, activate CREB. CREB activation is greatest in the CS quadrant of the olfactory bulb. Virally transfected mutant CREB prevents learning under normally effective conditions, arguing for a causal role of CREB activation. We now show cAMP is also causal in odor learning. Inhibition of cAMP breakdown induces learning to an ineffective US and extends memory duration. The beta adrenergic agonist isoproterenol (ISO) exhibits an inverted U-curve dose-response relationship as a US for odor learning. We ask if spatiotemporal properties of cAMP recruitment can account for this curve. We find odor and a learning effective ISO dose (2 mg/kg) produces cAMP peaks and troughs at 5 min intervals. Work at a single time point showed a lower ISO dose (1 mg/kg) did not effectively elevate cAMP, but a higher learning ineffective dose (6 mg/kg) did not differ from the optimal dose. With multiple measurements we show that 6 mg/kg ISO produces a linear increase in cAMP, while 2 mg/kg ISO produces oscillations. If odor is not paired with 2 mg/kg ISO, cAMP is elevated, but oscillations are absent. Odor alone does not elicit significant oscillations. We conclude cAMP is causal in odor preference learning and cAMP waves, probably dependent on an interaction of calcium and cAMP, are critical and account for the inverted U-curve relationship between ISO and odor learning.

62. Olfactory sensory physiology and behavior during rat ontogeny

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The rat olfactory system is critical for survival of the newborn. Odor detection, discrimination and memory all play important roles in the neonatal response to the mother. In adult rats, the olfactory system is closely intertwined with the limbic system, with strong interactions between primary olfactory structures, such as the olfactory bulb and piriform cortex, and major limbic structures, such as the amygdala and hippocampus. While most of the major excitatory connections between these structures are present by the end of the first postnatal week in the rat, there is extensive elaboration and interneuron development extending well after birth. For example, mitral cells extend axons to both the piriform cortex and amygdala near birth, yet neurogenesis of the major inhibitory olfactory bulb interneuron, granule cells, does not peak until late in the second postnatal week and continues throughout life. These often dramatic postnatal changes in olfacto-limbic circuitry suggest there may be equally dramatic postnatal changes in central odor processing and behavioral odor discrimination. Using single-unit and local field potential recordings in the olfactory bulb, piriform cortex, amygdala and hippocampus, as well as behavioral analyses, we have begun a description of olfactory processing during early postnatal development in rats. Our results currently suggest that while some aspects of central odor coding emerge slowly during the pre-weaning period, behavioral odor discrimination ability is relatively mature by the end of the first postnatal week.

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63. Experience-induced modulation of olfactory processing: a developmental perspective

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One of the major contributions of olfactory research has been the finding that previous experience heavily modifies information processing at several levels of olfactory pathway, including associative cortices and so-called early stages such as piriform cortex and olfactory bulb. These observations contribute to the present general theory stipulating that memories are stored in sparse neural networks including limbic and sensory areas. However, this view mainly results from experimental data collected in adults. Since the postnatal period is associated with important changes in cortical circuitry, it is necessary to examine whether structures and mechanisms supporting olfactory memories evolve as a function of age. In this context the mammalian olfactory system offers a unique opportunity to raise this question. However, available data in rat pups and in adults are more often based on different behavioral paradigms and different measures of neural activities. The purpose of this work is to underline possible new approaches that could bridge the gap between the two sets of data. In agreement with what was found in pups, the ascending noradrenergic system originating from the locus coeruleus is of prime importance in adults for the expression of behavioral and electrophysiological correlates of olfactory learning. In adult rat, olfactory structures generate ongoing

prominent oscillatory activities which are modified in a clear-cut manner during olfactory sampling. Furthermore, olfactory learning amplifies these oscillatory responses. In adult, olfactory fear conditioning is very robust and associated with increased synaptic efficacy in several olfactory bulb projection sites. A better knowledge of developmental olfactory memory could thus rely on a simple olfactory learning task coupled with longitudinal studies of established neural correlates of learning in olfactory circuit.

Symposium 2: Gustation—from the periphery to the centers

64. The neural isoform of tryptophan hydroxylase is localized to a subset of taste bud cells

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Taste receptor cells are epithelial in origin and form a synaptic contact with innervating sensory nerves. The identification of the neurotransmitter(s) in these cells has, however, remained elusive, with several classical neurotransmitters being implicated. In addition, some of these transmitters may function as paracrine neuromodulators. A number of studies implicate serotonin as a candidate neurotransmitter, yet evidence for local synthesis in the taste bud is sparse. In the current study, reverse transcription polymerase chain reaction (RT-PCR) identified the neural isoform of tryptophan hydroxylase (TPH2) in rat foliate and vallate isolated taste buds and single taste bud cells. TPH2 is the rate-limiting enzyme that converts L-tryptophan to 5-hydroxy tryptophan, the immediate precursor of serotonin. Sequencing of 666 bp near the 3'-end of the taste TPH2 showed this product to have 99% identity with rat brain TPH2. The peripheral isoform, TPH1, could not be detected by RT-PCR in taste tissue, but was detected in control tissues. Immunocytochemistry localized TPH2 to a subset of taste bud cells located within the main body of the bud. Some of these appear to be type III cells. Little staining was seen in the perigemmal cells. Double immunocytochemical labeling demonstrated that gustducin and TPH2 very rarely label the same cell, whereas phospholipase C and TPH2 often overlap. The results demonstrate that serotonin is probably not synthesized in gustducin-containing type II cells, but may be synthesized in a subset of type III cells as well as in some perigemmal cells. Serotonin may, therefore, play both a paracrine and neurotransmitter role in taste.

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65. Amphipathic sweet and bitter tastants permeate taste cells *in vivo* and are inhibitors of G-protein-coupled receptor kinases (GRKs) *in vitro*: possible implications for delayed taste termination

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Some non-sugar sweeteners and bitter tastants produce a delay in taste termination, termed 'lingering aftertaste'. Although sugars

and non-sugar sweeteners appear to stimulate the same taste G-protein-coupled receptors (GPCRs), the delay in taste termination is uniquely related to the latter and to some bitter tastants. Interestingly, the same tastants can stimulate GPCRs that are not taste receptors (Zubare-Samuelov *et al.*, 2003, *Am. J. Physiol. Cell Physiol.*, 285: C1255–C1262.). These tastants are amphipathic and some were previously reported to rapidly permeate isolated taste-bud cells. We now report that when the entire tongue of anesthetized rats was stimulated by the sweetener D-tryptophan (30 mM) and by the bitter tastants quinine (2 mM) and cyclo(Leu-Trp) (2 mM) for 90 s, the resulting intracellular contents of these tastants in CV taste-bud cells were 9.9, 6.4 and 2.5 mM, respectively. Since GPCRs are often desensitized following phosphorylation by GRKs located in the cytosolic side of the plasma membrane, we tested the possibility that the amphipathic tastants inhibit GRK5 and GRK2, which are present in lingual cells. Depending on the kinase tested, some non-sugar sweeteners (e.g. cyclamate, saccharin, D-tryptophan, neohesperidin dihydrochalcone) and bitter tastants (e.g. caffeine, quinine, limonin, L-tryptophan, naringin), inhibit GRK2- and GRK5-phosphorylated rhodopsin (a GPCR) and protein kinase A-phosphorylated casein *in vitro*. Concentration dependence for certain tastants was evident, revealing 80–100% kinase inhibition. Due to the ability of tastants to permeate taste cells under physiological conditions, the hypothesis that they inhibit GRKs (and perhaps other kinases) directly and thus inhibit taste GPCRs desensitization and taste termination is proposed.

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66. What does your tongue smell?

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Odorant detection by olfactory receptors localized at the membrane of the ciliae of olfactory sensory neurons represents the first step of odorant perception, discrimination and coding. Similarly, perception of bitter, sweet, sour, salt and umami tastes involves taste receptors localized in the microvilli of sensory cells in gustatory papillae. All these chemoreceptors belong to the family of G-protein-coupled, seven-transmembrane segment receptors, with close to 1000 genes. Each olfactory receptor can detect odorants from a broad or narrow spectrum, and within variable ranges of odorant concentrations. An expression library constructed from rat olfactory epithelium was partly screened and five new olfactory receptors cloned. A comparison of these sequences, together with those of previously known rat olfactory and gustatory receptors from the ORDB, was performed. It provided figures for relative homology between the receptors, and the scores obtained for the distance between receptors allowed the building up of a phylogenetic tree. This analysis showed that some receptors could be both olfactory and gustatory, i.e. present both in the olfactory epithelium and in the tongue. Only some of the gustatory receptor families (GUST and TB) are concerned, the others being totally different. The tongue could thus participate with the nose in the detection of the same odors/savors.

67A. Tongue regional differences in responses to umami-tasting substances in mice

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Our previous electrophysiological and behavioural studies of umami taste have suggested that it is the glossopharyngeal nerve (GL) innervating the posterior tongue that plays an important role in umami-specific information rather than the chorda tympani nerve (CT) innervating the anterior tongue. To clarify the mechanisms underlying tongue regional differences in umami response, we investigated the response properties of peripheral taste system to monosodium glutamate (MSG) and 2-amino-4-phosphonobutyrate (L-AP4), an agonist for candidate umami receptor mGluR4, by electrophysiological and biochemical methods, and Ca²⁺ imaging in C57BL mice. The responses of the CT to MSG but not to L-AP4 were significantly suppressed by gurmardin, a specific sweet response inhibitor, indicating that MSG response may be partly mediated by sweet-best fibers. The umami information mediated by a recently cloned candidate receptor, T1R1/T1R3 heterodimer, might be conveyed by these fibers, because this receptor has been found to be predominantly expressed in the anterior tongue and coupled to gustducin. On the other hand, the GL responses to MSG and L-AP4 were hardly suppressed by gurmardin, suggesting that these responses may be conveyed by umami-best fibers, which are exclusively comprised in the GL. A biochemical study demonstrated that MSG stimulation significantly elevated inositol 1,4,5-triphosphate (IP₃) levels in taste papillae. Since both MSG and L-AP4 stimulation caused increment of [Ca²⁺]_i in taste cells, the increase in IP₃ levels may relate to intracellular signaling mediated by mGluR4. Whole-cell patch-clamp recording from isolated taste cells showed that L-AP4 induced not only outward currents but also inward currents with conductance increases at about resting potentials. These results strongly support the idea that phospholipase C activation and downstream signaling of mGluR4 are involved in the transduction mechanism for unique umami taste especially in the posterior tongue. It is also suggested that stimulation of mGluR4 might activate cation conductance as well as [Ca²⁺]_i elevation.

67B. Location of the primary gustatory area in humans, studied by MEG and fMRI

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Based on clinical studies of patients with lesions or epileptic foci in the parietal lobe, Bornstein and Hausser-hauw *et al.*, concluded that the base of the postcentral gyrus was important in gustation. Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies, however, could not observe activation in these areas, and have estimated the putative primary gustatory area (PGA) at the anterior–middle insula and the frontal operculum in humans, as in subhuman primates. Thus, there is a discrepancy between imaging results from PET and fMRI and clinical observations. Magneto-encephalography (MEG), on the other

hand, has fine temporal and spatial resolutions, effective in the localization of the primary sensory cortex. We measured gustatory-evoked magnetic fields using a tactile free taste stimulator, and found the shortest latencies of activation at the transition area between the parietal operculum and insula (area G) and at the bottom of the central sulcus (CS). These two areas are the most probable candidates for the PGA, in agreement with the aforementioned clinical observations. Area G was activated in different latency with different tastants, and the frontal operculum and anterior insula were activated long after area G activation. In addition, the magnitude of activity in area G increased in a tastant concentration-dependent manner to a greater degree than did perceived intensity. The latency, however, did not correlate with concentration. In another sensory modality, frequent repetition of stimulus is important for activation of the primary sensory area. We therefore tried to measure regional cerebral blood flow change under rapid and frequent taste presentation. The stimulation condition consisted of 16 trials of stimulations consisting of a pulse of 1 M NaCl (for 0.5 s) followed by three water pulses separated with an air bubble. The taste solution was replaced by water in the control condition. In 82% of the subjects significant activations (1% uncorrected) were found at area G on one or both sides of the hemisphere after individual analysis.

Symposium 3: Human olfactory memory

68. Age-related changes of chemosensory functions

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As already shown about 100 years ago, aging is accompanied by a decrease in intranasal chemosensory sensitivity. Numerous studies have confirmed this finding for various odorants and different olfactory tasks, e.g. odor identification, odor detection, odor discrimination or odor memory. Interestingly, these processes appear to exhibit differential changes in relation to age. These age-related changes are investigated by means of psychophysical, electrophysiological and imaging techniques. Anatomical correlates are found both at the level of the olfactory epithelium and, importantly, at higher centers involved in the processing of olfactory information as indicated by electrophysiological measures. Although considerably fewer data are available regarding age-related changes of intranasal trigeminal chemoreception, its function also appears to decrease in an age-related manner similar to what is seen in the olfactory system. Thus, aging of chemosensory perception seems to be a multi-factorial process with cognitive processes playing a major role.

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69. Odour-evoked memories are powerful, but only under the right conditions

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Autobiographical memories evoked by odours have the reputation of being more effective, longer lasting and more detailed than those cued by other sensory modalities. Our previous work has provided supporting evidence for these assertions but the issues of memory

accuracy and veracity are still crucial; how do we know that the greater amount of detail in odour-evoked memories is accurate detail? We presented participants with a series of naturalistic tasks and, after a delay of varying degrees, used equivalent cues from different modalities to probe memory for these tasks. Our results show that odours can indeed be powerful reminders of past experience, but also that there may be strict limitations on the circumstances under which odours can function effectively as autobiographical memory cues. Issues such as event selection and event participation are highlighted as being of importance in this regard.

70. Olfactory metamemory and odor identification

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Although many aspects of odor identification have received attention, the participants' own cognitions (metamemory) about their identifications have not. We demonstrate that the participants are over-confident in their odor identifications and further investigate the feelings of knowing often accompanying odor-naming failures. Maybe the best-known such aspect is the often-cited tip of the nose (TON) phenomenon (Lawless and Engen, 1977). The latter refers to the strong feeling of knowing an as-yet unidentified odor and is the olfactory parallel to the tip of the tongue (TOT) experience. We here present data showing that the TON experience does predict later recall, but is otherwise poorly related to any partial activation of the odor name or other information associated with the odor. A cross-modal investigation of naming of odors and pictures of famous people, matched for naming difficulty, revealed that even when people felt highly confident that they could immediately name the presented object, odors were to a much greater extent incorrectly named than pictures. Moreover, it seemed that the TON experience is more about trying to delineate the identity of an odor, rather than knowing what it is and searching for its name, as is typically the case with names of famous people. Although there might be a weak association between an odor and its name, as proposed in the literature, the data indicate that the poor naming performance is often due to an inability to even identify the odor. The results are discussed in relation to metamemory theory.

71. Why should durian be better recognized by French or American students than by Vietnamese students? The effect of typicality and culture on odor recognition memory

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Typicality and culture have a strong effect on face recognition: (i) atypical faces are better recognized than typical faces and (ii) faces of one's culture are better recognized than faces from other cultures. Can we expect similar effects in odor recognition? To address this issue, we designed two experiments with American, French and Vietnamese students using 20 fruit and 20 flower odorants. Some of the odorants were thought to be familiar to people of one culture and some were thought to be equally familiar to people of all three cultures. In the first experiment, participants from each culture rated

the familiarity and the typicality of the odorants on seven-point scales. In the second experiment, an independent group of participants from each of the three cultures performed a yes/no recognition task on the same set of odorants. A significant positive correlation between typicality ratings and false alarm rates was found for the French participants; whereas a positive correlation was found between typicality ratings and hit rates for the American and Vietnamese participants. For all three countries, no correlation was found between typicality and familiarity or between recognition performance and familiarity on the whole set. However, separate analyses for fruit and flower odorants showed a significant correlation between typicality and familiarity ratings for fruit odorants only. This pattern of results suggests that the relationship between typicality, culture and memory is more complex for odors than for faces.

72. The role of odours in odour memory

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In research on odour memory many approaches have been used that are mainly modeled on methods in verbal or visual memory. These approaches involved different encoding strategies and retrieval methods, varying from evaluative conditioning and simple recognition to complex autobiographical analyses and priming tasks. In all of these studies however, little attention has been given to the role that the odours themselves play in odour memory and via this memory in our normal daily life. Recent research on incidental learning in odour memory and in other perceptual areas that are also not as easily accessible by semantic interpretation as vision and audition seems to indicate that it is time to reconsider some of the basic principles on which most perceptual memory studies are based. Thus, it is clear that odour memory does not always depend on remembering earlier presented stimuli with any degree of precision, but rather depends on feelings of knowing and not knowing. Several recent findings in research on differences in odour and food memory between young and older subjects show that the differences found cannot be attributed to deterioration of working memory in the elderly as is often assumed in other fields of memory research. Since much effort is spent on precisely localizing the brain processes involved in odour perception and odour memory, it seems useful to reconsider some of the concepts and methods that have been borrowed from these other fields of memory research and to see what the limitations of their applicability in olfaction are. Some of the assumptions on which the search for brain mechanisms is based will be critically reviewed.

Symposium 4: Vomeronasal chemoreception

73. Morpho-functional investigations of the murine vomeronasal organ

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The vomeronasal organ of the mouse was investigated in embryos, and in prepubertal and adult individuals by means of immunohistochem-

istry, using antibodies against nitric oxide synthase type 1 (NOS-I), several neuropeptides, neurofilaments (NF) and α -gustducin. Results show that NOS-I is expressed in the nerve supply to the vomeronasal organ (VNO) as early as the embryonic (E) day 16 and in the vomeronasal nerve at E15. NOS-I immunoreactivity increases in the VNO as it develops in the pre- and postnatal life; it shows a peculiar distribution within the vomeronasal pump. Neuropeptides (Substance P, CGRP, NPY, ANP) appeared at different ages in the postnatal VNO; VIP was found also in embryos starting from E18. These results indicate that there is a timing of expression of neuropeptides in the VNO, which starts in the perinatal period and is possibly related to VNO functional maturation. The timing of expression of NF proteins in the developing vomeronasal and olfactory neuroepithelium was: internexin, then nestin, followed by NF-M. In the nerve supply of accessory structures of the VNO, nestin was followed by internexin and peripherin, and in turn by NF-L and NF-M. At the time of sexual maturity NF-M was only expressed in vomeronasal neurons. NF-M, NF-L and peripherin were expressed in extrinsic nerves supplying VNO structures. α -Gustducin is present in some bipolar cells within the proliferative VNO zone. These data offer new insights into the molecular maturation of VNO neurons and VNO supplying nerves. More recently the VNO was characterized by means of nuclear magnetic resonance (NMR) spectroscopy. Results demonstrate that several chemical constituents in the VNO show age- and sex-associated changes in concentration. Preliminary experiments also suggest modifications in the VNO constituents after pheromonal stimulation. NMR spectroscopy can thus serve as a useful technique to investigate vomeronasal chemoreception.

74. Some comparative morphological aspects of the vomeronasal system in mammals

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The vomeronasal nerves and the accessory olfactory tract establish the corresponding connections between the three elements that constitute the vomeronasal system. Among these elements the vomeronasal organ (VNO) and the accessory olfactory bulb (AOB) are the most studied from all points of view, and both structures show interesting morphological differences among mammals. Some of these differences are discussed here. In relation to the VNO, the possibilities of communication between the vomeronasal receptors and the outside are not uniform, and this represents a critical issue in the way the animals detect odours through the VNO; at the same time the soft tissue of the VNO is enveloped by a bone or cartilaginous sheet, which surely has a specific function. Concerning the AOB, the main difference is probably the size, form and general disposition of its cells and consequently of the appearance of its layers.

75. Natural ligands of hamster aphrodisin

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The chemical nature of vertebrate pheromones remains largely undeciphered. Hamster aphrodisin is considered as a rare instance of a mammalian sexual pheromone. This protein, found in vaginal secretions, facilitates the mounting behaviour of males via activation of a specialized sensory structure, the vomeronasal organ, which activates the

accessory olfactory bulb. Since it might carry small pheromonal ligands due to its lipocalin structure, we analysed organic extracts from natural aphrodisin. We identified five predominant compounds specifically bound onto natural aphrodisin as 1-hexadecanol (44.7%), 1-octadecanol (19.5%), *Z*-9-octadecen-1-ol (18.2%), *E*-9-octadecen-1-ol (15.4%) and hexadecanoic acid (2.2%). Interestingly these compounds are also described as insect sex pheromones, revealing an amazing coincidence of chemical communication shared by mammals and insects.

76. Pheromone receptors in vertebrates: the rule of diversity

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Vertebrates express a specific class of seven transmembrane receptors, from the V1r superfamily, in the olfactory system. These receptors are involved in the perception of pheromones, at least in the mouse. In this latter species, the V1r repertoire contains >100 different genes which pertain to multiple isolated families. The comparison of the repertoires of different species reveals that species-specific V1r genes, or even families, are found among closely related vertebrate species, indicating a very rapid evolution of V1r genes. These remarkable differences in the V1r repertoires may play a major role in pre-mating interspecies barriers.

77. Signal transduction mechanism in vomeronasal sensory neurons

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The mouse vomeronasal organ (VNO) plays an essential role in the detection of pheromonal cues mediating social behaviors and neuroendocrine changes. Vomeronasal sensory neurons (VSNs) respond to chemostimulation with a receptor-mediated excitation leading to increased action potential firing and elevated intracellular Ca^{2+} . Efforts to identify the molecular basis of this excitatory response have focused on a member of the TRPC subfamily of ion channel genes, TRPC2, which is expressed uniquely in the VNO, with a strikingly restricted localization to the sensory microvilli of VSNs. In an attempt to define the mechanism underlying TRPC2 activation and pheromone transduction, we show that TRPC2 is essential for a novel, 42-pS cation channel that is gated by the lipid messenger diacylglycerol (DAG), independently of protein kinase C and Ca^{2+} . Inside-out patch recordings from the dendritic tips of VSNs demonstrate that this channel is Ca^{2+} permeable but shows none of the hallmarks of store-depletion-activated Ca^{2+} entry channels. Activation of the DAG-gated channel is severely impaired in mice with a targeted deletion in the TRPC2 gene. Several lines of evidence indicate that pheromone- and DAG-activated membrane currents share the same properties. Using a pharmacological approach, we demonstrated that the activation of pheromone-gated conductance depends critically on phospholipase C but not on phospholipase A2 or DAG lipase, ruling out an essential role for arachidonic acid or other polyunsaturated fatty acids. On the basis of these findings we conclude that TRPC2 encodes a principal subunit of the DAG-gated channel and that this channel is the

primary conductance pathway in the chemoelectrical transduction machinery of mouse VSNs.

Keynote lecture 2

78. Activity dependence and functional significance of neuronal replacement in the adult olfactory bulb

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The olfactory bulb (OB) retains the ability to acquire newly generated neurons throughout life. Neural progenitors originate from stem cells located in the subventricular zone lining the lateral ventricles. They migrate rostrally before reaching their final position in the OB where they differentiate into local interneurons and establish connections with their neuronal targets. First, neural progenitors migrate tangentially, along the entire extent of the rostral migratory stream (RMS), and once in the bulb, turn to migrate radially out of the RMS and start to ascend to the outer layers. Here, we shall describe how the newly generated neurons integrate into a pre-existing neural network and how olfactory function is maintained when a large percentage of neurons are subjected to continuous renewal. Migration of neuroblasts in the core of the OB is partially orchestrated by a gradient of extracellular cues within the OB. The extent of the expression of this gradient correlates strongly with olfactory sensory activity. Furthermore, sensory-driven activity controls proliferation of neuronal precursors as well as the differentiation, maturation and survival of newly generated cells in the OB. Finally, the activity-dependent recruitment of newborn neurons is also related to changes in olfactory behavioral function. This demonstrates that olfactory neurogenesis may adjust the neural network functioning to optimize odor information processing. The persistence of a high level of inhibitory interneuron production within the OB throughout adulthood, and its conservation throughout evolution suggests that this process is of fundamental biological significance. Analyzing adult bulbar neurogenesis in different ethologically relevant contexts should provide new fuel to assess its functional role.

Oral session 3: Olfaction—central processes and learning

79. Functional properties of adult-born periglomerular cells in the mammalian olfactory bulb

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The subventricular zone (SVZ) produces neuroblasts that migrate to the olfactory bulb and differentiate into periglomerular (PG) and granule cells throughout postnatal life. While such postnatally generated interneurons have been characterized morphologically, their physiological differentiation has not been thoroughly described. Combining retroviral-mediated labelling of newly generated neurons with patch-clamp electrophysiology, we demonstrate that soon after

new cells enter the olfactory bulb they display voltage-dependent currents typical of more mature neurons. We further show that these 'newcomers' establish synaptic contacts with the existing neuronal network, and are responsive to GABA and glutamate. A significant fraction of PG cells is known to be dopaminergic (DA), but the functional properties of these cells have never been investigated. Using transgenic mice expressing a reporter protein (eGFP) under the tyrosine hydroxylase promoter we show that among the cells added in adulthood in the glomerular layer there are also DA neurons, of two different types. We have studied their properties, and we show that most of them (~60%) fire spontaneously in the absence of synaptic inputs. Both in slices and after enzymatic dissociation DA neurons generated action potentials in a highly rhythmic fashion at ~4–8 Hz. We found that interspike depolarization was driven by substantial components of TTX-sensitive, non-inactivating Na^+ current and voltage-dependent Ca^{2+} currents (L- and T-type), whereas no contribution of hyperpolarization-activated cation current (I_h) could be evidenced. Finally, the pace-making currents have been kinetically and pharmacologically characterized, and their interplay in the generation of rhythmic pattern has been reconstructed in numerical simulations.

80. Labelled lines in the fish olfactory system

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The long olfactory tracts found in species of some fish families (carp, catfish, cod) permit experimental manipulation. Discrete ablation experiments combined with studies of the behaviour in crucian carp reveal distinct loss of particular behaviours. The olfactory tract can be divided in three bundles, the lateral olfactory tract (LOT), the lateral bundle of the medial olfactory tract (IMOT), and the medial bundle of the medial olfactory tract (mMOT). When the LOT was sectioned the fish lost their feeding behaviour. When only this part of the tract was intact the fish performed normal feeding behaviour (Chem. Senses, 2001, 26: 1133). Sectioning the IMOT led to loss of the male reproductive behaviour. In fish with the IMOT intact the reproductive behaviour was present (Chem. Senses, 2003, 28: 293). The alarm reaction disappeared when the mMOT was cut and it was present when only this part of the olfactory tract was intact (Chem. Senses, 2000, 25: 103). These findings are congruent with the results from electrical stimulation of the different bundles of the olfactory tract in cod (Science, 1980, 207: 559). The results of these experiments suggest that each of the three bundles of the olfactory tract mediates particular behaviours attributed to fundamental life processes, namely nutrition, reproduction and predator avoidance.

81. Involvement of cortical and medial nuclei of the amygdala in olfactory memory for lamb

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Maternal behaviour in sheep is characterized by the rapid formation of a selective bond with the lamb. Within 1 h after parturition, mothers learn to recognize the individual olfactory signature of

their own lambs and subsequently allow them to suck while rejecting any alien young (maternal selectivity). The aim of this study was to investigate the implication of the cortical and medial nuclei of the amygdala in this olfactory learning using pharmacological reversible inactivation through lidocaine perfusion. Just before parturition, ewes were perfused for 8 h either with lidocaine (4%, 10 $\mu\text{l/h}$) or artificial cerebrospinal fluid (aCSF, control) either in the cortical, medial or basolateral nuclei of the amygdala. At 2 h, the proportion of selective mothers was lower in the cortical and medial groups (respectively 2/9 and 2/8) than in the basolateral (5/6, $P < 0.05$) or in the aCSF group (9/11, $P < 0.05$). This lack of selectivity still persists at 4 and 8 h although some degree of recovery was observed. Moreover, inactivation of these nuclei 2 days later, once the mothers were selective, has no effect on lamb recognition. Therefore both medial and cortical nuclei of the amygdala are involved in formation but not in retrieval of lamb olfactory memory. Our results emphasize recent data indicating that the amygdala could be a keystone in the memory circuitry of socially relevant olfactory recognition.

82. Associative learning of the plant odorants racemic linalool, β -ocimene and β -myrcene in the moth *Heliothis virescens*

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The importance of olfactory learning in host plant selection is well demonstrated in insects, including the tobacco budworm moth *Heliothis virescens*. In the present study olfactory conditioning of the proboscis extension response was performed to determine moths' ability to learn and discriminate three plant odorants: β -ocimene and β -myrcene, which activate the same receptor neuron type, and racemic linalool, which activates two different types (Røstelién *et al.*, 2000, J. Comp. Physiol.; Stranden *et al.*, 2003; Chemecol *et al.*, 2004, in preparation). An air puff with each odorant blown into a constant air stream directed over the antennae was used as conditioned stimulus, and sucrose solution applied first to the antennal taste sensilla, then to the proboscis, was used as unconditioned stimulus. Conditioning with increasing odour concentrations induced increased learning performance. The dose–response curves obtained for the three odorants in learning experiments correlated with those obtained in EAG experiments, showing the highest sensitivity for linalool. This also correlated with previous imaging studies of the antennal lobe (Skiri *et al.*, 2004, Chem. Senses). The ability of the moths to discriminate between the odorants was evaluated in differential conditioning tests with odour concentrations eliciting the same percentage of conditioned responses. The moths could discriminate all three odours, which was surprising since β -ocimene and β -myrcene activate the same receptor neurons. However, these two odours seemed to be more easily confused in generalization tests than the other odour pairs. The best discrimination was found with myrcene as the rewarded odorant and racemic linalool as the unrewarded. The opposite combination gave lower discrimination, indicating a higher salience for myrcene than for racemic linalool. The present results show that moths can be

used to answer precise questions about how olfactory learning performance in insects relates to odour detection, processing and perception.

Oral session 4: Taste, smell and trigeminal perception—psychophysics, interactions, plasticity

83. Clinical and sensory applications of the sniff magnitude test

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The sniff magnitude test (SMT) is a new approach to assessing the olfactory ability of humans based on changes in sniffing behavior that occur in response to an odor. The test is rapid, automated and simple, requiring only sniffs of canisters filled with odorized or non-odorized air. Sniff responses are measured using a piezoelectric pressure transducer that converts pressure changes at the external nares to digitized signals that are averaged across trials and compared for odorant and no odorant trials. Stimulus ratings or verbal responses are not evaluated. The test was developed as a clinical measure that is equally valid for young adults, children and the elderly owing to its minimal reliance on cognitive abilities. This characteristic also makes the test suitable for olfactory studies of people from diverse linguistic and cultural backgrounds. The utility of the SMT across cultures, with children and for the evaluation of medical patients has been demonstrated. Its use in fragrance and flavor research is also considered.

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84. Influence of the trigeminal component on odor detection processes

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In the nose, the ability to detect volatile chemicals is mediated by two separate but interrelated sensory pathways: the olfactory and trigeminal systems. Because most odorants stimulate both olfactory and trigeminal receptors, it is relevant to ask how these anatomically distinct systems could interact. In an effort to assess the influence of trigeminal activation on odor perception processes, two studies were performed basing on olfactory detection threshold (ODT) measurements. A first study (Brain Res., 2004, 1002: 51–54) explored the implication of the trigeminal component in the conscious and unconscious odor detection process. Three methods were used to determine, for three odorants, the ODT: psychophysical thresholds (using the double forced-choice method), psychophysiological thresholds (by skin conductance response measures) and thresholds based on subjects's self-evaluation (conscious detection thresholds). Nasal stimuli were chosen for their different levels of hedonic valence and trigeminal activation. Results showed, for each odorant, significant differences between psychophysical and self-evaluated thresholds, which suggested the existence of unconscious odor detection. Otherwise, it would appear that an odorant with intranasal trigeminal properties had the ability to produce a physiological response

while the subjects were not aware of the stimulus. A second study (C. R. Biol., in press) evaluated, for two odorants with different trigeminal properties, modifications of psychophysical thresholds, after trigeminal stimulation with allyl-isothiocyanate. ODTs were measured before and after trigeminal stimulation. Results showed that whatever the odorant used, trigeminal activation produced a decrease in ODT corresponding to an increase in olfactory sensibility. In physiological conditions the trigeminal system could modulate the activity of olfactory receptor cells. This function may explain the rise of olfactory sensitivity following irritant stimulation.

85. Setting up a labelled affective magnitude scale in several languages

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The nine-point category scale commonly used to rate consumer liking was developed by Peryam in the mid 1950s. It has the advantage of being simple to use but also has some disadvantages. First, it lacks sensitivity when comparing well-liked products. Second, it can produce biases such as the 'end avoidance effect'. Third, the intervals between categories may not be psychologically equivalent. In 2001, Schutz and Cardello proposed a Labelled Affective Magnitude (LAM) scale with 11 hedonic labels, nine of which came from the nine-point category scale. In this new scale, the hedonic terms were positioned along a vertical line proportionally to the average magnitude estimates given for each hedonic term. The LAM scale showed better discriminating ability for well-liked products. Using an intranet questionnaire, we evaluated inter-country and inter-individual differences in positioning of the hedonic terms on the LAM scale by speakers of Chinese, English, French, German, Italian, Portuguese, Russian, Spanish and Thai. We also compared results of respondents from different countries speaking the same language (e.g. USA and UK; French and Swiss-French; German and Swiss-German). In all, 1025 people completed the questionnaire with at least 40 respondents in each language. Not surprisingly, there were differences in perceived intensity of the translated hedonic terms between countries. For example, the term used to translate 'dislike very much' in Italian had a much weaker connotation than in other languages. There were also differences between English speakers in the USA and UK, with 'like moderately' expressing greater liking in the USA than in the UK. In contrast, French and Swiss-French speakers positioned the hedonic terms in a similar manner, as did German and Swiss-German speakers.

86. Experience-induced increases in taste identification for monosodium glutamate are reversible

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We showed previously that human taste identification ability for monosodium glutamate (MSG) varies with experience. Subjects identified MSG solutions at significantly lower concentrations after brief exposure each day for 10 days to shrimp crackers containing MSG than subjects who had been exposed to chocolate

candies without MSG (C. Kobayashi and L.M. Kennedy, 2002, *Physiol. Behav.*, 75: 57–63). Here we evaluated the temporal properties of such experience-induced change in a two-phase study. In phase I, two groups of 20 Americans and Europeans each underwent 10 days of brief exposure at home to treatment foods potato crackers with MSG (MSG group) or sweet potato crackers without MSG (control group). On day 11 or 12, all were tested in the laboratory with a forced-choice procedure. First, they tasted 5 mM MSG and were told ‘This is MSG.’ Then, they tasted three pairs of MSG and NaCl solutions of approximately equal taste intensity (0.63, 0.93, 1.25, 1.85, 2.5 mM) and indicated the MSG solution of each pair. The MSG group recognized MSG at lower concentrations than the control group did ($P < 0.05$, *t*-tests). For phase II, each group was subdivided: one subgroup continued the treatment food for 10 days (MSG- or control-continued group) and the other discontinued the treatment (MSG- or control-stopped group). On day 21 or 22, all subjects again were tested. Overall, there was a difference in the ability to recognize MSG among the four phase I and II groups ($P < 0.05$, ANOVA). The MSG-stopped group recognized MSG at significantly lower concentrations in phase I than phase II ($P < 0.01$), while the other three groups did not differ between phase I and phase II (contrasts, $P < 0.1$). These results confirm the phenomenon of experience-induced increases in the ability to recognize MSG and show that without continued experience, the effect is reversed.

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Poster session 2: Physiology and behaviour

87. Taste potentiated odor aversion in the rat: influence of rat strains and odor quality on learning

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When an olfactory cue (OC) is paired with delayed illness (DI), rats acquire relatively weak odor aversion. In contrast, rats develop strong aversion to OC paired with DI if presented simultaneously with a gustatory cue. Such a conditioning effect has been referred to as taste-potentiated odor aversion learning (TPOA). TPOA is an interesting model for studying neural mechanisms of plasticity because of its robustness and rapid acquisition. Nevertheless, as with many other behavioural tasks, some factors can influence both the acquisition and retrieval of TPOA. We investigated two critical factors: the nature of the OC and the influence of the rat strain on TPOA phenomenon. For this purpose we decided to induce TPOA in either Wistar (W) or Sprague–Dawley (SD) rats. For each type of rat we used either geraniol or benzaldehyde as OC. Benzaldehyde is widely used in aversive learning paradigms and geraniol is known as a non-trigeminal olfactory stimulus. As far as we know, no studies have used geraniol as a conditioned stimulus in TPOA learning. First, we checked if geraniol could induce a conditioned odor aversion (COA) with a delay of 30 min between OC presentation and DI. Geraniol was unable to induce COA with LiCl poisoning in the chosen delay. Geraniol could be used as OC for TPOA learning. Thirsty male rats were submitted to TPOA learning. Two days later, TPOA expression was assessed in presence of the odor that previ-

ously acquired an aversive value. The percentage of W rats showing aversion to geraniol as OC was higher than the corresponding percentage of SD rats (62% versus 40%). Comparison of the influence of the two OCs on TPOA expression in SD rats showed that benzaldehyde induced a larger TPOA percentage (80%) than geraniol. The present study showed that SD strain and benzaldehyde as OC are the optimum factors in TPOA learning.

88. Reactivation of an olfactory discrimination task and mapping of Fos expression activation in olfactory-related areas in the rat

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Fos protein immunodetection was used to investigate rat brain activation when an olfactory discrimination task which had been progressively acquired was reactivated 10 days later. Quantitative analysis of Fos expression was performed in the main olfactory cortical area—the piriform cortex (PCx)—as well as in the hippocampus and the ventrolateral orbito-frontal cortex (VLO), which are known to be involved in mnemonic processes. Trained rats (T) had to associate one odor of a pair with water-reward in a four-arm maze. Fos expression was studied either after completion of the acquisition (TA) or after reactivation of the task 10 days after learning (TR). At both stages, two groups of pseudotrained rats (P) were also used: Po+ rats were not water restricted and were submitted to the olfactory stimuli in the maze without any reinforcement, Pw+ rats were water deprived and systematically received water in the maze without any odorous stimulation. Home-cage control rats (C) were also used. When acquisition was completed, a significantly lower Fos labeling in TA rats compared to P rats was observed in most of the analyzed brain areas. This finding indicated a learning-related Fos immunoreactivity in these areas. Following reactivation, in both the anterior part of PCx and in CA3, TR rats were less labeled compared to Po+ rats. In VLO, TR rats exhibited a significantly higher Fos labeling than Pw+ rats. Comparison of Fos labeling between the two stages—acquisition versus reactivation—showed that TR rats exhibited a significantly higher Fos labeling than TA rats in most of the brain areas considered. In particular, an increased Fos expression was observed in TR rats in VLO. However, Fos expression in P rats remained almost similar on both stages. Thus, VLO area seemed to be specifically involved in reactivation. Our data support the assumption of a differential implication of brain areas in acquisition and reactivation of an olfactory discrimination task.

89. Evidence for a ‘stop’-mechanism for food-intake in man: sensory-specific satiety and unpleasant sensations limit ingested quantity prior to repletion

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Hedonic decline has been identified by three theories as a decisive factor in limiting food intake: alimentary alliesthesia (Cabanac); sensory-specific satiety (Rolls); conditioned satiety (Booth). However, the stimuli used in these studies were often of either prototypical (sucrose solutions) or complex (refined dishes or seasoned

mixtures of several foods) in nature. The objective of this work was to test olfactory food-selection and the coherence of taste and smell hedonics of plain foodstuffs, and to elucidate the importance of gastric distension, hedonic shift and specific satiety for a foodstuff, as well as unpleasant sensations in limiting food intake. We monitored taste and odour hedonics from 20 min before to 20 min after ingestion, using three parameters: olfacto-gustatory pleasure (OGP); specific appetite (SA); and stimulus-induced salivation (SIS). Fasted subjects ($n = 48$, age 17–53 years) were blindfolded and had to choose their favourite one out of six fresh foodstuffs (cucumber, tomato, pineapple, banana, peanut and pistachio) by their preferred odour alone. At 12 min before and 12 min following the meal, subjects were asked to rate the odour of the chosen food which was subsequently eaten *ad libitum* while reported taste ratings, ingested quantities and *spontaneous* unpleasant sensations were registered. Subjects then had to give up to five reasons why they thought they had stopped eating. They were again blindfolded and re-evaluated the smell of all six foods. The following results were obtained. (i) Initial olfactory and gustatory ratings correlated significantly but dissociated after the meal. (ii) The hedonic drop from before to after ingestion was very significant ($P < 0.001$) for all three variables (OGP, SA, SIS) and specific towards the foodstuff eaten. (iii) The appearance of unpleasant sensations was frequently reported: shift in taste quality, interdental residua, difficult swallowing, unpleasant oro-pharyngeal sensations to name but a few. (iv) The following reasons were given for stopping intake (overlapping): gustatory displeasure or neutral taste (66.7%); unpleasant sensations (58.3%); feeling of fullness (35.4% of the cases); specific satiety (12.5%). We conclude that these four phenomena—gradual shift from olfacto-gustatory pleasure to displeasure, unpleasant sensations, fullness and specific satiety—seem to be involved in the mechanism that makes us stop eating.

90. Prenatal ethanol exposure induces a conditioned preference for ethanol in the infant rat

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Previous studies have demonstrated that rats exposed to a moderate dose of ethanol during the last days of gestation (17–20) show higher ethanol consumption during infancy. It was hypothesized that this increased ethanol intake could reflect a conditioned preference established prenatally after the association between the chemosensory aspects of ethanol and its reinforcing properties mediated by the opioid system. Recent data from this laboratory indicate that when ethanol is administered to the pregnant dam together with Naloxone, the effect of enhanced ethanol intake is drastically reduced. However, taking into account that changes in the consumption patterns of a substance do not necessarily reflect changes in its palatability, the above-mentioned hypothesis was further investigated by evaluating not only ethanol intake but also the behavioral reaction to the taste of ethanol using a taste reactivity test adapted for infant rats. Wistar pregnant rats were intragastrically administered during gestational days 17–20 either water or ethanol (2 g/kg) followed immediately by a subcutaneous injection of either saline or Naloxone (10 mg/kg). On postnatal day 14 pups were tested in their behavioral reaction to the taste of either ethanol,

a solution of sucrose + quinine (which resembles the taste of ethanol in rat) or water. On the following day, the intake of those same substances was evaluated. Pups prenatally treated with ethanol and saline consumed more ethanol and displayed more mouthing in reaction to ethanol taste than those pups exposed to ethanol and Naloxone or than pups never exposed to ethanol before. Ethanol prenatal treatment also increased intake of the sucrose + quinine solution. These results, together with previous data from this laboratory, support the hypothesis of a conditioned preference to ethanol established *in utero* mediated by the opioid system.

Psychophysics/sensory interactions/chemistry

91. Perceptual interactions in binary odour mixture: a peri-threshold study

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In the field of aroma studies, and especially concerning wine bouquet, it is usually considered that the key odorants are those present above their odour threshold. However, in wine, a previous study suggested that the presence of odorants generated by oak wood at a concentration lower than their threshold values might have an impact on the global flavour of the product. In addition, it has been recently observed that woody notes in wine tend to dominate the fruity notes at supra-threshold concentration levels of both odorants. Starting from these observations, the aim of the present study was to evaluate the quantitative and the qualitative perceptual interactions in fruity/woody binary mixtures of wine odorants. Four successive dilutions of whisky lactone (woody note) at sub- or peri-threshold levels were mixed with one supra-threshold concentration level of isoamyl acetate exhibiting fruity notes. This supra-threshold concentration level was close to that usually found in wine. The perceived quality and intensity of nine stimuli (four concentrations of whisky lactone, one concentration of isoamyl acetate and their four possible combinations) were evaluated in five replications, by a trained panel of 13 subjects. From a qualitative point of view, the mixtures are perceived as fruity and woody simultaneously when peri-threshold concentrations of whisky lactone were mixed with supra-threshold concentration of isoamyl acetate. Concerning the odour–intensity interactions between two odorants, the results evidenced a significant synergic effect on the fruity notes, due to the presence of sub-threshold concentrations of whisky lactone. The observed perceptual interactions suggested that the knowledge of the odour threshold of a volatile component in the product is not sufficient to tell how much a particular component contributes to its total aroma in a mixture.

92. Assessment of trigeminal role on androstenone perception by means of lateralization judgments

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Different rates of specific anosmia to the odor of 5- α -androsten-16en-3-one (androstenone) have been reported in the general

population (E.A. Bremner *et al.*, 2003, *Chem Senses*, 28: 423–432.). Recent data in our laboratory suggested involvement of the trigeminal system in the perception of androstenone. The aim of this study was to determine whether the odor of androstenone has a detectable trigeminal component. Correct judgment of the side stimulated by a pure odorant when presented to only one nostril is not possible without stimulation of the trigeminal nerve (T. Radil and C.J. Wysocki, 1998, *Ann. NY Acad. Sci.*, 855: 641–644). Using a device that delivered an odorant to one nostril while providing equal airflow of unodorized air to the other nostril, subjects were given 40 stimulations (20 for each nostril) of androstenone (2.5 mg/ml diluted in propylene glycol), randomized for side of presentation. Twenty stimulations of full-strength cineole were used as a control for method validity since cineole is known to be a bimodal odorant (T. Hummel *et al.*, 2003, *Toxicol Lett.*, 140–141: 273–280). Interstimulus intervals of 60 s were used in order to allow the trigeminal system to recover fully. Two-tailed *t*-tests showed that when subjects' correct lateralization scores were compared to chance levels they correctly identified the stimulated nostril better than chance for both androstenone ($P < 0.01$) and cineole ($P < 0.01$). Implications of this finding include a possible explanation for the discrepancies regarding specific anosmia rates to androstenone. Specifically, the present data suggest that a non-detection rate when determined using crystal androstenone may be influenced by trigeminal stimulation, hence resulting in a high rate of detection, whereas methods using diluted androstenone and reporting anosmia according to detection thresholds are based on olfactory perception.

93. Prevalence of olfactory dysfunction: the Skövde population-based study

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Patients with olfactory dysfunction appear repeatedly in ear, nose and throat practices, but the prevalence of such problems in the general adult population is not known. The objective was therefore to investigate the prevalence of olfactory dysfunction in an adult Swedish population, and to relate dysfunction to age, gender, diabetes mellitus, nasal polyps and smoking habits. We therefore undertook a cross-sectional, population-based epidemiological study. A random sample of 1900 adult inhabitants, stratified for age and gender, was drawn from the municipal population register of Skövde, Sweden. Subjects were called to clinical visits that included questions about olfaction, diabetes and smoking habits. Examination was performed with a smell-identification test and nasal endoscopy. In total, 1387 volunteers (73% of the sample) were investigated. The overall prevalence of olfactory dysfunction was 19.1%, comprising of 13.3% with hyposmia and 5.8% with anosmia. A logistic regression analysis showed a significant relationship between impaired olfaction and aging, male-gender and nasal polyps, but not to diabetes or smoking. In an analysis of only individuals with anosmia, diabetes mellitus and nasal polyps were found to be risk factors, while gender and smoking were not. The sample size of this population-based study was adequate, with a good fit to the entire population, which suggests representativity for the Swedish population. Prevalence figures of various types of olfactory dysfunction could be given with reasonable precision and suggested risk

factors analyzed. The lack of a statistically significant relationship between olfactory dysfunction and smoking may be controversial.

94. Rational design of new cooling compounds

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Beside the fundamental tastes, the chemesthetic sensations generated by hot and cooling compounds contribute to the overall taste profile of a foodstuff. The use of hot and cooling ingredients in foods is widely used all over the world and the food industry has a great interest in their applications. Cooling compounds have also other important applications in the pharmaceutical, cosmetic and cleaning products industries. Two chemesthetic receptors for hot (VR1) and cooling compounds (CMR1) (D.D. McKemy, W.M. Neuhausser and D. Julius, 2002, *Nature*, 416: 52–58) have been identified, and their study is quite important not only for their role in gustation but also for their relationships with the mechanism of some neurological diseases and nociception. The leading cooling compound is (–)-menthol; it is the most widely used cooling agent in the pharmaceutical and food industry but its very low olfactory threshold for minty flavour is a serious limitation for its application as a food additive. Few other classes of compounds are known to have a cooling effect. Among them there are icilin, a synthetic compound discovered by Wei in 1983 (E.T. Wei, 1983, *J. Pharm. Pharmacol.*, 35: 110–112), and the cyclic α -ketoenamines recently isolated from roasted dark malt and then synthesized by Hofmann (H. Ottinger, T. Soldo and T. Hofmann, 2001, *J. Agric. Food Chem.*, 49: 5383–5390). We started a systematic study of all known cooling compounds in order to develop some structure–activity relationship of general application. We compared the molecular feature of many compounds in order to look for the functional groups that could be related to the cool sensation; we also looked for other characteristics, such as lipophilicity, electronic and steric parameters, that could be used in (Q)SARs. Based on the information obtained, some new compounds have been designed and synthesized. The new compounds have been analysed by means of sensory analysis. For this aim, a panel has been previously trained using menthol as a standard for the determination of the cooling activity.

95. Time-course of intensity and pleasantness perception in response to chemosensory stimuli presented with a paradigm designed for fMRI experiments

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Functional magnetic resonance imaging (fMRI) studies of taste and retronasal olfactory perception have benefited from the use of templates based on the time-course of perceived intensity during a functional run (Faurion *et al.*, 1999; Cerf-Ducastel and Murphy, 2001; Cerf-Ducastel *et al.*, 2001). However, as different cognitive tasks, such as pleasantness processing of taste and smell stimuli, are being studied with fMRI the question arises as to

whether different templates need to be considered to process the results of those experiments. The present study examined perception profiles based on the continuous evaluation of either intensity or pleasantness of taste and smell stimuli. Eight subjects (four female, four male) produced perception profiles using a linear potentiometer connected to a computerized interface. Stimuli included one odor, i.e. ethyl butyrate (EB, fruity), and one taste, i.e. sucrose (sweet), dissolved in water and presented to the subject's mouth according to a stimulation protocol adapted to fMRI testing (50 μ l/3 s, three ON-OFF cycles, 18 s ON stimulus, 75 s OFF water). For each subject, six intensity and six pleasantness profiles were recorded for each stimulus in two sessions using labeled magnitude scales (Green, 1997). All subjects produced intensity perception profiles with three peaks corresponding to the three ON periods for both EB and sucrose. However, for pleasantness profiles, only three subjects produced profiles with all peaks for EB and seven subjects for sucrose. This result probably reflects the moderate hedonic valence of the stimuli, at the chosen concentrations, and shows the limitation of profiles solely based on pleasantness. Repeated-measures ANOVA showed that there was no significant difference in the latency of peaks between intensity and pleasantness profiles for either EB or sucrose, which suggests that the time-course of intensity perception might be a satisfactory approximation of the time-course of pleasantness.

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96. Effect of air pollution on olfactory function in residents of Mexico city

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To our knowledge there has been no study of the effect of everyday air pollution on olfactory function. It was therefore the aim of this study to compare the olfactory performance of long-term residents of Mexico City (MC)—an environment with high air pollution—with the olfactory performance of residents of the Mexican state of Tlaxcala (Tx)—a region culturally and geographically similar to MC but with low air pollution. Healthy volunteers (MC $n = 82$, Tx $n = 86$), aged 20–63 years and balanced for gender, were tested for the perception of the odors of everyday beverages presented in squeeze bottles. When tested with ascending concentrations of stimuli in a three-way oddball paradigm, residents of Tx detected the odor of an orange juice preparation (Clight) and of Nescafé at significantly lower concentrations than residents of MC. They could also attribute a quality to and then finally correctly identify the stimuli at lower concentrations. However, differences between the groups decreased across the three tasks, suggesting the increasing participation of central, cognitive processes unimpaired by pollution. Residents of Tx also performed significantly better in discriminating between two similarly smelling Mexican beverages, horchata and atole, in oddball tests. Significant differences between the two populations were apparent even in the youngest subjects. No significant differences were found between sexes. Thus, air pollution in Mexico City appears to have a substantial impact on peripheral olfactory function, even in young adults.

97. Relationship between pleasantness and intensity in individual compounds and binary mixtures

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The nature of the relationship between intensity and pleasantness in olfaction is still controversial. According to one view, these are highly correlated, whereas according to another, they can be dissociated and varied independently. We explored this question using individual compounds and binary mixtures. In experiment 1, participants ($n = 20$, 10 female) rated 12 compounds in five concentrations each, for intensity and, independently, for pleasantness. We found that intensity and pleasantness were negatively correlated for unpleasant odorants, and for two neutral odorants. Whereas two other neutral and one pleasant odorant did not show any relationship between these two odor properties, two pleasant odorants displayed a positive correlation between them. In experiment 2, we selected six individual compounds, three pleasant and three unpleasant, that were iso-intense. We used a substitution method (Olsson and Cain, 2000) to create nine series of binary mixtures of each combination of pleasant and unpleasant compounds. Thirty participants (15 female) rated these binary mixtures for intensity and pleasantness. For four series of mixtures, we were able to demonstrate that perceived intensity remained constant while perceived pleasantness increased systematically with the increasing proportion of the pleasant odorant in the mixture. We concluded that the relationship between intensity and pleasantness may be different depending on an odor's pleasantness: while the intensity of unpleasant odorants is negatively related to their pleasantness, this is not necessarily the case with neutral and pleasant odorants. Our findings with binary mixtures confirmed that intensity and pleasantness can be clearly dissociated and experimentally manipulated in olfaction.

98. Cognitive effects on subjective intensity under intermittent presentation of short-duration odor

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Cognitive aspects of human odor perception and adaptation have been of interests to many researchers, and it has been reported that different instructional contexts for odors, such as 'healthy' or 'hazardous', affects the quality perception and adaptation/habituation process (Dalton, 1996; Sakai *et al.*, 2004). These previous studies have employed continuous odor exposure technique, so the odor was presented continuously during experiment. In the present study, a short-duration odor was presented repeatedly. We hypothesized that short-duration stimulation would allow for clearance of the odor at the receptor level, so central involvement of odor perception and cognition could be emphasized, excluding the peripheral involvement of odor perception. Odor was presented using the olfactometer developed by Kobal and his colleagues. Anethole, an odor unfamiliar to Japanese people, was presented 60 times within four sessions, and each stimulus duration was 200 ms. The inter-session interval was 3 min. The participants were given either 'healthy'

or 'hazardous' information on the odor, and then required to evaluate the odor intensity continuously using a sliding lever. Participants with 'hazardous' bias evaluated the odor as more intense as compared with those with 'healthy' bias. The 'hazardous' bias group perceived the odor as more intense than the 'healthy' bias group in the latter two sessions. In the present study, cognitive effects on subjective intensity under intermittent presentation of short-duration odor were investigated. The negative bias on anethole increased its subjective intensity. And the intensity did not depreciate across sessions compared to that of the positive bias group. These results are consistent with Dalton (1996). However, we obtained more robust effects of cognitive involvement by employing the short-duration, repeated-presentation method.

99. Solvation parameters: improvements in the definition and easy generation. Application to olfaction

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It has been known for a long time that the specificity of olfactory neuroreceptors in vertebrates is low (R.C. Gesteland, J.Y. Lettvin, V.S. Pitts and H. Rojas, 1963, in Y. Zotternam, ed., *Olfaction and Taste I*. Pergamon Press, Oxford, pp. 19–34). It has been recently demonstrated that the specificity of the protein olfactory receptors (ORs) is also low (B. Malnic, J. Hirono, T. Sato, L.B. Buck, 1999, *Cell*, 96: 713–723; W.B. Floriano, N. Vaidehi, W.A. Goddard, III, M.S. Singer and G.M. Shepherd, 2000, *Proc. Natl. Acad. Sci. USA*, 97: 10712–10716), confirming that the global olfactory recognition cannot result from a giant combinatorial of hundreds or thousands of highly specific signals, but rather from a limited number of keys of recognition numerically modulated (an alphanumeric coding: P. Laffort, 1994, in G. Martin and P. Laffort, eds, *Odors and Deodorization in the Environment*. VCH, New York, pp. 105–141). It has also been suggested for a long time that these keys of recognition could be the same as those involved in solubility phenomena and therefore could be experimentally determined. This approach, started by Dravnieks and Laffort in 1972, has been progressively refined by several authors, using various experimental measurements including gas–liquid chromatography (A. Dravnieks and P. Laffort, 1972, in D. Schneider, ed., *Olfaction and Taste IV*. Wissens-Verlag-MBH, Stuttgart, pp. 142–148). It has been shown that five independent solvation parameters—dispersion, orientation, polarizability-induction, acidity and basicity—are needed and are sufficient to account for the entire solubility phenomena. The aim of the present study is to: (i) test the several sets of solvation parameters already published and, if possible, improve them; (ii) update an easy method of generating optimized values, principally by using a simplified molecular topology; (iii) present an application to olfactory experimental data.

100. Perceptual interactions in odour mixtures: impact of ethanol on the perception of woody/fruity mixtures

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Perceptual interactions have been previously observed in binary mixtures of woody and fruity odorants of wines (B. Atanasova,

D. Langlois, S. Nicklaus, C. Chabanet, T. Thomas-Danguin and P. Etiévant, 2003, in A. Lonvaud-Funel, G. de Revel and P. Darriet, eds, *Oenologie 2003: 7e Symposium international d'oenologie*. Tec & Doc Lavoisier, Paris, pp. 560–562). These authors indicated a tendency of the woody notes to dominate the fruity ones. However, in wine many other compounds could have an impact on the bouquet. In this context, ethanol is particularly interesting because it is found at a constant level in wines and activates both olfactory and trigeminal systems. Therefore the aim of the present study was to evaluate perceptual interactions in ternary mixtures and especially the influence of ethanol on the perception of mixtures of woody and fruity odorants. Four concentration levels of whisky lactone (woody note or 'coconut') and isoamyl acetate (fruity note or 'banana') and their 16 possible combinations in which ethanol has been added at a 12% constant rate have been tested. A panel of 15 trained subjects evaluated overall intensity, quality and the intensity of each note ('coconut', 'banana' and 'alcohol') for each stimulus. The data are compared to results obtained in which samples were free of ethanol, but in which headspace concentrations of fruity and woody odorants were matched.

101. Quantification of olfactory perception based on psychophysical profiles—a detailed discussion with color perception as an analogue

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In recent work we projected an olfactory perception database onto a low-dimensional map. We developed a framework consisting of several data analysis methods, such as multidimensional scaling and self-organizing maps, to attain a topological conservation of the given data in low dimensions. On the way to understanding principal features of olfactory perception, we furthermore attempted to estimate the complexity of this sense, i.e. the dimensionality of the given data space, as accurately as possible. To date, most efforts, focusing on the organization of receptors or neural responses, have constructed maps based on general properties of chemicals. However, there is still no approach that succeeds in predicting olfactory perception based solely on the structure of the chemical stimuli. The hypothesis we continue to pursue—that human olfactory perception reflects the metabolic relationships between molecules in the natural environment—has been strongly supported by this new mapping framework and potentially represents a major step forward in understanding olfaction in general. To test how capable our framework is with respect to extracting significant features of human perception in general, we set up a psychophysical experiment in which human subjects were asked to describe their color sensations using words. These descriptors were chosen to be comparable to the descriptors used to describe olfactory sensations—including hedonic and emotional labels. We used the resulting data to describe color perception without any knowledge about the underlying physical or physiological processes, just based on the psychophysical description data. In this case, we were able to validate this map against everything we know about the perception of colors. These results appeared to be promising on the way to describing senses that we do not yet understand in such detail, particularly olfactory perception. We will present our data analysis framework and the application of this framework to both color and olfactory perception.

102. Ambient odors modulate the amplitude and time-course of visual attentional capture

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Sudden visual events capture attention involuntarily because they may signal potential threats. Some theoretical accounts consider that the biological significance of these events is established through the limbic structures. Thus, the manipulation of the limbic activity would affect attentional capture. Since these structures are directly linked to the olfactory system, we have tended to modulate their activity with olfactory stimulations. We have examined behavioral performance in two tasks of attentional capture by luminance under conditions of ambient odors (no-odor, PEA or AIC). In experiment 1, we found that the amplitude of attentional capture was indeed modulated by ambient odors, and in experiment 2 we found that this effect reflected the time-course of attentional capture as well. The modulation of visual attention by ambient odors depended on the odor's properties.

103. Olfactory function in children evaluated with psychophysical and electrophysiological measures

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The aim of the study was the evaluation of olfaction in children from 3 to 12 years old by means of psychophysical and electrophysiological examinations. A total of 146 children in this age group participated in the psychophysical examinations. Testing was completed in 111 children (52 girls, 59 boys). In 35 cases (14 girls, 21 boys) testing was aborted as children did not understand the testing procedure or were not motivated. Testing of threshold, discrimination and identification of odorants were performed by using the 'Sniffin' Stick' test battery. The psychophysical testing revealed a significant increase of the results with increasing age for threshold, discrimination and identification of odorants. Major differences were seen when considering the group of 3–5 year old children. Differences of gender were only seen when odorants were named spontaneously, as girls performed better in this task. Hyposmia in the 3–5 year old children was at 4.75 for threshold, 7 for discrimination, 6 for identification and 20 for the sum of threshold, discrimination and identification. In the 6–8 year old children hyposmia was at 9, 9, 7 and 25, and for the 9–12 year old children 9, 10, 9 and 27. Considering subjects of different age groups above the 50th percentile, the results were significant only for threshold testing. Within the scope of electrophysiological examination, olfactory event-related potentials could be recorded in 12 children from 3.5 to 10 years old (6 girls, 6 boys). H₂S was used for olfactory stimulation (odorant presented by an olfactometer into the left nostril, stimulus duration 200 ms, ISI 30 s). EEGs were obtained at midline recording sites. Children were divided into three groups (≤4 years, 5–7 years, 8–10 years), four children per group. The largest amplitudes PIN1 and N1P3 were seen in the youngest children. With increasing age latency times became longer, which was most pronounced for the P3 latency [F(2,11) = 3.96, P = 0.058]. These results indicate that psychophysical and electrophysiological measurements of olfaction

in children are possible. OERP-recordings indicate that from the 3rd to the 10th year of life important changes in the processing of olfactory information do occur. The results of the psychophysical testing seem to be strongly influenced through attentional changes of children of different age.

104. Monosodium glutamate and saltiness: biomolecular analysis of psychophysical evidence

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Monosodium glutamate (MSG)-induced enhancement of saltiness was evaluated using conventional psychophysical methodology (converging limits, CLs). Stimuli: aqueous solutions of NaCl, NaCl + MSG, NaCl + KCl, NaCl + MSG + KCl, KCl, MSG and KCl + MSG. Concentrations: NaCl 12.5, 25, 50, 100, 200 mM; KCl:NaCl ratio 2:1; MSG 4.32, 10.80, 27.00, 67.50, 168.75 mM (27 mM in mixtures). CL estimations were plotted against concentration, and psychophysical slopes (*B*, Steven's coefficient) were calculated. Results were analysed by two-way ANOVA followed by the least significant difference test (LSD). *B* values, *r*² (regression coefficient) were: 0.80 ± 0.06, *r*² = 0.98 (NaCl); 0.60 ± 0.01, *r*² = 0.99 (NaCl + MSG); 0.69 ± 0.04, *r*² = 0.99 (NaCl + KCl); 0.65 ± 0.04, *r*² = 0.99 (NaCl + MSG + KCl); 0.49 ± 0.05, *r*² = 0.97 (MSG); 0.66 ± 0.02, *r*² = 0.99 (KCl); 0.58 ± 0.04, *r*² = 0.99 (KCl + MSG). Concentration (*P* < 0.0001), panellist (*P* < 0.0001) and salt (*P* < 0.001) affected *S* and salt type interacted with concentration (*P* < 0.0001). KCl and (NaCl + MSG + KCl) were different to MSG, NaCl and NaCl + MSG (LSD, *P* < 0.05). Results showed that *B*_{NaCl} > *B*_{KCl} > *B*_{MSG}. MSG or KCl flattened the response to NaCl, and MSG specifically affected NaCl. Addition of MSG to NaCl + KCl mixtures did not enhance saltiness. *B*_{NaCl} decrease induced by MSG or KCl resulted from increased saltiness at low NaCl levels. Data analysis according to Beidler's equation (tastant–receptor binding model for taste) suggested a likely biophysical interaction with Na⁺ binding sites in taste cells resulting in increased binding affinity and saltiness enhancement by MSG. This effect was found to be NaCl specific. At present, the main alternatives to reducing Na⁺ content are KCl or MSG combinations with NaCl. Nevertheless only MSG appears to avoid significant flavor deterioration, a feature decisive at choice time. The characteristics of the biomolecular events underlying MSG effects linked to Na⁺ specificity are discussed in relation to flavor preservation in low-Na⁺ foods.

105. Comparing the information content of two large olfactory databases

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An expert's subjectivity in establishing an olfactory description can produce wide discrepancies. This could result in differences between trustworthy databases listing the odor profile of identical

compounds. To better assess these problems, several data mining methods (M. Pintore, K. Audouze, F. Ros and J.R. Chrétien, 2002, *Data Sci. J.*, 1: 99–110) were applied in this work on two selected molecular series (derived from S. Arctander, ed., 1960, *Perfume and Flavor Materials of Natural Origin*. Elizabeth; S. Arctander, ed., 1969, *Perfume and Flavor Chemicals*, Montclair, NJ; PMP, 2001, *Database of Perfumery Materials and Performance*. BACIS, The Netherlands). Each of these databases include ~2600 compounds and will be hereafter called ‘Arctander’ and ‘PMP2001’, respectively. A straight database comparison isolates ~1000 common compounds and 40% of them associate a totally different olfactory profile, in which no descriptor included in the Arctander description can be found in the PMP2001 one! Moreover, only 2% of these common compounds recover the same olfactory descriptors. This comparison indicates how much the databases differ, but gives no criterion for evaluating which is the ‘best’ one. To fulfill the latter objective, a deeper analysis on their information content was performed with help of structure–odor relationships developed by combining genetic algorithms and fuzzy logic (M. Pintore *et al.*, 2002). Several reduced data sets, regrouping some hundred compounds, were extracted from both databases. Each series include four or five classes that associate the same olfactory descriptors for both Arctander and PMP2001 descriptions. The robustness and the prediction power of the classification models established on these databases give a powerful criterion for evaluating the ‘quality’ of their information content. This modeling procedure can be then used as an efficient strategy to better evaluate the information content of any corporate database.

106. Cross-cultural odors throughout Europe: influence of ageing on identification?

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Because odor identification is influenced by a subject’s geographical origin, we developed a European test (ETOC) in which we selected odors in order to limit this influence (T. Thomas-Danguin, C. Rouby, G. Sicard, M. Vigouroux, V. Farget, A. Johanson, A. Bengtson, G. Hall, W. Ormel, C. de Graaf, F. Rousseau and J.-P. Dumont, 2003, *Rhinology*, 41: 142–151). We showed previously that in the case of ETOC, age and gender were more influential factors than geographical origin. Here we present results of 1330 European citizens from eight regions of six European countries (Germany, France, Sweden, The Netherlands, Denmark, Finland) who were submitted to the ETOC. Subjects had first to detect and then to identify 16 commonly encountered odors. We considered only the scores of people who succeeded at detecting the odorant sample prior to the identification step. The question under study was: does age influence performance in the same manner for each odor? A significant regional effect was found only with four odors, and a sex effect for six odors; for two odors, men performed better than women. The main identification differences were due to ageing, with a significant decrease from 60 years of age. Results show that ageing does not induce a homogenous decline for each odor in the sample. Age curves differ between odors: the youngest group is not always the best at identifying, while the oldest group is not always the worst. These results suggest cohort effects, implying that, in some cases, generation effects on identification are larger than cultural differences between regions of Northern Europe.

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107. Variety of time–intensity curves during offensive odor exposure and its relation to perceptual and cognitive aspects

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The perceived intensity during odor exposure has long been thought to decline as an exponential function. It was recently reported, however, that cognitive factors might influence the perceived intensity of odors during experimental exposures (Dalton, 1966). The purpose of the present study is to clarify the variety of intensity curves during odor exposure and its relation to perceptual and cognitive aspects of odor. Odor was presented by the olfactometer that we have developed to produce various fluctuating odors. Triethylamine was used as the offensive stimulus odor and was presented at a constant concentration for 10 min. The flow rate of stimulus was 2 or 4 l/s. The dilution rate was 1 ml/2 l (1 ml bubbling odor in 2 l air), 2 ml/4 l, 4 ml/4 l and 8 ml/4 l. The concentration at the odor outlet was 35.7 p.p.m. in the odor of 4 ml/4 l dilution rate and 58.0 p.p.m. in that of 8 ml/4 l. A participant sitting at a distance of 25 cm from the odor outlet evaluated the perceived intensity continuously using a sliding lever for 12 min including air introduced before and after the stimulus odor. Six healthy women aged from their twenties to forties participated 1–4 times in the 2 l/s flow rate experiments and 14 women in the 4 l/s experiments. Participants were asked to answer perceptual and cognitive aspects such as familiarity, unpleasant–pleasant and odor quality after intensity evaluation. Familiarity was evaluated from 1 to 5, unpleasant–pleasant from –4 to 3. Odor quality was evaluated by referring 28 descriptive words prepared for offensive odors and by estimating the suitability (0–5) to each descriptive. The 95 intensity curves obtained were visually classified into five typical patterns by three evaluators. These patterns are an exponential curve (hereafter called pattern A), an exponential and then constant curve (pattern A’), a fluctuating curve (pattern B), a constant high-intensity curve (pattern C), and a gradually increasing curve (pattern D). Patterns A, A’, B, C and D accounted for 19.0, 10.5, 50.5, 17.9 and 2.1% respectively. Pattern B occupied more than half, and the exponential curves only 29.9% including pattern A’. Pattern D was found only rarely in the stimuli of the present study. We investigated the relation of these patterns with perceptual and cognitive aspects. The difference was shown between a group of patterns A and A’ and a group of patterns B and C. The odor quality was most suitably expressed by ‘excrement’. However, the group of patterns A and A’ showed less suitability than that of patterns B and C. In addition, the group of patterns A and A’ showed less familiarity and less unpleasantness than that of patterns B and C. These results suggest that time–intensity curves during offensive odor exposure show various patterns including an exponential one, and these patterns are related to the perceptual and cognitive aspects of odor that a participant experiences.

108. Temporal aspects of hedonic and intensity responses

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Few studies have investigated temporal aspects of hedonic responses, and those few that have, have focused on the product. This study focused on the response itself and on the temporal aspects of hedonic responses as compared to intensity responses. The time–intensity scaling method was used and subjects were instructed to focus either on intensity or on pleasantness of three different concentrations of an orange lemonade stimulus in a within-participants design. The overall multivariate effect of condition (intensity or hedonic) was significant [$F(3,21) = 16.229, P = 0.000$]. We found a difference in the latency [$F(1,23) = 30.300, P = 0.000$] and the duration [$F(1,23) = 15.095, P = 0.001$] of the hedonic and intensity responses. The time of maximum response did not differ [$F(1,23) = 1.209, P = 0.283$]. As was expected, the onset of the intensity response was earlier than the onset of the hedonic response. Contrary to our expectations, the intensity response lasted longer than the pleasantness response. This may indicate that the processing of pleasantness occurs in part simultaneously with the processing of intensity aspects.

Psychology/sensory analysis

109. Hedonic categorization of smells in human development: the role of lexical knowledge

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Olfactory perception is influenced by prior linguistic knowledge. These ‘top-down’ influences originate from a high level of processing to generate expectations of categorization of the olfactory input. Here, we investigated the effect of lexical knowledge on hedonic categorization through a differential approach. Specifically, the issue of whether these ‘top-down’ effects are similar during childhood and adulthood is addressed. In experiment 1, adults ($n = 20$; age range 20–29 years) were tested under two conditions. In condition A, they judged the hedonic tone of pleasant, neutral and unpleasant smells (six odorants) twice in two different sessions separated by 5 min. All three categories of smells were rated as less pleasant during the session 2 ($P < 0.05$). In condition B, the same protocol was used except that during the session 2 all participants were told the name of each odor before its second presentation. In that case, no reduction of pleasantness was observed between sessions 1 and 2 ($P > 0.05$). Thus, it seems that lexical knowledge stabilizes hedonic categorization, which may be fluctuating on the perceptual level. In experiment 2, the same test was run with children ($n = 19$, age range 5–6 years). Similar results as in experiment 1 were obtained for condition A ($P < 0.05$). In contrast, a different pattern was observed in condition B whereby the pleasantness of a neutral odor increased following the presentation of a pleasant name during the second session ($P < 0.05$). This suggests a different susceptibility to lexical top-down effects for hedonic categories in children and adults: prior lexical knowledge may help to create and organize olfactory categories dur-

ing childhood, whereas it may only contribute to maintain them in adulthood. Ongoing studies are performed in order to replicate the effect with different odors.

110. The influence of the name or profile of a perfume on its assessment and the observed behaviour

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Two studies have investigated effects of the image (or profile) of a perfume on its assessment and handling. In a pilot study 130 subjects assessed the general image of eight different well-known and advertised perfumes in a questionnaire. Based on the pilot study, three perfumes were chosen for the main experiment: Chanel No. 5, Naomagic, Kölnisch Wasser. There are three experimental groups. Each perfume was used in its own bottle/packing and in the bottles of the other two. As a result we have nine experimental conditions. A total of 225 women took part in the main experiment. Each subject rated the perfumes in three conditions in random order on bipolar rating scales and on polar graphical scales, and answered multiple-choice questions. They were filmed by a video camera throughout the whole session in order to obtain behavioural data about the interaction with the perfume bottle and smelling strips. The handling of the packing and bottle were analysed for duration and frequency of touching. The four categories for the behavioural analysis were: applying on a paper strip, applying on skin, smell at the bottle, smell on cap. Tests using Kölnisch Wasser were the shortest. Perfumes in the Naomagic bottle were fanned much longer to the nose. The handling of the Naomagic package was longer than for Kölnisch Wasser. Kölnisch Wasser was rated as moderately pleasant. It was significantly rated as more pleasant in the packing of Naomagic and Chanel No. 5 than in its own package. The pilot study also revealed that there is a tremendous difference between Chanel No. 5, Kölnisch Wasser and Naomagic concerning the time of day someone would wear it, on which occasion and whom you intend to meet.

111. Measure of children’s attention to odours in everyday life: development and validation of a verbal method

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Individual differences in attention to everyday odours have rarely been investigated, even in children who are said to be less reserved than adults in reporting odour-based feelings. Here, we present the development and validation of a questionnaire designed to evaluate inter-individual differences in attention to food, social and environmental odours in 6–12 year old children. A previous study involving 82 children was performed to construct the items. In the present study, 50 children passed individually a 24-item version. An individual measure of attention to odours (IMAO, range 0–24) was computed by summing the item scores. Results showed: (i) between-subject differences, since IMAO was largely distributed (from 5.6 to 17.5) and (ii) between-group differences: IMAO tended to be lower for boys (10.7) than for girls (12.4) and IMAO increased with age ($r = 0.35; P < 0.05$). In addition, three measures of internal validity were performed: (i)

a 'test—retest' 2 months apart ($n = 18$) revealed a high reproducibility of the IMAO ($r = 0.75$; $P < 0.001$); (ii) the moderate correlations between the items ($-0.40 < r < 0.48$) confirmed their discriminant power; and (iii) the relatively low internal consistency (Cronbach's $\alpha = 0.65$) suggested that the questionnaire has a multi-dimensional structure. Moreover, the correlation between self-reported and parent-reported attention to odours based on nine items ($r = 0.51$; $P < 0.001$) provided evidence for the external validity of the questionnaire. This questionnaire provides a first evaluation of the relevance of everyday odour cues in school-age children. This verbal approach is currently being completed with behavioural observations.

112. Familiarity breeds content: effects of familiar odors on pain responses in term and preterm newborns

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We report here a study that focuses on the effects of familiar odors on pain responses in full-term and preterm newborns. We hypothesized that smelling a familiar odor during a painful event would promote self-regulation in infants. In the first experiment, full-term newborns were exposed to a familiar odor (maternal or non-maternal odor), a new odor or no odor while undergoing a routine heelstick. Infants' crying, grimacing and head movements were recorded before, during and after the heelstick. No group difference was found in overall distress during the heelstick. However, infants who smelled a familiar odor displayed less distress during the recovery phase compared to during the heelstick phase. By contrast, infants exposed to an unfamiliar odor or no odor showed no significant decrease in distress after the heelstick. Moreover, infants presented with their mother's milk displayed significantly fewer head movements during the heelstick compared to the other groups. In a second study, preterm infants were observed during a blood draw on the heel (heelstick) or a venipuncture on the hand. During the procedure, one-third of the infants were presented with a familiar odor, one-third of the infants were presented with a new odor, and one-third were presented with no odor. In general, heelsticks elicited more behavioral distress (crying, grimacing) than venipunctures. When exposed to the familiar odor, the venipuncture group showed no significant increase in distress during the procedure compared to baseline levels. By contrast, all the other groups displayed a significant increase in distress between baseline levels and the blood draw. These results support the mediating role of familiar stimuli in the soothing process, especially during milder pain. They also point to a potentially 'special' role for maternal odors in soothing infants.

113. The odour of human milk: conditions for stability of its attractiveness to newborns

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Human milk has been shown to be olfactorily attractive to human newborns. However, milk is highly evolutive in biochemical and physico-chemical terms after its ejection from the breast. Here, we examined how long human milk holds its potency to elicit neonatal responses indicative of attraction and appetite. With that aim, relative head orientation movements and oral activities were quantified

in breast-fed newborns ($n = 48$; age 4 days) exposed to olfactory tests pairing fresh milk (freM) either with milk standing at ambient temperature (atM) for various delays or with milk stored in a freezer (froM; -20°C for 3 h). The odour of atM standing for 30 min was shown to be as attractive as the odour of freM, but atM standing for 3 h appeared to lose its appetitive properties. Thus, the attractive value of human milk odour seems to be short-lived after ejection. However, the neonatal behaviour was similar towards the odours of freM and of froM, with the exception that oral activity is reduced after freezing. These results indicate that the odour of human milk rapidly loses its power to release appetitive responses in newborns, and that even freezing is not able to completely preserve the initial attractiveness of the odour of fresh human milk. This loss of olfactory attractiveness of milk may result from biochemical alteration or from volatilization of the attractive cues from milk.

114. Relationship between odour sensitivity, food behaviour and temperament in infants: development of a procedure

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The extent to which sensory sensitivity influences food preferences and food use is still a matter of debate. Infancy is described as a crucial period for elaboration of preferences, but surprisingly there have been few attempts to study the relationship between sensory sensitivity and food preferences in young children. The aim of this study is to investigate the relationship between odour sensitivity, food behaviour and temperament in 6–12 month old infants. To evaluate responsiveness to odours, we used a procedure described by Mennella and Beauchamp (J.A. Mennella and G.K. Beauchamp, 1998, *Chem. Senses*, 23: 11–17), which consists in observing the facial and bodily reactions of infants as they explored successively different scented rattles. The rattles were visually identical but different in odours (no odour, rose, rancid butter and vinegar) and in intensity (low, intermediate, high). Different behaviours were analysed, including the proportion of time that the infant manipulated, looked at or sucked the rattle and the frequency of vocalizations. Mothers completed two different questionnaires, one to assess children's temperament (S. Bertrais, B. Larroque, M.H. Bouvier-Colle and M. Kaminski, 1999, *Rev. d'Epidémiol. Santé Pub.*, 47: 263–277) and one to assess food behaviours. The food behaviour questionnaire assessed in particular three dimensions of feeding problem: inadequate appetite, finickiness and neophobia. Methodological considerations concerning the assessment of olfactory sensitivity via observation and food behaviour in infants via questionnaire will be discussed. This study gives the first insight into the relationship between sensory sensitivity, temperament and food behaviour in infants.

115. Are we able to recognize a regularly consumed drink among flavor distractors?

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The food behavior of humans largely depends on previous food experiences. In fact, we have argued that one of the main functions of food memory is to recall both before and during consumption of

a food previous experiences associated with that food. However, food memory studies have generally assessed participants' memory for a target food learned at one occasion in a laboratory context, which limits the use of these results to gain an understanding of daily food behavior. Thus, the present experiment was designed to assess consumers' ability to recognize a food that they are used to consume in their daily life among flavor variants of this food. One hundred and forty-four consumers who used to consume a soft drink X at least several times a month were recruited. They were presented a monadic random series of 12 samples, namely six samples of the usual drink X and six samples of X with a modified flavor (e.g. less sweet than X). After tasting each sample, participants indicated whether this sample was identical or different from the product that they were used to consuming. The impact of two factors was studied. First, we compared participants who exclusively consume the soft drink X versus participants who mainly consume X but also consume other brands of this soft drink. Our hypothesis was that exclusive consumers of X are less 'variation tolerant' and thus produce fewer false alarms (modified X wrongly perceived as identical to the usual product) than non-exclusive consumers of X. Second, we compared participants who were told that the study aimed at analyzing the flavor of soft drinks (neutral context) versus participants who were told that the study aimed at comparing the effect of an 'industrial' process versus a 'traditional' process on the flavor of soft drinks (spur context). Our hypothesis was that participants in the spur context produce more hits (X correctly perceived as identical to the usual product) and fewer false alarms than participants in the neutral context.

116. Chemo-sensorial expertise and categorization: the case of beers

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How do experts and novices categorize beers? Can they verbalize their categories? In recent years it was suggested that categorization tasks might help novices to describe their perceptions. We further examine this claim by comparing the stability, repeatability and pertinence of beer experts' and novices' performance in two categorization tasks. The categorization tasks included two phases separated by a 15 min break. In the first phase of the first task, the experts and a group of novices had to sort freely 12 beers. In the second phase they had to sort again the 12 beers using the same criteria. In the first phase of the second task, the experts and a second group of novices were asked to sort the 12 beers and to provide a descriptor for each group. In the second phase, each participant was given the descriptors he/she used in the first phase and had to indicate to which beers these descriptors corresponded. Results were analysed both at the group level, using multi-dimensional scaling, and at the individual level, using symmetric difference distances. To evaluate the similarity of performance between the two groups of participants and the stability of the categorization within each group we compared the MDS solutions obtained for experts and novices in the first phase of each categorization task. The categorizations performed by both groups of participants are comparable and the categorizations performed by each group are stable from one task to the other. To evaluate the repeatability and the pertinence of participants' performance we compared the symmetric dif-

ference distances between the first and the second categorizations performed by each participant in each task. A significant difference was observed between experts and novices in the second task but not in the first one. This indicates that novices' performances are as reproducible and stable as experts' performances, but the verbalization of experts seems to be more pertinent than that of novices.

117. Modeling perceived intensity and quality of binary mixtures

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Four concentrations of pyridine, n-butanol and amyl acetate were mixed into 16 binary mixtures for each pairwise combination. The three sets of mixtures were presented along with their single constituents to participants by means of a constant-flow olfactometer. After learning the single constituents of the mixtures, the participants judged perceived odor intensity and quality of each stimulus six times. Intensity was judged using the method of free magnitude estimation. Quality was assessed by asking the participants to identify whether the one, the other, or both constituents were in the mixture. Odor intensity and quality of mixtures were compared between the three pairs of substances. A vector model for the prediction of odor intensity and quality based on the perceived intensity of the unmixed constituents (Olsson, 1998) were tested.

118. The effects of low-level exposure to peppermint and lavender scents on school-task performance in elementary-school children

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We used the treatments of school premises with low concentrations of essential oils recommended for medicinal purposes, to study the effects of ambient odors of peppermint oil and lavender oil on the students' performance in math and language school tests. Five classes of elementary-school children (3rd–7th grades, 135 subjects in total) participated in this study. During the 'scented' lessons an odor-delivering system maintained very low concentrations (<0.03 mg/m³) of the ambient odor in the classroom. Treatment with an odorant, if compared with similar lessons without odor, produced statistically significant effects on the performance. The latter was estimated by using the school marks given by the teachers, and also by counting the number of errors. The following effects were observed. (i) Peppermint improved the students' performance of the word-dictation spelling test so that the mean count of uncorrected errors decreased and the mean school mark increased. A similar increase of the mean mark and decrease of the mean error count was observed in the arithmetic dictation test. However, peppermint did not affect the performance of the written word-copying test and the written arithmetic test, which both rely more on attention and concentration and less on memory compared with the two dictation tests. This may indicate that the effects of peppermint are mainly realized through enhancing working memory. (ii) Lavender, like

peppermint, improved the score in the word-dictation spelling test, but affected negatively the students' performance in both math tests by decreasing the mean school mark. These experiments have shown for the first time that even very weak ambient scents can modify the performance of children at school, and that some of these effects are beneficial for learning.

119. The impact and interaction of cognitive dissonance, reactance and food neophobia in the evaluation of novel sour candies

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The impact of psychographical variables such as food neophobia, variety seeking, etc., on the acceptance of novel sensory stimuli has been investigated by numerous authors in recent years. Psychological research has shown that—according to the theory of reactance—individuals tend to avoid stimuli more strongly when they are restricted in the freedom of their choice behaviour. Furthermore, judgements tend to be increased when cognitive dissonance can be decreased. In this experiment 157 participants tasted extremely sour candies which were new for the German market. The respondents whose degree of food neophobia had been previously measured by the Food Neophobia Scale were divided into three groups: the 'reactance group' (group R), the 'dissonance group' (group D), and a control group. Whilst the control group members received no particular information on the test procedure, members of group R and group D were presented with different information: Group R: 'You're going to taste four different candies. Unfortunately you cannot choose among the products you'll get, although most of the other participants can.' Group D: 'You're going to taste four different candies. Two of them will be extremely sour. They're so sour that other participants could not continue with the test and stopped it. You can now decide if you really want to participate in the test.' The results show a significant impact of the information on the evaluation of the sour candies. In addition, the degree of the individual food neophobia interacted significantly with the type of information provided in the different groups. Neophilics judged the sour candies significantly better in the reactance condition, whereas neophobics in the dissonance group preferred the candies compared to those who were in the reactance group.

120. High school students' beliefs about health effects from exposures to environmental odors

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Many adults have pre-existing hypotheses about the health effects of exposures to environmental odors, which may guide their reactions to actual odor exposure events. A study was conducted in high school students to explore such beliefs in adolescents. Two hundred and sixty high school students (15–17 years) from the Philadelphia area were presented with six brief exposure scenarios, which varied over factors such as source of the odor ('natural' versus 'chemical')

and frequency ('regular' versus 'accidental'). They were asked to imagine each of the scenarios and their odors, rate their pleasantness and the intensity with which they expected to experience each of a list of 34 health symptoms. PCA analysis was carried out on the symptom ratings to examine which symptom clusters emerged. Pleasantness ratings varied across scenarios, with natural odors (e.g. pig) being rated as significantly more unpleasant than chemical odors (e.g. refinery; $P < 0.01$). Analyses of expected health symptoms showed very low ratings in general (medians 0–1, on a scale from 0 to 4). PCA analyses revealed similar results for all six scenarios. PC1, with 50.2% explained variance (EV), was composed of diffuse symptoms reflecting psychological and physical malaise (e.g. feeling confused, feeling weak, lower back pain), while PC2 (EV = 7.2%) was composed of focused symptoms (e.g. coughing and gastrointestinal). Symptoms loading on PC2 received higher ratings in general than symptoms loading on PC1. There was no relation between ratings of odor pleasantness and symptom ratings. PC analysis reflected the distinction between direct (i.e. to be expected) health effects from exposure to odorous emissions versus indirect (or unlikely) symptoms often attributed to odorous emissions. By endorsing the direct symptoms rather than the indirect symptoms, many students' beliefs may be considered as rather appropriate.

121. Using latent semantic analysis to assess different representations of olfactory sensations in various professions

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Some professionals use a common knowledge which seems to be founded on the odours' utility during their activity. This knowledge supposes that professionals share common representations of the sensations being considered. Professionals can be divided in two categories: (i) those confronted with pleasant odours (cooks, chefs, sommeliers, etc.) or (ii) those confronted with unpleasant odours (firemen, forensic surgeons, etc.). The aim of this study is to quantify the degree of similarity of language used when speaking about odours between and within professions. We use the latent semantic analyses model, which extracts a vector representation of the meaning of numerical texts (here interviews with professionals), and calculates the degree of similarity between members of the same profession and between professions. Initial results match the conclusions of an anthropologist who is an expert in the field of odours. There is a common factor which leads professionals confronted with the same kind of odours to use a semantically similar language. Moreover, perfumers use a specific language different from all other professions, while gardeners and sommeliers use the most shared language.

122. Sensory evaluation of 'organic' building material emissions: effects of information on sensory evaluation

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Emissions from indoor building materials containing linseed oil ('organic') ($n = 6$) and comparable synthetic products ($n = 3$) were

evaluated by ~35 subjects for odor intensity and odor acceptability, in low background climate chambers (CLIMPAQ), using constant climatic conditions. As observed in earlier studies, high odor intensity is associated with low odor acceptability, implying that the consumer considers odor from building materials to be negative. Emissions from 'organic' products were judged to have higher odor intensity and lower odor acceptability than their 'synthetic' counterparts. Thus for odor intensity: linoleum > PVC, linseed oil emulsion paint > polymer emulsion paint, linseed oil based floor oil > modified polymer based floor oil. These results clearly indicate that modern synthetic products can have a lower odor impact than 'organic' products. When the emission sources were identified and labelled as either 'organic' or 'synthetic' (information condition), and the emission evaluations compared to those with blind evaluations (non-information condition), odor intensity was significantly lower for all the 'organic' products, as well as the substrate beech wood and the empty chamber (control air). Acceptability was increased significantly for all samples except linoleum no. 1 and the 'synthetic' ones: PVC, polymer based emulsion paint, modified polymer based floor oil. The major effect here is probably that acceptance of odors is increased when we are informed of their origin and believe them to be harmless. This effect might be reinforced by classifying the products as 'organic'. Since it is postulated that unknown odors can contribute to the tendency of office workers to report building-related symptoms, it is possible that dissemination of information about building renovation (or building materials, before the occupation of new buildings) might decrease the levels of the occupants' building-related symptoms associated with odor.

123. When odors matters: odor cuing of emotional events

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The aim of this research was to examine olfactory cuing of emotional and neutral events. In study 1, subjects were exposed to a series of photo slides depicting an emotional event (car accident) along with a congruent odor (i.e. petrol) or no odor. Five days later participants were asked to recall details of the event. Results indicated that both central details and circumstantial information of the emotional event were better retained following olfactory cuing. In study 2, we explored the role of olfactory congruency and retention interval on recall proficiency of emotional events. Subjects were presented with series of photo slides showing either emotional or neutral events in conjunction with a context congruent odor, a context incongruent odor, or no odor. We expected that olfactory cuing in general enhances recall performance as compared to the no-odor condition, and that memory for emotional events with olfactory congruent processing will be better than that for non-congruent processing. Results will be presented both with respect to memory performance (e.g. number of details, accuracy), assessed 24 h and 8 days after exposure across all cue formats, and experiential measures (e.g. arousal, intensity).

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124. The effects of labelling and repeated consumption on desire and liking over time

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This study assessed the impact of labelling and repeated consumption on desire and liking of different chicken bouillons. Two hundred subjects were allocated to two groups: a freshly prepared bouillon group ($n = 100$) and a cube version bouillon group ($n = 100$). All subjects received the same bouillon over the whole period. For each group, half of the group tasted the bouillon blind and the other half tasted the bouillon labelled. For example, when the freshly prepared bouillon was tasted with labelling, it was labelled as a 'freshly home made chicken bouillon'. Subjects drank 100 ml of the bouillon 12 times over a period of 4 weeks. Desire and liking was measured at each consumption. Liking of the bouillons was quite high and remained constant over time. There were no differences in liking among the bouillons (both blind and labelled). However, the label 'fresh' had a massive positive impact on the desire ratings: the desire was significantly higher for the freshly labelled product, whereas the label 'fresh' did not influence actual product liking. This study demonstrates the power of expectations as measured with desire: a product labelled as 'fresh' is more desired by consumers even though the product itself is not liked more. The difference in results between desire and liking emphasizes once again the importance of clarifying the difference between questions about 'desire to eat' a food and 'liking' for a food.

125. How come I cannot remember the beers I drink? Expertise level and memory performance

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Despite its obvious importance in understanding expertise, memory has been scarcely studied in relation to chemical sense expertise. Most of the work carried out in this domain has focused on perceptual and verbal performance without evaluating explicitly experts' memory abilities. The principal aim of this study was to evaluate the difference in the memorization of beers and odours between beer-trained assessors and novices. The second objective of this study was to evaluate if memory performance for beers could be predicted by memory performance for beer odour compounds. To address these issues, we compared the memory performance of 19 beer-trained assessors and 22 novices. This experiment included two yes/no recognition tasks with learned and new stimuli, one performed with 16 beers as stimuli and the other with 20 beer odour compounds, and a same/different discrimination task performed on the same beers. We found that beer-trained assessors outperformed novices in the yes/no recognition task performed both on beers and on odour compounds present in beers, but only for beers they have been trained on and regardless of the type of odours. Moreover no significant difference was found between experts and novices in the beer discrimination task. This suggests that the expert advantage cannot be attributed to a better perceptual ability, but to an efficient coding and retrieval of information in long-term memory. In parallel, no significant correlation was found between discrimination and memory performance. Subjects with good odour memory

performance did not always succeed at memorizing beers and vice versa. This suggests that during training experts might develop independent memory structures for odour compounds and beers.

126. Flavor enhancement versus provision of variety as a means to increase food intake in the elderly with varying olfactory and mood status

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Age-related olfactory impairment and depression are suggested to decrease food intake in the elderly. Methods of prompting intake in elderly subjects ($n = 60$, age range 61–86 years) with varying olfactory and mood status were studied in two experimental conditions. The enhancement group ($n = 28$) received regular smoked ham and smoked ham flavored with extra strong smoke aroma or with pepper mix for home use. The variety group ($n = 32$) received four different cold cuts: smoked ham, cooked ham, pepper ham and sausage for home use. Both groups participated in two successive home use periods over 6 days. They received 600 g cold cuts/period, and were allowed to consume them *ad libitum*. The samples were rated at the beginning and at the end of the study for odor and flavor intensity (tasting sessions) and liking (tasting sessions and home use). Consumption was measured by weighing the returned leftovers of the samples. Olfactory capabilities were measured with the Scandinavian Odor Identification Test (SOIT), and mood status with the Profile of Mood States (POMS, bipolar) scale. The scores of the measures were used in dividing subjects into normosmics and hyposmics (SOIT, cut-off score = 11) and into 'low' and 'high' mood groups (POMS). The enhancement group liked both the regular ham and ham enhanced with pepper mix similarly, while added smoke aroma decreased liking. Despite the olfactory capabilities, the subjects >75 years of age were less responsive to the flavor changes than those aged 61–74 years in their ratings. The variety group liked smoked ham the most and sausage the least. Total consumption of the samples was similar in both groups, but contrary to our hypothesis, hyposmics consumed the samples more than normosmics. Mood status (specifically elated-depressed dimension) affected consumption, interacting with olfactory capabilities: the hyposmics with 'low mood' consumed more than those with 'high mood', while the effect was the opposite in the normosmics. In conclusion, both olfactory and mood status along with age contributed to the consumer responses, while the flavor enhancement was by no means superior to variety in increasing the intakes.

127. Weaning practices: a cross-culture comparison

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Weaning practices depend on cultural and social norms, and are influenced by economic and time constraints. The present study describes current infant feeding practices for samples of mothers in two European countries with the aim of better understanding the process of the transition from milk to non-milk foods. The first group (A) comprised 140 infants (71 male, 69 female); and the second group (B), 160 infants (81 male, 79 female) aged 3–12 months. Clear differences in weaning practices were found between the two

groups. For infants in group A, 36% were never breast-fed compared to 11% in group B. Mothers of group B who breast-fed (exclusively or not) their infants did so for a longer period than those of group A. At 4 months, weaning had started for 65% of group A infants and for 20% of group B infants. Mean (\pm SEM) weaning ages were 4.2 ± 0.1 and 5.5 ± 0.1 months in groups A and B, respectively. The mean number of vegetables introduced during the first month of weaning was higher for the group A (6.2) than for group B (2.3) infants. Moreover, even before beginning to wean, 46% of group A mothers offered their baby a variety of foods 'just for taste', whereas this was the case for only 26% of group B mothers. These intercultural differences will be discussed in the context of weaning recommendations in the European Union.

128. Preference for astringent foods: is individual variability related to physiological parameters, and how does it evolve with repeated exposures?

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The contribution of sensory physiology to individual variations in food preferences has not been much explored. The objective of this study was twofold: on the one hand, the preferences of 10–11 year old children for astringent fruit juices were studied in relation to their oral physiology and to their sensitivity to astringency, and on the other hand, the evolution of food preferences and of some oral parameters were studied following a period of ~30 days of exposure to an astringent fruit juice. To explain individual variations in food preferences for astringent fruit juices, the following parameters were examined: papillae number, saliva flow before/after the consumption of the juice, proline-rich protein (PRP) content in the saliva before/after the consumption, saliva pH before/after the consumption and perceived astringency intensity in fruit juices. To examine the evolution of food preferences following the consumption of the astringent juice, children were provided with bottles of an astringent fruit juice they were requested to drink at home, five times per week for 6 weeks. During this period, children came at the laboratory one week out of two to rescore their preference for the astringent fruit juice. The results will show the relation between individual variations in food preferences to individual variations in sensory and oral parameters. Moreover, the evolution of the preferences for the astringent juice will be discussed in relation to the evolution of the oral parameters, with a particular emphasis on the possible induction of PRP secretion and of the saliva flow modulation following the repeated consumption of astringent juice.

129. Psychosocial studies on the impact of odour pollution

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Environmental odours emanating from waste can produce marked annoyance in neighbouring populations. This study aims at better understanding the perception of malodours from garbage dumps by nearby populations. Ten garbage dumps were selected in France as a function of the nature and amount of waste products, and of environmental conditions (permanent residences, country houses, farms, etc.). The study used sample surveys (groups of 50–100

individuals), individual interviews and meetings with corporations involved in garbage treatment. Although neighbouring populations can sometimes report tolerance of olfactory annoyance, they describe garbage odours as an important source of discomfort. In all cases such odours are felt as an intrusion into the private space and a source of ownership depreciation. The sampled population never considers the way their lifestyle influences the subjective impact of the odours. In sum, people living in the neighbourhood of sources of annoying odours react to them as a function of (i) the odour itself, (ii) their involvement with the source of wastes, and (iii) local behaviour and attitudes.

Symposium 5: Chemoreception, food preference and food choice

130. Sensory quality, a driving force or an adaptive covariant of food intake?

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The perceived sensory and hedonic quality of foods is often considered an obvious and therefore not even an interesting predictor of food intake. This view is dramatically supported by studies in which the willingness to consume a previously unfamiliar food is greatly affected by the very first sensory exposure to this food. Further evidence is provided by studies in which the development of children's food preferences has been monitored; the observation of children also reveals the plasticity of preferences. Consumer studies show that repeated food choices are highly predicted by the perceived hedonic quality, and a high correlation is typically seen between the rated frequency of use and ratings of liking of a food. However, in spite of diminished chemosensory capabilities, elderly consumers do not necessarily prefer enhanced flavors in foods, which implies that small sensory changes are acceptable if the food has maintained most of its familiar characteristics. The relationship between perceptions and intake becomes even more complex when the effect of cognitive input, such as health claims associated with foods, are empirically tested. In such cases, the responses need to be examined against the beliefs, expectations, previous consumption patterns, individual traits and motivation of the respondent. It seems that there are situations in which sensory input has a major impact on food choice and consumption, while, on the other hand, there are other mechanisms leading to indifference of sensory-hedonic aspects.

131. Food preferences, food variety seeking and liking for unfamiliar foods in subjects aged 4–22 years: link with food choices in early childhood (2–3 years)

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To what extent are early food choices related to further food preferences and variety seeking? This question, with considerable theoretical and practical interest, has rarely been tackled. The present study has evaluated the relation between food behaviour at two different stages: in early childhood (2–3 years old) and later in life (4–22 years old). Food choices of toddlers were continuously recorded be-

tween the ages of 2 and 3 in a nursery canteen, where children were free to choose the composition of their lunch from among a varied offering of eight dishes. A total of 418 children were monitored, about 25 per year between 1982 and 1999, over 110 lunches per child on average. The same subjects were contacted in 2001–2002 and asked to participate in a study aimed at evaluating their present food preferences, variety seeking, food neophobia and liking for unfamiliar foods. Eighty-two per cent of the subjects ($n = 341$) participated; their ages were then 17–22 ($n = 91$), 13–16 ($n = 68$), 8–12 ($n = 99$) to 4–7 ($n = 83$). For most food categories, individual present preference was highly linked to individual preference at 2–3 years old. A strong link was found for cheeses, and also to a lesser extent for animal products and vegetables. Analyses by specific foods confirmed these findings and revealed in particular that strongly flavoured foods (such as mature cheeses and vegetables) elicited stable patterns. Concerning present variety seeking, it significantly increased with variety seeking at 2–3 years old, with age and with breastfeeding duration and significantly decreased with present neophobia. The influence of age and gender on present preferences and food variety will be discussed, revealing in particular behavioural changes at puberty. In contrast, actual liking for unfamiliar foods was not related to the early variety-seeking behaviour, even for the youngest subjects.

132. Influence of colour on perception of wine aroma

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The mental processes that enable a wine connoisseur to identify a favourite vintage have received little systematic study. Two experiments explored wine expertise by investigating perceptual processing in judgements of wine aroma. Specifically, we investigated olfactory perceptual bias, a cognitive construct concerned with how what we already know influences what we smell. Colour-induced olfactory bias was investigated in wine experts (experiment 1) and in social drinkers (experiment 2). We hypothesized that colour-induced perceptual bias was more likely to occur in wine experts than in social drinkers, leading experts astray. The task simulated a wine-evaluation situation where colour and aroma were open to evaluation by visual and olfactory senses. Experts were able to discriminate white wines that had been masked with colour to simulate an aged white wine and a red wine, although they did succumb to a degree of colour-induced olfactory bias. That is, experts' aroma judgements to the white wine that was coloured red were more accurate when the wine was presented in opaque glasses than when presented in clear glasses. Social drinkers found the task extremely difficult, demonstrating indiscriminate behaviour in some conditions. The data suggest that wine experts do indeed differ cognitively from novices in their approach to evaluating wine aroma. Theoretical and applied implications are discussed.

133. Attending to the sensory characteristics of foods during eating: the effects of distraction

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As a food is eaten, the hedonic evaluation of the sensory characteristics of the food declines; this is known as sensory-specific satiety. This effect is thought to be adaptive since it promotes intake of

a varied diet. However, splitting attention between eating and another task (e.g. watching television) appears to increase food intake. It is not clear how this occurs, and we therefore compared food intake during different conditions when subjects paid specific attention to the sensory characteristics of the food during eating, or consumed the same foods while distracted. In the first study 36 subjects attended the laboratory on four occasions, to eat a snack of sweet or salted popcorn in counterbalanced order: A (baseline: no distraction); B (same taste: interrupted to taste and rate the pleasantness of the eaten popcorn); C: (congruent taste: interrupted to taste and rate a congruent food—cheese or chocolate—to match sensory characteristics of preferred popcorn); D (incongruent taste: interrupted to taste and rate a food which differed in sensory qualities to the eaten popcorn). Males ($n = 12$) demonstrated reduced intake in condition B relative to the distraction conditions [$F(3,33) = 3.238, P = 0.04$]. This finding was not evident in females who consumed the same weight of food in each condition. Thus, for men at least focusing on the taste of the food as it is eaten reduced intake relative to that during distraction. In the second experiment, distraction involved the presence of others. Subjects ($n = 21$) visited the laboratory on four occasions to eat a meal. Subjects ate alone, ate alone with a distraction (interactive television game show), ate with two friends or ate with two strangers. In this way, the effects of distraction and the presence of others were examined independently. A buffet-style meal was presented on trays consisting of a variety of sandwiches, sweet and salty foods (3328 kcal). A significant effect of condition [$F(3,36) = 3.854, P = 0.02$] on food intake (kcal), was found only for men. This indicated that eating with friends enhanced food intake by 28% compared to eating alone ($P = 0.025$). Men also ate 15% more when watching television than when eating alone. These effects were not found for women, who tended to eat a similar amount of food under each condition. In conclusion, men in both experiments ate less when they had no other competing task. Indeed, being asked to focus on the sensory characteristics of the food as it is eaten seemed to reduce intake in men, but not women. Overall, these preliminary studies suggest that distraction may enhance food intake by drawing attention away from the normal progression of sensory-specific satiety during a meal.

134. Sensory responses to foods of obese and normal weight subjects: the issue of sensory specific satiety

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On average obese people eat more than normal weight subjects. One important question is whether or not the higher intake of obese subjects is related to differences in their sensory responses to foods. From various studies it is clear that obese and normal weight subjects do not differ to a large extent with respect to their liking for fat, sugar or other sensory factors. A number of recent studies have focused on the issue of sensory specific satiety. Sensory specific satiety is the decline in the reward value of a food during its consumption. The results of three recent studies indicate that obese subjects may be less sensitive to sensory specific satiety than normal weight subjects (Epstein *et al.*, 1996; Jansen *et al.*, 2003; Snoek *et al.*, 2004). This lower sensitivity was especially expressed in an higher degree of 'wanting' for foods in obese subjects, while there were little differences in the degree of 'liking'. This would imply that average meal

size in obese subjects may be higher than in normal weight subjects, because the decline in the desire to eat during meal consumption is lower. The issue of sensory specific satiety and obesity is an important future research area.

Symposium 6: Evolution of pheromonal communication

135. Evolution of pheromonal communication in *Drosophila*

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Sexual communication may be at the heart of many instances of speciation. It requires that the genetic control between the emission and the reception of a signal must be tightly co-adapted or the system would collapse during evolution if either component were to diverge too greatly. There has been very little experimental investigation of the genetic architecture of such systems. In organisms such as frogs, crickets, moths and flies, studies have been based on statistics and suggest that the emission of the signal and its reception are coded by different genes. In *Drosophila melanogaster*, we found that a mutation—resulting of the insertion of a single transposon—in the *desat1* gene simultaneously altered several phenotypes that are all involved in mate recognition and choice by female and male flies. In particular, the mutation changed the production and perception of sex pheromones before and during mating. Moreover, *desat1* expression was found in the peripheral tissues that produce and detect sex pheromones. Excision of the transposon could rescue all altered phenotypes, but not always in a coincidental manner. This indicates that emission and perception of sex pheromones are not coded by the same products, and may depend upon the pleiotropic activity of *desat1*. Pleiotropy could be related to the quantitative variation of one or several of the five *desat1* transcripts in the relevant tissues. Our current genetic, molecular and behavioral data support this hypothesis and suggest that subtle change in the regulation of *desat1* could participate to the evolution of pheromonal communication in *Drosophila* flies.

136. The scent of speciation: fruit odor discrimination and sympatric host race formation in the apple maggot

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Speciation in sexual organisms occurs as inherent barriers to gene flow evolve between previously interbreeding populations. To elucidate the origins of species requires understanding how and why new traits arise to reproductively isolate taxa. The *Rhagoletis pomonella* sibling species complex is a model for sympatric speciation via host plant shifts. The recently derived apple (*Malus pumila*)-infesting population of *R. pomonella*, which originated via a shift from hawthorn (*Crataegus* spp.) in the mid-1800s, represents an example of host race formation in action, the hypothesized initial stage of sympatric speciation. Host-specific mating is a key feature of *Rhagoletis* biology since these flies mate exclusively on or near the

unabscised fruit of its host plants. Differences in host preference translate directly into mate choice and premating reproductive isolation. Studies on the characterization of key host volatiles for derived apple-, ancestral hawthorn- and dogwood-infesting host races show that the flies use fruit odor as a key olfactory cue to help distinguish among their respective plants. In flight-tunnel assays and field tests, apple, haw and dogwood flies preferentially oriented to, and were captured with, chemical blends of their natal fruit volatiles. Flies of mixed apple and hawthorn ancestry were also studied to initiate studies on the physiological basis for odor preference. A major question in this study of host races is whether there are changes in the odor receptors in the antenna or in the way that input signals are integrated and interpreted in the insect's brain. Single cell electrophysiology was used to determine if differences in peripheral chemoreception could contribute to host preference and fidelity in the pomonella group. The results suggest that differences in peripheral sensitivity and adaptation may contribute to behavioral discrimination of host fruit and influence host fidelity in the pomonella group.

137. The evolution of host affiliation and chemical defense in chrysomeline leaf beetle larvae

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Larvae of *Chrysomelina* leaf beetles secrete defense toxins, stored in pairs of exsertile, thoracic and abdominal glands. Three main classes of toxins are observed. Iridoid monoterpenes are synthesized *de novo* by the insects. Salicylaldehyde is derived from *Salicaceae* phenolglucosides, salicin and salicortin. Esters of 2-methylbutyric and isobutyric acids ('butyrates') have a mixed plant-insect origin, the acids being synthesized by the insects and the alcohols being derived from the host plants. Molecular phylogeny demonstrates that *de novo* synthesis of iridoids is the ancestral condition from which the ability to derive salicylaldehyde evolved. This evolution only requires small changes in the enzymes involved in the biosynthesis of iridoids, and in specific transports of plant glucosides from the gut to the glands, the latter being already present in iridoid-secreting species. The secretion of butyrates is the most recent evolution, and appeared first as a dual defense, salicylaldehyde and butyrates in admixture, in species feeding on *Salicaceae*. This new defense allowed the insects to shift from *Salicaceae* to *Betulaeae*. Specialist natural enemies were probably instrumental in promoting changes in chemical defense and shifts of host plants.

138. Structural principles and evolutionary aspects in chemical signalling

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The sexually deceptive European orchid *Ophrys sphegodes* and its pollinator, *Andrena nigroaenea*, use a unique mixture of 14 ubiquitous hydrocarbons to produce the signal triggering mate-finding

and pollination. This use of quantitatively well-defined blends of common compounds in specific proportions may offer evolutionary flexibility. In the system *Ophrys speculum/Campsoscolia ciliata* the volatile signal produced by the flowers and the females of the pollinator species comprises 9-hydroxy-decanoic acid (in a distinct enantiomeric composition) and the corresponding ketoacid, which are structurally very close to the honey-bee queen substance, (*E*)-9-oxo-2-decenoic acid. Whether these similarities reflect evolutionary processes or convergencies is a matter of speculation. The Australian orchid *Chiloglottis trapeziformis* and its pollinator, *Neozeleboria cryptoides*, represent the opposite extreme to a versatile multicomponent mixture: they produce a chemical signal that consists of only one single compound, 2-ethyl-5-propylcyclohexan-1,3-dione. The production of this unique substance requires a rigid biosynthetic process and a highly specific receptor: a system with seemingly limited evolutionary flexibility. Pheromone structures of several caddisfly species and those of phylogenetically related primitive moth species like *Eriocrania* or *Nepticula* are (almost) identical: short-chain saturated and unsaturated straight-chain methyl carbinols or methyl ketones with uneven numbers of carbon atoms. Species of the limnephilid family produce branched compounds that have also been identified as components of volatile signals (attractants or repellents) in spiders, ants, bark beetles and weevils. The striking similarities among all these structures point to common biosynthetic routes. It may be interesting to speculate whether endosymbionts may be involved in the production of these compounds that show typical features of microbial polyketides.

139. Occurrence of olfactory lobes across arthropods, with emphasis on the evolution of sex-specific glomeruli

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Ball-, wedge- or islet-like units of neuropil, often referred to as glomeruli, comprise the first synaptic neuropil of olfactory systems, both in vertebrates and arthropods. Within the latter, certain species of insects possess specialized sex-specific olfactory glomeruli that are supplied by the endings of olfactory receptors tuned to components or blends of components of pheromones emitted by females of the same or competitively related species. Such prominent neuropils contain unusually large neurons (for an insect) that are readily accessed using intracellular recordings and dye fills. They have thus provided ideal 'model systems' on which to research the circuitry underlying olfactory integration. But do insects generally have macroglomeruli? And does sexual dimorphism exist in other arthropods, such as crustaceans and chelicerates? If other taxa have specialized receptors tuned to sex-specific odors, how are these represented in the brain? I will present a brief survey across the arthropods that suggests specialized olfactory glomeruli are characteristic of specific groups of insects, but not others. Among the chelicerates, anatomical observations identify enlarged and structurally specialized glomeruli in scorpions and amblypygids. These examples are of special interest regarding convergent evolution because in this group glomeruli have taxon-specific locations, being present in the neuromere of the segment from which arise the olfactory receptor organs.

140. It came from the sea—olfaction in the giant land crab *Birgus latro*

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An important step in the evolution of the olfactory sense has been the transition from sea to land. Although terrestrial and aqueous olfactory systems share many characteristics, marked differences exist. These distinctions probably reflect the differences in the ligands that the systems need to detect airborne, mostly hydrophobic volatiles on land and water-soluble molecules in the sea. The olfactory system of land crabs, whose terrestrial existence is a comparatively recent evolutionary development, represents an excellent opportunity to investigate the effects of the sea to land transition. Have land crabs come to the same solutions as other terrestrial animals, or is their olfactory sense characterized by unique innovations? Here we show that the terrestrial robber crab (*Birgus latro*) has evolved an olfactory sense that displays a high degree of resemblance to the insect system. The similarities extend to physiological, behavioural and morphological characters. The insect nose of the robber crab is a striking example of convergent evolution, and neatly illustrates how similar requirements result in similar end-products.

Keynote lecture 3

141. Pheromones and mouse reproduction: from receptor to behaviour

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Mice show a number of different responses to male urinary chemosignals that influence their reproductive success, including oestrus induction, puberty advancement and a block to pregnancy. These effects have a number of features in common. They all require the vomeronasal accessory olfactory system, act via the neuroendocrine hypothalamus and result in prolactin suppression which effectively brings females into oestrous and prevents implantation. All of these effects require a signal that conveys 'maleness' (brevicornin/thiazole), while the block to pregnancy additionally requires components that signal the male's identity (MUPs/MHC) since only strange males block pregnancy. The former cues signal via the V1Rs to the anterior accessory olfactory bulb (AOB), while male identity involves V2R signalling via the posterior AOB. The biological significance of male identification in the context of pregnancy block is in ensuring that pheromones from the male that mates do not terminate his own pregnancy; only those from a strange male can activate this neuroendocrine signal. If primer pheromones that act via the vomeronasal organ (VNO) convey individual identity, then this relatively primitive neural system has the capacity for individual recognition. A number of anatomical, pharmacological and electrophysiological studies show that the AOB is itself integral to this recognition process, and this recognition has much in com-

mon with main olfactory and hippocampal learning. Recent studies from the laboratories of Dulac (Harvard) and Mombaerts (Rockefeller) have also shown that the vomeronasal projections have the capacity to influence male sexual and aggressive behaviour. More notably, genetic ablation of a TRPC2 ion channel that localizes to VNO sensory microvilli impairs the male's ability to engage these behaviours in a sexually specific manner. Although complete ablation of the main olfactory receptors prevents males from distinguishing or showing a preference for female urine, males with genetic ablation of VNO receptor signalling are not sexually demotivated, but the behaviour occurs inappropriately to the sexual context in which the male is placed. Sexual dimorphisms in the neural connections of the vomeronasal projections occur under the influence of testosterone, and require activity in intrinsic GABA-ergic neurons. This raises the question as to whether signalling in the VNO may not only influence reproduction and behaviour, but also influence the developmental wiring of this system to the brain.

Oral session 5: Oral communication—pheromones

142. The rabbit mammary pheromone as a learning agent for neonates

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Postnatal adaptation of newborn mammals depends in large part on their ability to rapidly acquire new information that optimizes their behaviour. Depending on the species, various stimuli can act as neonatal incentives, e.g. thermal, tactile, vocal and visual inputs from mother and littermates, milk and the act of sucking itself. All these stimuli can facilitate the encoding of contingent, novel stimuli as cues to the reinforcing consequences of behaviour. Rabbit newborns are mainly driven by somesthesia and chemoreception in their search for milk. They are directed by various signals, including the mammary pheromone (MP) which is emitted in milk and is especially efficient at eliciting oral grasping (OG) and sucking. This latter behaviour itself appears to establish associative learning of any contingent odorant carried by the mother's abdomen (Hudson *et al.*, 2002). This work examines whether the MP could eventually be a reinforcing agent mediating this olfactory learning. In experiment 1, two groups of 20 pups (age 2 days) were exposed to the MP paired either with neutral odorant A (conditioned group) or with odorant A alone (control group). They were then tested for OG to odour A. It resulted that odour A itself elicited OG at the same level as MP, i.e. in >90% of the conditioned group. Thus, the OG response initially released only by MP became also associatively linked with odour A. This novel odour-response coupling was clearly due to MP since control pups did not display any response to A. In experiment 2, the generality of this MP-induced odour learning was positively established with a second odorant which was qualitatively distant from odour A. These results make it clear that MP not only constitutes a strong releaser of neonatal OG behaviour involved in milk ingestion, but also constitutes a reinforcing agent acting in one trial to induce an appetitive olfactory learning.

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143. Is the rabbit mammary pheromone the same as the rabbit nipple-search pheromone?

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The abdominal skin of sexually mature female rabbits is the carrier of undefined odour cues which release in rabbit pups a very specific searching behaviour, guide pups to nipples, and finally allow grasping, attachment and sucking of nipples. The odour cues have been termed nipple-search pheromone (Hudson and Distel, 1983). The first drops of rabbit milk are carriers of odour cues which, when presented on a glass rod, release in rabbit pups search-like head movements and grasping of the rod. The cues have been termed 'mütterliches Pheromon' (Schley, 1976) and later equated with the nipple-search pheromone (Keil *et al.*, 1990). 2-Methylbut-2-enal (2MB2) is the sole compound in rabbit milk which, when presented on a glass rod or from a GC outlet, releases in rabbit pups search-like head movements and grasping. 2MB2 has been termed rabbit mammary pheromone (Schaal *et al.*, 2003). However, milk can be excluded as the odour source for the release of nipple-search behaviour by female rabbits, and, unfortunately, there is little evidence as to the presence of 2MB2 on the abdominal skin of females (Moncomble *et al.*, 2002). We will therefore review the presently available behavioural and physiological data for 2MB2 which allow the mammary pheromone to be regarded as functionally equivalent to the nipple-search pheromone. Most findings of the Schaal group are in line with the earlier findings of Schley, Hudson and co-workers, while other findings still await their confirmation.

144. Emotional priming of facial perception and the startle-reflex are modulated by the odour of anxiety

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Many socially living vertebrates communicate anxiety via chemosensory signals. So far, some rating studies reported on a similar phenomenon in humans. Here, we investigated whether subliminal face perception and the startle-reflex amplitude can be pre-attentively modulated in the context of anxiety odour. Axillary odour samples were taken from 12 males before an academic oral examination (anxiety condition) and during a sport situation (control condition). Subjective ratings reveal that all donors experienced more anxiety and less pleasure during the anxiety condition ($P < 0.001$ each). An emotional priming study, using happy, fearful and sad facial expressions as primes (12 ms) and neutral faces as targets (47 ms) was carried out with 16 subjects. In the context of the control odour, judgements of targets primed by the happy face were more positive than judgements of targets primed by negative facial expressions ($P = 0.02$). In the context of the anxiety odour, the priming effect of the happy face was reversed in females ($P = 0.02$). The perception of the negative facial primes was not affected by the anxiety odour. Additionally, acoustic startle probes (100 dB, 50 ms bursts of white noise) were presented to seven subjects during and between the presentations of the anxiety odour, the

control odour and the odour of unused cotton pads. The anxiety odour enlarged the startle-reflex amplitude significantly, compared to the odour of the control condition ($P < 0.02$) and compared to the odour of unused cotton-pad ($P < 0.02$). The results of the priming experiment indicate that in socially relevant ambiguous situations human perception might be predominated by chemosensory signals. Additionally, the results of the psychophysiological study refer to a pre-attentional withdrawal activation through the perception of human anxiety odour.

145. Latent alarm signals in tadpoles of the common frog, *Rana temporaria*

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It has been assumed that chemical alarm signals ('Schreckstoff') are present in tadpoles of toads (like *Bufo bufo*), while they are lacking in tadpoles of several frog species (like the common frog, *Rana temporaria*). In prey species where alarm signals are present, predators may also be detected in a particular way. 'Predator labelling' is the mechanism by which aquatic animals may detect predators indirectly by sensing alarm signals from ingested conspecific prey. Recently, a new type of chemical alarm signal has been discovered in invertebrates based on 'predator labelling'. These signals, called 'latent alarm signals', do not induce instant alarm behaviour when released by injured conspecific prey. Latent alarm signals are inactive while present in live prey tissue, and must pass through the gastrointestinal tract of a predator to become active. The process of signal activation is probably carried out by intestinal bacteria. Since tadpoles of the common frog are reported to respond to predator odours, the presence of latent alarm signals in this vertebrate species was investigated. Experiments were designed where fish were fed either *R. temporaria* tadpoles or control feed, and later allowed to condition test waters. Extracts were also made from homogenized tadpoles, where the homogenates were used for making either fresh extracts, or were allowed a 3 h respite at room temperature before extracts were made. When extracts were prepared for behavioural testing, they were frozen immediately after being made. Based on studies in behaviour, morphology and life history, the presence of latent alarm signals in tadpoles of the common frog were subsequently tested. Tadpoles responded to test waters containing conspecific scent by avoidance behaviour, and prolonged time to reach metamorphosis combined with lower weight at metamorphosis, compared to control waters. Accordingly, it is concluded that latent alarm signals seems to be present in tadpoles of the common frog.

146. The influence of predator odours on reproduction in rodents

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Reproductive traits in rodents are affected by a number of environmental, social and chemosensory factors. The majority of

studies on reproductive inhibition have focused on intraspecific influences of semiochemicals and how they influence reproductive output and behaviour in females. A few studies have focused on between-strain influences or interspecific influence, although the source odour generally is still confined to rodents. We examined the influence of predator chemical cues derived from geographically sympatric and allopatric predators on the reproductive output of rats, mice and voles. Naïve laboratory animals respond to predator chemical cues with reduced litter size and skewed sex ratios. In mice we observed block of pregnancy. This indicates an innate nature of the response. Reduction in litter size was brought about by resorption of the embryos during the early part of gestation. The reduction in litter size in rodents exposed to predator urine was attributable to suppressed progesterone levels affecting the implantation of embryos and not to stress or fear. Chronically high corticosterone levels did not affect litter size. Exposure of Norway rats to cat urine also affected the number of corpora lutea. Seasonal changes in sensitivity to predator odour were observed in Norway rats and in house mice. The suppression of rodent reproduction also occurred when rodents were exposed to urine of conspecifics housed under high population densities. The evolutionary adaptive response for reduced litter size is to produce high-quality offspring in an environment where food resources are scarce. The fact that rodents respond to certain chemical signals in predator urine in a similar fashion may be fortuitous, and may have more to do with the coincidence that the urines contain similar chemical cues resulting from protein digestion in carnivores and protein catabolism in nutritionally deprived rodents rather than specific predator-prey adaptations.

Poster session 3: Anatomy/histology/development

147. Vomeroolfaction in Squamata

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Vomeroolfaction plays a major role into the interactions between squamates and their environment. Tongue flicking behaviour in both sister-groups (Iguania and Scleroglossa) occurs to gather chemical information for detection of food, conspecifics (kin and sex recognition) and predators. The tongue plays the major role in obtaining chemicals from the environment and transmitting molecules toward the vomeronasal organ through the buccal cavity. Morphological analysis permitted a comparison of the mechanism of the tongue protraction-retraction cycle involved in each type of tongue flicking in representative species of both sister-groups. Kinematic analysis based on high-speed video (250 frames/s) allowed us to determine performance in these tongue flicks. Both analyses are used to suggest differences into the neuromotor control of tongue flicking in iguanians and scleroglossans and relate such difference to the behavioural properties of the vomeroolfactive behaviour in these squamates. Finally, relationships between lingual structure and performance are examined to investigate the mechanism involved in vomeroolfaction and show that evolutionary constraints (phylogenetic versus adaptive) play a dominant rule in both sister groups.

148. Immunocytochemical demonstration of γ -amino-butyric acid (GABA) and neuropeptides in the antennal lobe of the noctuid moth *Heliothis virescens*

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The antennal lobe of the moth brain is the primary olfactory centre processing information about pheromones and plant odours. As described in other insect species, there are two main populations of antennal lobe neurons: local interneurons and projection neurons (Homberg *et al.*, 1988, *Cell Tissue Res.*, 254: 255). Whereas the local interneurons communicate within the antennal lobe, the projection neurons carry information to higher brain centres, including the mushroom bodies and areas of the lateral protocerebrum. In the noctuid species *Heliothis virescens* the antennal lobe consists of ~65 glomeruli, including a male-specific macroglomerular complex (MGC) (Berg *et al.*, 2002, *J. Comp. Neurol.*, 446: 123). The importance of olfactory communication has been thoroughly studied in *H. virescens* by characterizing the significance of the individual MGC units via functional tracing of the pheromone receptor neurons as well as of the antennal lobe projection neurons (Hansson *et al.*, 1995, *J. Comp. Physiol.*, A177: 535; Christensen *et al.*, 1995, *J. Comp. Physiol.*, A177: 545; Berg *et al.*, 1998, *J. Comp. Physiol.*, A183: 669). To obtain further knowledge about the neural networks underlying olfactory processing, we have used immunocytochemical techniques to map the antennal lobe of *H. virescens*. Stainings for γ -amino-butyric acid (GABA) and the neuropeptides allatotropin, allatostatin, locustatachykinin and Phe-Met-Arg-Phe (FMRF) amide, were carried out by the indirect peroxidase-antiperoxidase (PAP) technique. In addition, double immunofluorescent stainings combined with confocal microscopy were performed. Each antiserum labelled a particular set of antennal lobe neurons with soma located in the lateral cell cluster. The results indicate that the majority of the labelled neurons are local interneurons. The double immunostaining experiments showed co-localization of GABA and the neuropeptides.

149. Early development of the human vomeronasal organ

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The human vomeronasal organ (VNO) remains an enigmatic structure, with no consensus about its physiological significance. Here we re-examined embryonic and fetal human VNO development with special emphasis on the presence of neuron-like cells. At early embryonic stages (5.5 gestation weeks), before the presence of a fully developed VNO, cells expressing calretinin (CalR), GAP43 and Reelin (a glycoprotein involved in cortical neuronal migrations) were observed in the stream of cells leaving the placodal epithelium. Interestingly, Reelin expression was not detected during later stages of development, neither in the epithelium nor in migrating cells, suggesting a special role for this

glycoprotein in the migration of early cells. Earlier stages have to be studied to clarify this point. From 7 to 12 weeks of gestation, vomeronasal and olfactory epithelia show the same expressions of CalR and beta III tubulin, adding information about the neurosensorial-like phenotype of some human VNO cells, as previously suggested in other studies. The Notch signalling pathway is involved in determining cell fates in a variety of tissues, and, depending on the context, either inhibits or induces differentiation. Here, we compared Notch1 expression in both olfactory and vomeronasal epithelia during development in human fetuses and fetal rats. In both species, there was a small number of Notch1-positive cells in olfactory epithelium, as compared to the number of CalR- and tubulin-positive cells. In the vomeronasal epithelium, few cells were Notch1-positive too, suggesting that during early development, differentiation of some cells of both epithelia could imply the Notch-signalling pathway. Moreover, among migratory neurons detaching from the human VNO at 7–12 weeks, Notch1-positive cells were probably LHRH neurons. The role played by Notch1 signalling in olfactory and vomeronasal epithelia formation has to be determined.

150. Viral tracing of trigeminal neurons innervating the nasal cavity of mice

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The trigeminal nerve (V cranial) is the major mediator of sensations from the mammalian head and comprises neurons that transduce mechanical, thermal and chemical stimuli. It conveys information from the meninges, the cornea and conjunctiva of the eyes, the facial skin and the mucous membranes of the oral and nasal cavities. Thereby single neurons mediate sensory input from selective areas of the head. Differential physiological features of peripheral neurons depending on their function and area of innervation remain largely unclear. The identification of cultured trigeminal neurons primarily innervating the nasal cavity is a basis for subsequent activity recordings on this chemosensory subpopulation. Viral tracing was performed to identify trigeminal neurons that mediate sensory information from the murine nasal cavity. After application of high titered Pseudorabies virus (PrV, expressing GFP, RFP or β -galactosidase) into the nose, marker protein and immunohistochemistry based investigations were carried out. Paraffin-embedded sections and whole-mount preparations were used to describe viral spread. Histochemical investigations revealed an ipsilateral spread via the ophthalmic division of the trigeminal nerve to the gasserian ganglion (GG). Infected GFP/RFP-labeled ganglion neurons could also be identified after dissociation and plating, allowing electrophysiological- and calcium-imaging-based characterization of functional properties. PrV constitutes a powerful tool to perform rapid transneuronal tracing of the murine trigeminal system and affords the possibility of selectively labelling neurons innervating the nose. Electrophysiological- and calcium-imaging-based characterization of these neurons concerning their specificity for selected chemical compounds will be the subject of future research.

151. A three-dimensional atlas describes the evolution of the projection fields in the main olfactory bulb of *Xenopus* during metamorphosis

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The olfactory system of *Xenopus* uniquely features two subunits: one involved in detection of water-borne odorants, the other involved in detection of air-borne odorants. These two systems are anatomically distinct. The olfactory sensory neurons (OSNs) from the aerial olfactory epithelium localized in the principal cavity (PC) are thought to project exclusively to the mediodorsal part of the main olfactory bulb (MOB), whereas OSNs belonging to the aquatic olfactory system localized in the medial cavity (MC) would project to the ventrolateral part of the MOB. By contrast, *Xenopus* tadpole only has an aquatic olfactory system whose OSNs project to the entire MOB. Therefore, a huge reorganization of olfactory projections takes place during metamorphosis (J.O. Reiss and G.D. Burd, 1997, *Cell Dev Biol.*, 8: 171–179). Using DiI injections and SBA stainings, we revealed subdivisions in the aquatic olfactory system and studied their evolution during metamorphosis. We have investigated the evolution of OSN projections from stages 47 to 66 and built a three-dimensional atlas of these projections at four representative stages. We showed that the aquatic MOB is divided into seven main projection fields conserved throughout metamorphosis. While new PC axons invade the dorsomedial part of the olfactory bulb, thus forming a steadily growing new field, the seven conserved fields of the MOB progressively shift to its ventrolateral part. This three-dimensional atlas will support further developmental and functional studies.

152. Two closely located areas in the suboesophageal ganglion receive projections of the taste receptor neurons located on the antennae and the proboscis in *Heliothis virescens*

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The nectar feeding moth *Heliothis virescens* can learn odours, as demonstrated by the use of the proboscis extension reflex, i.e. sucrose stimulation of the taste sensilla elicits extension of the proboscis. By pairing odour stimulation with sucrose stimulation, the moths extend the proboscis when subsequently stimulated with the odour alone. In order to reveal the neuronal connection between the olfactory (conditioned) and taste (unconditioned) pathways we have studied the taste sensilla and the projections of the associated receptor neurons in the primary taste centre, the suboesophageal ganglion (SOG). Fluorescent dyes were applied to the antennal (*s. chaetica*) and the proboscis (*s. styloconica*) taste sensilla. Examination in a confocal laser scanning microscope (CLSM) revealed the labelled primary axons in the brain. The axons from *s. chaetica* ran tightly together, bypassed the antennal lobe posterior-laterally and terminated ipsilaterally in two areas, first in a fan-shaped pattern in the antennal mechanosensory and motor centre (AMMC), and then extending in a fingerlike pattern reaching from the deutocerebrum into the dorsal SOG. Projection of a single fibre in the

ventral SOG was also found in some preparations. Axons of the receptor neurons in *s. styloconica* on the proboscis entered the SOG via the maxillary nerve and terminated in the dorsal neuropil in a pattern parallel, anterior and medial to the projections of the antennal taste neurons. A few single fibres projected both ipsi- and contralaterally in the SOG. A three-dimensional reconstruction of the SOG with precise location of the taste areas was made by the use of the software AMIRA.

153. Evidence for the involvement of serotonin on the development and innervation of mice taste buds

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Taste buds are assemblies of slender epithelial cells that receive and process sapid information from the environment. In mammals, their development and innervation takes place during the late phase of embryogenesis but also in the adulthood. In fact, in the adult mouse tongue, an average of 11% of the gustatory receptor cells are replaced each day. However, the cellular factors and the molecular mechanisms that control the differentiation and renewal of taste cells remain largely unknown. Several lines of evidence indicate that the neurotransmitter serotonin (5-HT) behaves as a differentiation and axon guidance signal during midgestation. Based on the existence of serotonin and its reuptake system in the developing tongue epithelium, we have examined in the present study the potential involvement of this neurotransmitter in the development and innervation of the taste buds. Time pregnant C57BL/6J mouse were injected subcutaneously with *p*-chlorophenylalanine (pCPA), a synthesis inhibitor of 5-HT, from gestational day 13 to 19. The number and innervation pattern of the taste buds in neonatal mice was then examined by immunolabeling using the monoclonal antibodies Troma-1 and 2H3 as markers for taste buds and gustatory nerve processes respectively. Neonatal offspring from pregnant C57BL/6J mice treated with pCPA showed a 52% reduction in the number of taste buds as compared to the offspring of saline-treated animals. In addition, *in utero* exposure to pCPA impaired the innervation of the remaining taste buds in the anterior part of the tongue without affecting either the density or the innervation profile of the posterior taste buds. These anatomical changes are associated with a drastic reduction in the brain content of serotonin as determined by HPLC analysis. Our observations suggest that 5-HT might act as a neurotrophic factor for the developing taste bud.

154. Neurogenesis in human olfactory epithelium

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The birth and differentiation of neurons has been extensively studied in the olfactory epithelium (OE) of rodents, but far less is known about this process in humans. We aimed to characterize the cellular profile and expression of molecular markers useful in characterizing early stages of neurogenesis in human OE, *in vivo* and *in vitro*. The rodent OE is a highly laminar structure in which

cells progress up from the basal lamina as they divide and differentiate. In the human OE, the organization of proliferating, early and later precursors and mature neurons is not organized in a laminar distribution. Horizontal basal cells, which are actively dividing cells along the basal lamina in rodents, are not evident, and proliferating cells labeled with ki-67 are rounded cells in the second layer above the basal lamina. Nearly all cells in contact with the basal lamina, as well as some ki-67-positive cells, express P75NGFR, which, in rodents, is only expressed during embryogenesis. Gap-43- and OMP-labeled cells were also β -tubulin reactive, and could be found at any depth in the OE. We further assessed OE neuronal differentiation *in vitro* in primary cultures. Neuronal cells derived from human OE biopsies express markers for immature and mature neurons, grossly recapitulating neuronal differentiation of olfactory neurons *in vivo*. As seen in OE, cells appeared *in vitro* that were immunoreactive for both ki-67 and P75NGFR. A subset of cells double-labeled for BrdU and mature cell markers, indicating maturation of neuronal cells born *in vitro*. These data highlight species-related differences in OE anatomy, suggest a role for P75NGFR in maintenance of the adult human OE and demonstrate the utility of primary cultures for experimental studies of human olfactory neurogenesis.

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155. Expression of NogoA protein in the rat olfactory system during development and neuronal regeneration

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Regrowth of injured axons in the adult central nervous system (CNS) of mammals is very limited, unlike the spontaneous axonal regeneration observed in the peripheral nervous system (PNS). NogoA and other myelin-associated neurite-growth inhibitors are in part responsible for the lack of axonal regeneration in the CNS. In the olfactory system, primary receptor neurons constantly regenerate throughout adult life and newly formed axons reinnervate their target, the olfactory bulb (OB). The presence of an axon growth-permissive environment and guidance cues ensures correct targeting of olfactory axons both during development and epithelial regeneration. In this study, we examined the spatiotemporal expression pattern of NogoA protein in the rat olfactory system by immunohistochemistry. We analysed different stages of embryonic and postnatal development and several delays of axonal regeneration following unilateral bullectomy. We used a rabbit anti-NogoA antibody (kindly provided by M. Tessier-Lavigne, USA). NogoA is strongly expressed by growing olfactory axons from embryonic day E13. This strong expression persists during embryonic and postnatal development. From postnatal day (P) 6 onward, we observed a decrease in the intensity of NogoA labelling in axon fascicles coursing through the lamina propria. Following unilateral bullectomy, NogoA was seen to be up-regulated in growing olfactory axons at post-surgery times, corresponding to a massive renewal of olfactory receptor neurons. In the developing OB, broad NogoA expression is detected at early embryonic stages. After birth, isolated fibres in the external plexiform and glomerular layers show strong NogoA expression. Only a low and diffuse staining persists

in the adult OB. Unlike the known function of NogoA as an axon-growth inhibitor, our data suggest an additional function for NogoA in the olfactory system. Expression of NogoA by olfactory receptor neurons could support olfactory axon outgrowth both during development and axonal regeneration.

156. Taste bud innervation patterns and hyperinnervation in BDNF over-expressing mice

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The peripheral taste system probably maintains a specific relationship between ganglion cells that signal a particular taste quality and taste bud cells selectively responsive to that quality. We have explored a measure of the receptoneural relationship in the mouse. By injecting single fungiform taste buds with retrograde neuroanatomical markers, the number of labeled geniculate ganglion cells innervating those buds was identified. On average, we found that 3–5 ganglion cells innervate a single bud. By injecting neighboring buds with different color markers, we also found that the buds are primarily innervated by separate populations of geniculate cells (i.e. multiply labeled ganglion cells are rare). In other words, each taste bud is innervated by a population of neurons that apparently only connects with that bud. This finding, that ganglion cells send sensory fibers that converge selectively on a single bud, is a new-found measure of specific matching between the two principal cellular elements of the peripheral taste system. Since growth factors and their receptors appear critical for the establishment of taste buds and their innervation, it is conceivable that the expression of these factors may influence peripheral innervation patterns, as in other sensory systems. We therefore explored this in transgenic mice that overexpress BDNF in the lingual epithelium (including cells of fungiform papillae) and that show 193% more geniculate ganglion cells and 65% fewer buds. Consistent with the relative numbers of neurons and buds, we observed 4–5 times more innervation of taste buds. In other words, the innervation from the larger population of ganglion cells deploys evenly among the smaller pool of target buds. Moreover, as with wild-type mice, the mutant buds tend to have their own non-overlapping group of innervating ganglion cells. Thus, both wild-type and mutant mice exhibit an apparent fundamental receptoneural pattern—indicative of the degree and exclusivity of gustatory innervation.

Biology/physiology/hormones/ecology

157. Necrophobic behavior in termites induced by chemoreception

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When alates of *Pseudacanthotermes spiniger*, an African fungus-growing termite, were kept in a Petri dish for a few days, an in-

teresting necrophobic behavior could be observed, as soon as some individuals died. Alates first groomed and covered with saliva the cadavers, then buried them with sand and soil in order to isolate them physically from their partners. Under our experimental conditions (1 cadaver, 10 alates in a 11 cm diameter Petri dish, with moistened sand), this necrophobic behavior required a contact with the antennae or the maxillary and labial palps, and, therefore, was induced by contact chemoreception. In order to identify the semio-chemicals triggering this behavior, solid-phase microextracts (SPME) and liquid extracts of the integument of dead alates were analyzed by GC-MS. Fifty-one substances have so far been identified. The major compounds (C14–C18 saturated and unsaturated linear fatty acids, phenol and indole) were tested on a lure (a piece of filter paper). When individually tested at a concentration of an alate equivalent, no burial behavior was induced. However, if these compounds were mixed together, the necrophobic behavior was clearly induced in the alates. This necrophobic behavior was interpreted as a strategy to prevent the spreading of pathogenic organisms. This can have a significant biological sense before dispersal flight, as alates remain for a few weeks in small chambers inside the nest, or during the phase of a new colony's foundation, as pleometrosis can occur (foundation with several individuals). For the first time in termites, the chemicals responsible for this necrophobic behavior were identified.

158. Morphofunctional reports of taste in cetaceans (Odontocetes)

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The sense of taste is little understood in dolphins; however, some morphological features of the brain and cranial nerves suggest that dolphins may be endowed with taste sensations to some extent (S.F. Komatsu and Yamasaki, 1980, *J. Morphol.*, 164: 107–109; P.E. Nachtigall *et al.*, 1984, *Acta Zool. Fennica*, 172: 147–148). Many aspects of the topic are still unresolved, either because information available is incomplete and contradictory, or because data has been obtained only from captive animals. Also, it is still unclear whether dolphins possess taste glands which are known to contribute to the sense of taste in mammals. In order to evaluate the presence of a taste receptor, the tongue of different species of odontocetes has been examined for the distribution of taste buds. Samples of tongue and oral cavity mucosa have been dissected from five dolphins of different ages and both sexes found stranded and dead along the coasts of Corsica and Sardinia. The samples have been dissected out within 24 h of the finding and divided into two lots: the former was formalin buffered-fixed, paraffin embedded and stained for morphological analysis; from the latter taste buds were isolated after enzymatic digestion of the extracellular matrix of the lingual tissue and put in saline with the aim of designing and setting up suitable electrophysiological experiments. The morphological data paralleled the results obtained by behavioural bioassay. Morphofunctional issues on the taste structures in odontocetes will be discussed.

159. The influence of oral physiology and product characteristics on the swallowing threshold

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The urge to swallow food might be triggered by a threshold level in both food particle size and lubrication of the food bolus. Thus, both oral physiology and product characteristics may influence the swallowing threshold. We quantified the swallowing threshold in a group of 87 healthy adult subjects (age 42 ± 12 years) by counting the number of chewing cycles needed to prepare food for swallowing. We determined the influence of oral physiology on the swallowing threshold by measuring salivary flow rate, maximum bite force and masticatory performance. We used $\sim 9 \text{ cm}^3$ of bread, toast, Melba toast, breakfast cake, peanuts, cheese and carrot to determine the influence on the swallowing threshold of various food characteristics, e.g. hardness, moisture and fat. Furthermore, we tested the effect on the swallowing threshold of buttering the bread, toast, Melba toast and breakfast cake. Salivary flow rates were significantly and negatively correlated with the number of chewing cycles of Melba toast and breakfast cake. Hence, subjects with more saliva needed fewer chewing cycles for these dry products. Maximum bite force and masticatory performance had an influence on the swallowing threshold for the hard products only (Melba toast, peanuts and carrot). We found significantly different numbers of chewing cycles for the various foods, ranging from 17 for cake to 63 for carrot. Hard and dry products needed more chewing cycles before swallowing. Buttering the food significantly reduced the number of chewing cycles needed before swallowing. This was especially true for the dry products cake, Melba toast and toast. Hard and dry products require more chewing cycles and longer time in the mouth before swallowing for sufficient breakdown to take place and for enough saliva to be added to form a coherent bolus safe for swallowing. Consequently, more saliva, higher maximum bite force and better masticatory performance were correlated with a smaller number of chewing cycles. In addition, butter enhanced lubrication and bolus formation of dry products. In conclusion, product characteristics as well as oral physiology affect swallowing threshold.

160. Impacts of ecology and morphometry on average and specific olfactory sensitivities in the marine and terrestrial mammalia

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Nasal cavity morphometry and chemical ecology were evaluated as correlates of olfactory sensitivity. The following hypotheses were tested. (i) The specific olfactory sensitivities of a mammal for compounds that are common in its natural habitat are elevated relative to other compounds and other mammals. (ii) Average olfactory sensitivity is related to morphometric features of the nasal cavity that increase delivery of odorant to the olfactory epithelium. Olfactory thresholds of 13 terrestrial mammals for a variety of naturally occurring carboxylic acids, alcohols and esters were obtained from the literature. In addition, thresholds for a subset of these com-

pounds were behaviorally measured in five captive sea otters. Nasal cavity specimens of all 14 species were imaged using computer tomography scanning and conventional histology. The surface area, lumen volume, length and position of the respiratory and olfactory region of each specimen were measured and a simple flow model used to provide from these measurements a morphometric estimate of olfactory uptake efficiency. Volatile chemical profiles for 210 natural sources including animals (scent glands, urine, body tissues), plants (roots, stem, bark and leaves), flowers, fruits, grains, honeys and fungi were obtained from the literature. These were separated into marine and terrestrial guilds and further subdivided by taxon, biome and geographical region. The thresholds for widely tested compounds were combined to generate a standardized olfactory threshold for each species. These standard thresholds were used to evaluate olfactory uptake efficiency as a predictor of general olfactory sensitivity. Specific thresholds of each species for each compound were normalized by the standard threshold for the species and the standardized specific thresholds pooled among compounds and species to evaluate a regression of specific olfactory sensitivity on ecological relevance (frequency of occurrence in the species natural habitat).

161. Oral administration of unsaturated fatty acids triggers a rapid rise in bile flux and protein content of pancreatic juice in rats with ligatured esophagus

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Recent evidence suggest that lipids, especially long-chain fatty acids, might constitute gustatory stimuli in mammals. In humans, the presence of fat in the oral cavity is enough to affect blood lipid parameters (Mattes, *Physiol. Behav.*, 2001, 74: 343–348). In esophagostomized rats, oral stimulation with fats produces a rise in the enzyme secretion by the exocrine pancreas (Hiraoka *et al.*, *Physiol. Behav.*, 2003, 79: 713–717). Collectively, these data suggest the presence of a lipid detection system in the oral cavity which might be responsible for digestive anticipation allowing an efficient absorption of dietary fat. To further explore this hypothesis, bile and pancreatic juice were collected separately from rats with ligatured esophagus subjected to an oral load with either a medium-chain fatty acid, a saturated or an unsaturated long-chain fatty acid in suspension in water [e.g. caprylic acid (C8:0), stearic acid (C18:0) and linoleic acid (C18:2,n-6) respectively]. Control animals received water alone. No change in either bile or pancreatic juice secretions were noticed after oral stimulation with caprylic and stearic acids compared to control animals. By contrast, a significant increase in the bile flux occurred 5 min after introduction of linoleic acid in the oral cavity. The maximal effect was detected at $t = 10$ min and was followed by a progressive return to the basal secretion level. Simultaneously, the protein content of pancreatic juice was enhanced without any corresponding change in the exocrine pancreatic flux. These results demonstrate that oral stimulation by free fatty acids leads to a rapid change in flux and composition of digestive secretion. This effect is selective since it is found only with unsaturated fatty acids. Taken together, these new data provide additional evidence for the role of free unsaturated fatty acids as potential gustatory stimuli.

162. Parvalbumin α : a proteinaceous chemoattractant recognized by the vomeronasal organ of *Thamnophis marcianus* (Colubridae)

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The vomeronasal organ is involved in many behaviours such as territory recognition, partner courtship or prey recognition. In *Thamnophis*, the latter depends on the presence of phagostimulating compounds in the preys' cutaneous mucus. With the aim of understanding the structure–function relationships of the phagostimulating compounds and the working mechanisms of the vomeronasal system, an extensive effort has been made to isolate, purify and characterize the chemoattractive agents present in the cutaneous mucus of some prey species of *Thamnophis marcianus*. Three proteinaceous complexes with Mr 14, 24 and 30 kDa, which elicit attack by snakes, have been isolated from the mucus of *Rana temporaria*. The 14 kDa complex seems to contain only two proteinaceous entities coeluting in RP-HPLC and of similar molecular weight, suggesting that they are isoforms of the same protein. Moreover, this protein is able to give rise to two proteinaceous bands at 24 and 30 kDa, where phagostimulating activity was originally observed. Finally, an antibody raised against the 14 kDa protein recognizes two proteinaceous bands at 24 and 30 kDa. Amino acids microsequencing and immunological characterization have shown that this phagostimulating protein belongs to the parvalbumin α family and is produced in the mucous gland of the frog's skin. This calcium-binding protein is generally found in muscle and nervous cells and the present results constitute the first evidence of the extracellular localization of a parvalbumin. This work is in accordance with previous studies on the earthworm, suggesting that proteins are the chemical signal for prey recognition in *Thamnophis* snakes. We are currently investigating the phagostimulating properties of frog muscular parvalbumin and its structural resemblance to mucus parvalbumin. We also evaluate to what extent the present observations could be generalized to other prey of *Thamnophis*.

163. Organization of the septal organ: olfactory receptor expression and nerve fiber projection

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The septal organ (SO) is a small patch of olfactory epithelium located as an island in the respiratory epithelium on the nasal septum. Although discovered decades ago, its functional relevance remains enigmatic. Here, we have identified a repertoire of chemosensory receptors expressed in the SO. The results demonstrate that SO neurons express receptor genes belonging to class-II olfactory receptors that are also expressed in the main olfactory epithelium. In the SO, no topography analogous to the receptor expression zones of the main olfactory epithelium was evident. The majority of identified receptors correspond to genes with restricted expression in the medial and lateral zones of the main olfactory epithelium. Most of the receptor types were found to be

expressed in only few SO neurons, except for mOR244–3, which was observed in a very high proportion of cells. Although a large fraction of SO neurons expressed mOR244–3, we found no evidence for the co-expression of different receptors in individual cells. Analyzing the projection pattern of SO neurons using the OMP-GFP transgenic mouse line revealed that axons navigate in highly variable fiber tracks across the main olfactory epithelium towards the main olfactory bulb. All SO axons cross through the cribriforme plate at a spatially defined site and terminate exclusively in the posterior, ventromedial aspect of the bulb. Here, one portion of axons forms a dense network on the medial side where they apparently enter glomeruli which are mainly innervated by axons of olfactory sensory neurons from the main olfactory epithelium. Another significant portion of the axons targets a few glomeruli which appear to receive input exclusively from the septal organ neurons.

164. Metabolite levels in mouse urine: the importance of not averaging over individuals

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A previously described multi-analyte mouse urine analysis method requiring only 20 μ l per sample was used to determine methyl disulfide, 2-*sec*-butyl-4,5-dihydrothiazole, geraniol, indole, *trans*- α -farnesene and *trans*- β -farnesene levels in the urine of individuals that had been housed in groups under various grouping conditions (males only, females only, males mixed with females, etc.). Preliminary results show significant differences in analyte levels between the sexes and among males, but only relatively small differences among females.

165. Odorant-induced signaling pathways controlling human sperm chemotaxis

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Fertilization is still one of the best-kept secrets of nature. After more than a century of investigations we still have only rudimentary ideas about how spermatozoa finally make their way to find and fertilize the egg. Recently, we provided evidence that an olfactory receptor, hOR17-4, plays a key role in navigating human spermatozoa. A variety of floral odorants, especially the scent of 'lillies of the valley' (bourgeonal), are capable of activating this 'unconventional' sperm receptor. Based on these findings, numerous questions arise. Most notably, we ask which molecular mechanisms link hOR17-4 activation to such significant changes in spermatozoa behavior. Do the signaling mechanisms in male gametes resemble those long-studied pathways employed in nasal olfactory neurons or is the physiological difference of these two unrelated cell types also reflected in individual signaling cascades? Introducing a new approach to protein identification in mature spermatozoa that is based on mass spectrometry, we provide evidence for expression and participation of specific

receptors, G-proteins and membrane-bound adenylate cyclases (mACs) in the underlying signaling cascade. Spatial distribution patterns of the identified signaling components largely correspond to the spatiotemporal character of odorant-induced Ca^{2+} changes viewed via single-cell, high-resolution imaging techniques. In further imaging experiments, as well as behavioral assays, we show here that a mAC couples OR activation to changes in spermatozoa swimming behaviour, such as chemotaxis, chemokinesis and hyperactive flagellar beating. In summary, our work provides new insights into the important initial steps that transduce detection of chemical signals into ordered changes in spermatozoa swimming behaviour. Thus, the present data provide a basis for further investigations in the important field of fertility and contraception.

166. Stinky mushrooms and rotting cacti—drosophilid olfaction outside the *Drosophila melanogaster* subgroup

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For the drosophilid fruitflies olfaction is a crucial commodity and serves as the primary mode through which resources are located. We have previously investigated the olfactory tuning of the members of the *Drosophila melanogaster* subgroup and have shown that the olfactory system in these species is highly conserved. We have now extended our investigation into the evolution of the olfactory code to encompass species outside the *melanogaster* subgroup. We have primarily investigated American cactophilic and European fungiphilic fruitflies. Through extensive use of linked gas chromatography–electroantennodetection we here show that the olfactory system of these distantly related species share distinct characteristics with the *melanogaster* siblings; however, specific shifts in the code are present that probably reflect adaptations to unique hosts.

167. Importance of heat and odour production in the reproductive strategy of *Helicoverpa muscivora*

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Several flowering plants have evolved elaborated means to deceive insects into acting as unrewarded pollinators. The dead horse arum, *Helicoverpa muscivora*, native to the western Mediterranean area, fools flies by emitting a smell like a dead animal, an important oviposition resource for these insects. The unpleasant smell is accompanied by a meat coloured, hairy appearance. We have previously shown that the olfactory cues are crucial to attract the pollinators during the first day when the inflorescence blooms, produces odour and is thermogenic. Blowflies enter the protogynous floral chamber carrying pollen they picked up from a previous visit. Some of them stay into the floral chamber until the next morning when the male florets have already started to produce pollen, which coats the flies during their escape. We have also investigated whether plant thermogeny has a direct effect on pollinator behaviour. By manipulating heat and odour release from the plant, we have shown that the heat produced along the appendix is important

to lure the flies to this structure. In fact, flies coming from the appendix are more prone to enter the trap chamber. The produced heat is significant for the success of the plant's reproductive strategy and plays an important role in guiding flies into the trap chamber that houses the female and male florets.

Pheromones/biological signals

168. Aphrodisiacs and sexual selection in cockroaches. Do *Leucophaea maderae* (Blaberidae, Oxyhaloinae) females ingest male secretions?

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Sexual behavior in cockroaches has been described in detail and it is generally admitted that three chemical cues are involved in mating: a volatile sex pheromone, emitted by the male or the female, attracts the partner from a distance; a contact sex pheromone is responsible for female recognition by males; and the so-called aphrodisiac secretions produced by male tergal glands enhance the final copulatory acts. These glands, exposed during the characteristic male wing-raising posture, urge the female to climb onto the male's back to lick the secretions. The female is then in a proper position for mating. Our studies on *Leucophaea maderae* demonstrated that the tergal secretion contains characteristic male proteins. The quantity of proteins produced is maximal on the median part of the second tergite. As in all investigated species of cockroaches, it was never demonstrated that the male secretions are really ingested by the female. Additionally, the physiological or behavioral roles of the secretions were never investigated. Using surgical ablations of various chemoreceptive organs, coloration or radiolabeling of the male secretion, and behavioral observations, we proved that females do really ingest the male secretions. The quantity and the nature of the secretion affect the duration of the various precopulatory acts. Labial and maxillary organs are involved in the chemoreception of the secretion and in the success of copulation.

169. Isolation of a fraction inducing activity in neurons of the 'alarm centre' in the olfactory bulb of the crucian carp, *Carassius carassius* L.

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In an attempt to isolate the alarm substances in cyprinids we have used the olfactory system of the crucian carp as an in-line neurophysiological detector (NPD) under chromatographic separation of the skin extract of this species (J. Chromatogr. B, 2004, 800: 41). In the initial analytical separation of fish skin extract, the active fraction displayed a characteristic UV three-dimensional spectrum. Further isolation of active fractions was made in two steps: (i) with a preparative adsorbosphere nucleotide–nucleoside reverse -phase column and (ii) with a preparative C18 reverse-phase column. In both cases the active fractions in the chromatograms were recognized by matching the new spectra with the previously collected UV three-dimensional analysis spectral data. These fractions, from preparative HPLC, were applied to the olfactory epithelium while recording the spike activity from neurons in the 'alarm centre' of

the olfactory bulb in crucian carp. The selected fractions were as potent as the crude fish skin extract from the crucian carp. These experiments demonstrate that by combining chromatographic and neurophysiological methods it is possible to isolate the active alarm substances.

170. Neonatal responses to the lactating breast: a first attempt at fractionating odours *in situ*

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The breasts of lactating mothers release a mixture of odour cues that elicit oral activity or positive orientation in newborns. These cues emanate from various sources including milk, areolar secretions, sweat or sebum. Previous studies have related maternal areolar skin glands to neonatal behaviour and growth. To sort out the gross source of the active cues, we examined here the responses of 3 day old infants to their mother's breast surface made completely or partially olfactorily accessible through selective covering with a transparent plastic film. The infants were videotaped while they were positioned, without contact, in front of their right mother's breast for two periods of 90 s. For each subject, the 'baseline breast' (i.e. fully covered breast) was compared with a 'treated breast'. Five conditions were tested where the baseline was contrasted with: (1) the fully exposed breast, (2) the breast and areola covered, and nipple exposed, (3) the breast and nipple covered, and areola exposed, (4) the breast covered with anointment of milk; and (5) the breast covered with anointment of areolar secretions. The first results indicate increased oral activity in contrast 1 ($P < 0.05$) and no differential response between both stimuli in contrast 2, confirming that the whole breast odour elicits appetitive responses, but that the nipple alone does not explain this attraction. The whole series of results will be presented and discussed.

171. Crab urine sex pheromones: plume search and dummy females

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During the last decades, a number of studies have focused on crustacean sexual pheromones, and evidence that several species rely on these is accumulating. However, an in-depth understanding of the mechanisms of pheromone communication is still lacking in this group as well as in marine systems in general. Several authors have confirmed the presence of a sex pheromone in the crab *Carcinus maenas*, but detail is lacking. This study was directed to examine direct effects of the female urine-borne pheromone using two methods that as far as possible eliminate other cues or dependence on innate excretory rhythms. The first assay used controlled flow delivery of the pheromone plume, and the second a pheromone-treated palpable target, i.e. a polyurethane-sponge dummy female. When males were run in a controlled flow olfactometer, they were provided only with an odour plume containing pre-moult female urine at different dilutions. Responding males showed increased activity time and number

of search runs. Furthermore, time from onset of stimulus delivery to the start of search behaviour was shorter. Several males showed the characteristic posing and tiptoe-walking search. Crabs responded to plumes containing 1 µl urine/l water. No significant increases in times or distances moved occurred at higher concentrations. Males provided with a dummy female target treated with pre-moult urine displayed other behaviours. When the dummy was presented to the male, he rose up and grabbed it with dactyls of the second or third pereopods, and cradle-carried the sponge as if it was a female. Female urine diluted 100 times or less generated cradle-carrying. Cradle-carrying times were longer at higher concentrations. Claw-stroking was displayed at 10 times dilution. Male posing was observed already at a dilution of 10⁴. This study indicates that the natural sequence of behaviours leading up to mating can be generated using only different concentrations of pre-moult female urine, and the presence of a target.

172. Coding of sex pheromones in the olfactory bulb of the crucian carp *Carassius carassius*

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Fish use the sense of smell to locate food, to avoid predators, to migrate and to reproduce. These behaviours are evoked by particular odours. A crucial question is how the olfactory system is organized in order to distinguish between the different odours. Recent studies showed that each of the three morphological types of olfactory sensory neurons in fish project to bulbar neurons whose axons make up a specific bundle of the olfactory tract. Because each bundle of the olfactory tract mediates a specific behaviour pattern, it is suggested that the morphology of the sensory neurons has functional implications. In the present study we have investigated the possible projection of the crypt sensory neurons into the lateral bundle of the medial olfactory tract, which mediates reproductive behaviour elicited by prostaglandin PGF 2 α in crucian carp. Furthermore, we have recorded the responses of bulbar neurons responding to four types of pheromones known in carp, i.e. 17,20 β -dihydroxy-4-pregnen-3-one; 17,20 β -dihydroxy-4-pregnen-3-one-20-sulfate; androstenedione and prostaglandin PGF 2 α . Our results indicate that the secondary neurons in the ventral part of the olfactory bulb do discriminate these different pheromones. These findings are congruent with the observation of different sexual behaviours evoked by these four pheromones. Our results also reveal that there is a sexual dichotomy in response to these pheromones.

173. The effects of skin extract exposure and availability of hiding material on alarm behavior and brain monoaminergic activity in crucian carp

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The crucian carp performs a typical behaviour when exposed to olfactory cues from injured skin of conspecifics. The fish swim rapidly to the bottom and hide in available material. This work examines

the effects of skin extract exposure and availability of hiding material on this avoidance behaviour, and concomitant changes in brain monoaminergic activity in crucian carp. Individual fish were exposed to skin extract in aquaria with or without hiding material. Exposure to skin extract resulted in the expected rapid movement towards the bottom of the aquarium. This avoidance behaviour lasted 1–2 min, whereupon activity decreased below the level observed before exposure, suggesting a ‘freezing’ type of avoidance behaviour. This behaviour was independent of the availability of hiding material. Brain dopaminergic activity increased in telencephalon and decreased in the brain stem following skin extract exposure, again independent of the availability of hiding material. However, fish kept in aquaria without hiding material showed an elevation of serotonergic activity in the brain stem and the optic tectum compared to fish with available hiding material. This effect was also observed without exposure to skin extract. Absence of hiding material, therefore, elicits a higher arousal or fear level. In fish with hiding material, the fish steered up a cloud of fine sediments and showed a more pronounced decrease in locomotor activity in agreement with this being a more efficient freezing or immobile avoidance behaviour. These results show that basic components of avoidance behaviour and related brain changes are present also in the fish brain, pointing to phylogenetic roots of avoidance behaviour also in higher forms.

174. Assessment of olfactory function and androstenone odor thresholds in man with or without functional occlusion of the vomeronasal duct

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While the function of the mammalian vomeronasal organ (VNO) has been shown in numerous studies, the functionality of the human VNO is a matter of debate. In previous studies we have demonstrated that a vomeronasal duct (VND) can be detected in ~50% of subjects. Similar numbers of adults are able to perceive the odor of androstenone, which is frequently regarded as a ‘human pheromone’. Considering that the effect of these putative ‘pheromones’ is often used in the context of activation of the VNO, the aim of this study was to look at thresholds for androstenone in adults with or without covering of the VND. In addition, the study aimed to look for correlations between sensitivity to androstenone odor and general olfactory function. A total of 34 subjects (19 men, 15 women, age range 18–78 years) participated. In addition to androstenone odor thresholds (concentration 0.1 μM to 10 mM), the subjects’ general olfactory abilities were measured using the ‘Sniffin’ Sticks’ test battery, which consists of tests for odor identification, odor discrimination and phenyl ethyl alcohol odor threshold. Measurements were performed with and without covering the VNO. In each subject the tests were performed on one side only. As established with the olfactory test battery, 6% of the subjects were anosmic; however, 24% of the subjects had a specific anosmia for androstenone. Covering the VND had no effect on olfactory test results of androstenone thresholds. Compared to men, female subjects had higher olfactory test scores (indicating higher sensitivity) and lower androstenone thresholds. In addition,

olfactory test scores were found to correlate with androstenone thresholds. These results indicate that the presence of a VND does not seem to play a major role in the perception of androstenone odor.

175. Context-dependent mood effects in men during estratetraenol exposure caused by sex of experimenter

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Exposure to the endogenous steroid estratetraenol has previously been shown to influence cortical metabolism and behavior in a sex-specific way. Other studies have shown that effects of putative pheromones also can depend on the sex of the experimenter. A previous study of whether the sex of the experimenter influences effects of exposure to estratetraenol in men could not be observed (Jacob *et al.*, 2001). The aim of the present study was to further investigate the effects of sex of experimenter for this particular substance. Seventy-eight males were exposed to either an experimental solution consisting of 250 μM estratetraenol in propylene and masked with eugenol, or a control solution consisting of propylene glycol and eugenol. The solutions were masked to prevent discrimination between the experimental and control solutions. Participants were tested by either a male or a female experimenter using a double-blind, between-groups design. Effects of exposure to estratetraenol on mood, heart rate and skin conductance were assessed. No significant overall effects of exposure on aggregated mood scales or the psychophysiological measurements were found. Individual subscales showed statistical tendencies ($P < 0.10$) for the experimental group to feel more focused, sensual and energetic than the control group. There was a significant interaction between experimenter sex and substance in that the experiment group felt fewer overall negative emotions when tested by a male. This result calls for further scrutiny with regards to the sex of experimenter as a relevant context when effects of putative human pheromones are investigated.

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176. The nipple of the lactating rabbit as an odor source: converging evidence

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Newborn rabbits are directed to their mother’s nipple by specialized odor cues whose exact sources remain elusive. Prior studies indicate that the active cues are distributed either on the nipple surface, or in the lumen of the lactiferous ducts (LD) as milk becomes behaviorally efficient only after passing through them. Nipples of lactating does (New Zealand–California breed) were thus screened for glandular sources known to be involved in chemical communication in other species. We searched for skin glands (i) diffusely

distributed on the nipple surface, (ii) situated around the pores of the LD, from where they could stain both the skin surface and the milk; and (iii) located in the LD and releasing their secretion in the flow of milk. Histology, light and electron microscopy, as well as X-ray microtomography, were used. It emerged that: (i) exocrine glands of sebaceous type opened only at the base of the nipple. (ii) No exocrine structures were found around the ostium of the LD. (iii) Apparently no exocrine structures are located in the LD lumen, but electron microscopic images revealed differential functional figures in the cells lining the ductal lumen in lactating and in virgin females. Further, tomography showed the highly circumvolved structure of the luminal surface of the LD, suggesting that their distal part may be involved in secretory/excretory exchanges with the flowing milk. In sum, while classical skin glands were clearly visible at the base of the nipple, no such structures could be pinpointed close to LD openings and in the nipple, which nonetheless are a known source of active cues.

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177. Circadian and developmental fluctuations in the releasing and reinforcing potencies of the rabbit mammary pheromone

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Newborn rabbits display a typical searching pattern under the mother's abdomen before grasping a nipple. Such responses are also released by the mammary pheromone (MP) emitted by lactating females, or by any odorant after it has been associated with the MP. Two studies addressed whether the releasing and reinforcing properties of the MP are modulated by internal factors related to satiation and early maturation. (i) The releasing activity of the MP was tested on 2- or 5-day-old pups ($n = 232$) either 5 min, 3 h and 6 h before the daily sucking, or 5 min, 3 h and 6 h after. (ii) The reinforcing impact of the MP on an initially neutral odorant was applied in 2- or 5-day-old pups ($n = 135$) at the same time-points as above and checked subsequently by counting pups expressing oral responses to the odorant alone. An age-related alteration of the releasing power of the MP became apparent: 2-day-old pups responded strongly to the MP ($\geq 95\%$) all along the 24 h cycle, while 5-day-old pups showed an inflexion of their response to the MP after sucking ($< 45\%$) before recovering high response levels prior to the next sucking. The ability of MP to induce odour-learning was also affected by both prandial and developmental factors. On day 2, the MP-induced learning was maximal ($> 80\%$) 3 h before and 5 min after sucking, but it dropped ($< 42\%$) 3–6 h after. On day 5, the MP was inefficient at inducing odour learning at any time. These results unveil two related processes of internal control on the functional roles of the MP: (i) a transition from an automatic to a prandially regulated response to the MP; (ii) a progression from optimal MP-reinforced odour learning around feeding time in the first postnatal days to the waning of this reinforcing power on day 5, delimiting a sensitive period for the reinforcing impact of the MP. These early ontogenetic changes will be discussed in the background of the unique constraints faced by rabbit newborns in the postnatal environment.

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178. Crucian carp (*Carassius carassius*) male response to ovulatory female in the wild

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Various experiments with goldfish have shown that sex hormones released from females act as olfactory signals detected by mature males and induce behaviour and endocrine responses. The same hormones, $17\alpha, 20\beta$ -dihydroxy-4-pregnen-3-one and prostaglandin F_{2a}, are also detected by the olfactory sense in mature males of the closely related crucian carp, inducing the same kind of responses. No studies have, however, demonstrated the priming and releasing responses in the wild, neither in goldfish nor in crucian carp. In the present study we demonstrate increased blood plasma levels of gonadotropin and volumes of strippable milt in crucian carp males kept together in the wild with conspecific females during their final maturation of eggs or with females injected with the egg maturation hormone $17\alpha, 20\beta$ -dihydroxy-4-pregnen-3-one.

179. Odour preference of newborn rabbit pups for the mother may be induced by the mammary pheromone

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Newborn rabbits display preferential orientation to lactating females on the basis of abdominal odours. In standard breeding conditions, they do not seem to discriminate their mother (M) from unfamiliar lactating does (UF), probably because in that species there is no selective pressure to do so. Here, we assessed first whether a maternal preference can be induced by odour learning in association with normal nursing. Three groups of newborns ($n = 3 \times 20$ pups) were exposed during the 3 day sucking period to an odorant A painted on the M's abdomen. The next day, they were given a preference test between the odours of two females who were scented with odour A or not. The pups oriented (i) longer to the scented M than to a control UF; (ii) equally to the scented M and a scented UF; and (iii) longer to a scented UF than to a control UF. Thus, rabbit pups can be induced to orient preferentially to a lactating doe (M or UF) bearing an odorant they have learned previously in the nursing context. Second, we evaluated whether the mammary pheromone (MP) emitted by lactating rabbits may be involved in that olfactory learning. The MP is known to release unconditionally the pups' searching-grasping response and to confer its power on any odour associated with it. Thus, pups ($n = 2 \times 20$, 3 days old) were conditioned to odour A in association with the MP, and then tested for their preference between two females odorized or not with odour A. They oriented longer to their M scented with A as opposed to a control UF, and no difference was noted between the scented M and a scented UF. In sum, although they seem normally non-selective regarding maternal odours, neonatal rabbits can be experimentally boosted to develop early preferences for given lactating does after odour learning. The above results designate the MP as one reinforcer underlying this phenomenon.

180. Spatial activation of the newborn rabbit olfactory bulb induced by the mammary pheromone: a 2-deoxyglucose study

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The newborn rabbit's survival is dependent upon its capacity to orally seize the mother's nipples for suckling. The success of the suckling behaviour of pups is partly dependent on a pheromonal signal emitted by the mother, which has been identified as 2-methylbut-2-enal and named 'mammary pheromone' (MP) (Schaal *et al.*, 2003). Peripheral electrophysiological responses to the MP have been reported in the olfactory system. Here, we used the 2-deoxyglucose (2-DG) method that allows to show the glomerular activity pattern in the olfactory bulb evoked by an odorous stimulus. Applying it in newborn rabbits, we explored responses of the olfactory bulbs (OBs) following an exposure to MP. After injection of radioactive 2-DG, newborn pups (2–3 days old) were stimulated by either MP or ethyl acetoacetate (EAA), an odorant that does not elicit grasping. A glass rod dipped in water dilutions (10^{-2} or 10^{-4} v/v) of either odorous substance was repetitively presented in front of the pup nose. Control animals were treated in the same way, without any odorant exposure. After a 40 min exposure, the OBs were dissected, frozen and serially sectioned. Autoradiographic films and the corresponding sections of the OBs were scanned for quantitative analysis. Exposure to the MP elicited a reproducible glomerular activation pattern which was mainly located symmetrically in the posterolateral aspect of both OBs. This pattern did not overlap that evoked by EAA which was tended to be in the medial part of the OBs. Moreover, the extent of the 2-DG patterns of activity appeared concentration dependent.

181. The role of chemical cues in reproductive isolation of closely related *Mus* species

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The *Mus musculus* s. lato species group includes closely related taxa at different stages of divergence: sympatric species (*Mus musculus*–*M. spicilegus*; *M. domesticus*–*M. macedonicus*; *M. domesticus*–*M. spretus*); parapatric taxa which hybridize in zones of contact (*M. musculus*–*M. domesticus*–*M. castaneus*); and allopatric species (*M. spicilegus*–*M. macedonicus*; *M. spicilegus*–*M. spretus*). As a result the *M. musculus* species group has served as an excellent model group in studies of microevolution (Sage *et al.*, 1993). In laboratory conditions three experiments were conducted: preference of conspecific and heterospecific urine odours in two-choice and four-choice situations and responses to these odours in a 'habituation–dishabituation' test. In all tests individuals of sympatric (*M. musculus*–*M. spicilegus*) and allopatric (*M. macedonicus*–*M. spicilegus*) species distinguished between the urine odours of con- and heterospecifics. Both males and females investigated conspecific urine odours more than heterospe-

cific odours in two-choice tests and preferred the arms of the experimental chamber with urine odours of conspecifics in four-choice tests. We recorded c-fos expression in main olfactory bulb (MOB) and in accessory olfactory bulb (AOB) in males in response to stimulation with urine from either con- or heterospecific oestral females. *M. musculus*, *M. spicilegus* and *M. domesticus* males showed clearly elevated c-fos expression in both MOB and AOB in response to stimulation with urine from conspecific oestral females. *M. spicilegus* males responded with elevated c-fos expression only in MOB to stimulation with urine from oestral *M. musculus* and *M. domesticus* females. In *M. musculus* and *M. domesticus* males also we did not observe c-fos expression in AOB in response to stimulation with oestral female urine from *M. spicilegus*. The data obtained support the hypothesis that chemical cues play a critical role in reproductive isolation of closely related *Mus* species.

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Clinical studies/brain imaging

182. Perception of freshness of food by humans: sensitization effects shown in a chemosensory evoked related potential paradigm

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Although some effects of learning on olfactory discrimination performances have been reported, applications in the domain of food odors are very unusual. The aim of our study was to look for physiological effects in the perception of freshness of foods. Chemosensory evoked related potentials (CSERPs) were recorded while human subjects were submitted to olfactory stimulations, using a computer-controlled olfactometer. Binary mixtures of compounds were made to mimic the alteration of a food odor, i.e. rancid butter: Four stimuli were delivered, a solution of a butter aroma (diluted at 10^{-2} v/v in mineral oil) and three binary mixtures of the aroma and the butyric acid at different dilution levels (10^{-5} , 10^{-7} , 10^{-9}). In addition, three mixtures of the butter aroma and dilutions of vanillin (10^{-1} , 10^{-2} , 10^{-3}) were used as controls. Right-handed healthy subjects (4 females and 4 males) divided in two balanced groups were tested on three consecutive days. On day 1, CSERP baselines were recorded in response to passive stimulations of butter aroma and to the three possible rancid mixtures. On day 2, subjects were sensitized by repetitive odor stimulations while completing a set of psychophysical assessments (intensity, pleasantness, familiarity and edibility). Half of the subjects (sensitized group) were trained to butter and rancid butter mixtures while the remaining subjects (non-sensitized group) were trained to butter and binary mixtures of vanillin–butter. On day 3, CSERPs were recorded while subjects compared butter and rancid butter, randomly delivered and producing a similarity judgement where they had to evaluate if the presented odors were similar or different. Results show sensitization effects on psychophysical assessments, and CSERP recording shows differences in cortical brain activity between the two groups (sensitized versus non-sensitized). The difference was paired with a facilitation in discrimination performances (butter versus rancid butter).

183. Olfactory dysfunction after traumatic brain injuryC. Eloit¹, J.L. Bensimon¹, S. Puget² and D. Trotier²¹ENT Department, Hôpital Lariboisière, Paris, France and²Neurobiologie Sensorielle, Massy, France.

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Olfactory dysfunctions are often consequences of traumatic brain injuries (TBIs). The physiopathology underlying this dysfunction is thought to be due to damage, stretching or disruption of the olfactory fibres going through the cribiform plate as a consequence of displacement of the brain, leading to a progressive atrophy of the olfactory bulbs. Olfactory bulb and orbitofrontal cortex damage have been reported in TBIs, using magnetic resonance imaging (MRI) of the brain. We have examined patients with olfactory deficiencies and presenting a first-time TBI. The investigations took place 2 months–20 years after the head trauma. The aims were (i) to specify the criteria for accurate brain imaging study by using MRI; and (ii) to present different aspects of TBI-induced endocranial lesions using MRI and match them to psychophysical measurement of olfactory disabilities. Clinical history, severity and mechanism of the brain injury, as well as olfactory detection and identification values, were measured and related to MRI aspects. MRI investigation is the only method that is both efficient and affordable (although it remains expensive). However, the MRI has to be T_2 high-resolution coronal and axial incidences, focused on the anterior part of the skull base (including fronto-orbital cortex, olfactory gyrus, anterior commissure and temporal lobes). MRI reveals anatomical lesions of the olfactory tract clearly. The most specific lesions are located in the orbitofrontal areas (corticocortical and subcortical tissue). It should be noticed that olfactory loss may coexist with anatomical presence of olfactory bulbs and tracts.

184. Trigeminal function in congenital anosmia

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To evaluate trigeminal function in congenital anosmia, 20 patients with idiopathic congenital anosmia (ICA; 19 females; 18–68 years, mean age 44 years) were investigated and compared to 20 sex- and age-matched healthy controls. Congenital anosmia was confirmed by psychophysical ('Sniffin' Sticks'), electrophysiological (olfactory event-related potentials) and imaging (MRI) methods. The following measures were compared between the two groups: (i) ability to lateralize unilaterally presented intranasal trigeminal stimuli; (ii) detection threshold for CO₂; (iii) intensity ratings for CO₂ stimuli of three different concentration; (iv) trigeminal event-related potentials; and (v) negative mucosa potentials. No significant difference was detected in the ability to lateralize between the two groups. However, healthy subjects were found to detect CO₂ at lower levels than patients ($P < 0.001$). In addition, patients rated weak CO₂ (40% v/v) stimuli as less intense compared to healthy subjects (patients 9%, controls 17%; $P = 0.001$), no difference could be detected at CO₂ levels of 50 and 60% v/v. Interestingly, the electrophysiological measures revealed a longer latency of the early event-related potential (ERP) component P1 in patients compared to healthy controls; no other measure showed a significant difference. The findings of the pres-

ent study indicate differences between congenital anosmic patients and healthy subjects in the perception of weak CO₂ stimuli. No difference between both groups could be detected with regard to lateralization, negative mucosa potential measurements, and, with the exception of the P1 latency, ERP measurements.

185. Does coverage of the vomeronasal duct elicit different cortical activation patterns in response to a putative pheromone and other odorants?J.C. Gerber¹, J.N. Lundstrom², J. Frasnelli³, M. Knecht³, M.J. Olsson² and T. Hummel³¹Neuroradiology, University of Dresden Medical School, Dresden,Germany, ²Psychology Uppsala University, Uppsala, Sweden and³Otorhinolaryngology, University of Dresden Medical School,

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To challenge the common belief that pheromonal communication is necessarily mediated by a vomeronasal organ (VNO), we tested the reverse hypothesis by covering up the vomeronasal duct (VND) in female subjects. We assessed cortical response patterns following stimulation with phenylethyl alcohol (PEA) and the putative pheromone androstadienone (AND), with and without coverage of the VND. We hypothesized that (i) cortical activation is independent of the VND's status and (ii) both odorants would show no difference in cortical representation. After detailed olfactory function tests, 16 women (age range 21–27 years), fertile and all presenting with a VND, underwent functional magnetic resonance imaging. Both AND and PEA were presented in suprathreshold concentrations (3 mol/l and 20% respectively) in a pseudorandomized block design (30 s on, 30 s off; 1 s stimulus duration, 3 s interstimulus interval). Subjects were blind to the status of the VND and the nature of the odorants. Imaging data were analysed with SPM2. In a group analysis, separately for both odorants, coverage contrasted with non-coverage showed overlapping cortical activations. When assessed in a combined analysis ([AND uncover–cover] > [PEA uncover–cover]), the differences were limited to the dorsal part of the superior temporal sulcus (STS) (BA 21/22) and the precentral gyrus (BA 4) on the right. Both areas are understood not to be a part of the olfactory network. The STS seems to be involved in stimulus integration, as described by others. Irrespective of the status of the VND, differential activation (AND > PEA) was seen in the right middle frontal gyrus and in the subcallosal area. This finding points to a higher involvement of the limbic system in the processing of AND. In conclusion, the human VND does not play a major role in olfactory processing as hypothesized. The substances used mainly account for the differences seen in cortical activation.

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186. Transport of orally inhaled agents into the blocked nasal cavity

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Human responses to airborne chemicals may result from systemic absorption and toxicological mechanisms as well as from sensory perception and associated cognitive processing. Isolating the nasal cavity can contribute to specifying the response mechanism, and

simple nasal plugs have been used for this purpose in a variety of studies involving inhalation or gustation. However, we find that nasal plugs do not prevent orally inhaled agents from reaching the nasal cavity. In the present study, subjects breathe a synthetic air, with 10% helium as a tracer and a depleted level of argon (0.04%), compared with natural air. Customized nasal plugs containing an air-sampling mechanism are fitted to each subject. After oral breathing through a non-rebreather valve for a selected period, a small sample of gas from the anterior nasal cavity is analyzed with a mass spectrometer. Techniques for focal intranasal gas sampling in the occluded nose have also been developed and can be used to follow the evolution of tracer gases at multiple locations between the anterior nasal cavity and the nasopharynx. Transport of the orally inhaled tracer into the anterior nasal cavity is rapid and increases as respirations continue. Significant tracer levels are detected within a few breaths and may exceed 50% of the inhaled concentration within one to several minutes. The concentration of argon, initially present as a component of natural air, is observed to decrease, providing a simultaneous measure of reverse transport. Effects of perturbations such as swallowing and coughing have also been studied. Transport into the occluded nasal cavity appears to be driven by convection resulting from the exhaled air stream impacting on the static column of air in the nasopharynx. Other potential mechanisms, including diffusion and transport via the bloodstream, and implications of these results for chemosensory studies, will be discussed.

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187. Hedonic positive and negative pure odors alter breathing pattern in a different way

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Pleasant odors are regarded as stimulating inspiration whereas unpleasant odors may shorten it. Fifty-three normosmic subjects had to breathe through one nostril. Odors were applied in front of the nose with a Kobal flow-olfactometer OM 2S. Hydrogen sulfide (H₂S) as an unpleasant pure odor and phenyl ethyl alcohol (PEA) as a pleasant pure odor were used in weak, moderate and strong concentrations. The stimulus duration was 200 ms. A trial consisted of 16 stimuli. The stimulus was triggered manually in late exhalation during regular breathing pattern. The interstimulus interval was at least 3 min. From the data, the duration and the areas below the respiration curves of inspiration and expiration were calculated with Lab VIEW-software. The changes of these four respiratory parameters were estimated using the ratio between the first breathing cycle after stimulus and the average of the five regular breathing cycles prior to stimulation. Only changes of >+20% were defined as a respiratory odor-related reaction. Short H₂S stimuli evoke three times more frequently a decrease of duration of expiration than an increase. Due to the forced exhalation an increase of the area below the expiratory curve is observed in these cases. Changes of inspiration do not show any trend. The subjects exhale quickly to breath fresh air in the following cycle. Short PEA stimuli evoke an increase about three times more frequently than a decrease of duration of inspiration. The area below the inspiratory curve is enlarged. The subjects inspire the pleasant odor deeply and long lastingly.

188. Olfactory sensitivity of subjects working in odorous environments

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The major aim of the present studies was to investigate olfactory function of people working in odorous environments. In experiment 1 we investigated the effect of occupational exposition to agricultural odors on olfactory function in a total of 120 subjects. Sixty were employees of both dairy and pig-breeding farms (FARM subjects); their results were compared to 60 healthy controls living in the same rural area. Both groups were matched for age and sex. Subjects underwent olfactory testing using 'Sniffin' Sticks'. Repetitive measurements were performed before and after the first day of a working cycle; in addition, FARM subjects were also tested in the evening of day 5 of this period. Interestingly, both groups were not different in terms of their overall olfactory sensitivity. In experiment 2 we investigated whether people working in perfumeries would exhibit an alteration of olfactory sensitivity. Here we studied 58 subjects employed in perfumeries and compared their olfactory sensitivity to 58 controls matched for age and sex who did not work in such odorous environments. Again, olfactory function was assessed using 'Sniffin' Sticks', which includes tests for phenyl ethyl alcohol odor threshold, odor discrimination and odor identification. Subjects working in perfumeries scored higher in odor-discrimination tests compared to controls. Working in an odorous environment for a full day also had no major effect on general olfactory abilities, as indicated by measures performed at the beginning and end of a working day. Taken together, results from the present study do not support the idea that odorous environments are deleterious to general olfactory function. In fact, as concluded from experiment 2, working in an odorous environment may even enhance olfactory abilities. However, other interpretations may relate to the idea that subjects with relatively high olfactory sensitivity would be drawn more to professions involving certain olfactory abilities.

189. Recording the human electro-olfactogram (EOG) with external electrodes

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The aim of this investigation was to explore the possibility of recording electro-olfactograms (EOGs) using external electrodes placed on the root of the nose on either side of the bridge and at the medial termination of the eyebrows. The EOG is considered to be the summated generator potential of olfactory receptor cells and therefore represents peripheral olfactory events. Recording of human EOGs is technically difficult due to poor access to the olfactory mucosa and the nasal irritation is tolerated by few subjects. The evoked potentials at these sites in response to two odorants, n-amyl acetate and benzaldehyde, were recorded simultaneously with the EOG, recorded conventionally with an

intranasal electrode, and the olfactory event-related potential (OERP) recorded using scalp electrodes. The extranasal potential recorded at the root of the nose, 0.5–1 cm below the nasion, 1 cm from the ridge and ipsilateral with the stimulus, had the highest degree of correlation with the intranasal EOG. We refer to this site as N1 (left side) and N2 (right side). Further analysis demonstrated that the latency, the time constant of the rising phase and the amplitude of the evoked potential recorded at N1 also had a higher correlation coefficient with the EOG than did those potentials recorded at other sites. Statistical analysis indicated that the latency and time constant of the response recorded externally at N1 were the same as those of the EOG recorded intranasally. We conclude that an olfactory evoked potential, with many of the characteristics of the EOG recorded from the olfactory mucosa, can be recorded externally at a site close to the bridge of the nose. This non-invasive method of recording the EOG will have benefits for the objective assessment of olfactory function.

190. Anosmia: more than a sinonasal symptom

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Anosmia is generally considered a symptom of chronic sinonasal disease. Consequences of olfactory loss are not as striking as those due to blindness or deafness. Unlike vision and audition, human olfactory physiology is far from being understood. This might be one reason for the lack of medical interest in anosmia. However, there is growing evidence that olfaction plays an important role in interpersonal communication. The aim of this study was to assess the incidence of anosmia within 1240 subjects on a population-based sample. Subjects reporting sinonasal complaints were excluded. Participants received ear, nose and throat and olfactory testing. Five per cent of these subjects had nasal polyposis in the absence of sinonasal complaints, confirming previously reported high nasal polyposis incidence rates. Within the remaining 1182 subjects (603 men, 579 women), anosmia occurred in 4.7% with no sex-related differences in terms of the frequency. Most anosmic subjects were unaware of their poor performance; accordingly, they could not indicate its origin. Like others, we found age to be a main factor leading to anosmia. However, the present data also revealed anosmia to occur in up to 5% of subjects under 65 years of age. This incidence is similar to that of diabetes. In comparison, blindness is estimated to occur at a frequency of 0.7–1.5% (WHO databases). In conclusion, the current findings suggest anosmia to occur much more frequently than previously assumed. Whether this reflects a high vulnerability or decreasing importance of the olfactory system in humans remains an open question. The results further indicate that anosmia is not simply a consequence of sinonasal disease. Consequences of anosmia are hedonic alteration of nutrition, permanent feeling of insecurity due to the missing alert system, and mood changes sometimes leading to mental depression. Most anosmic patients experience a lack of interest on the part the medical profession and feel that their condition is not being taken seriously. Medicine in developed countries is increasingly confronted with problems related to quality of life or lifestyle,

which are both considerably affected by anosmia. Considering its high incidence, olfactory dysfunction should be taken into account in the global care of patients. Since olfactory deficits have been shown to occur in several general pathologies and especially in neurodegenerative diseases in their early phase, anosmia must be considered more than a trivial symptom of sinonasal disease and deserves attention from general practitioners. The present data show the need of further research into treatments of olfactory disorders.

191. Parosmia and phantosmia: frequency and clinical significance

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This study aimed to investigate the frequency and prognostic significance of qualitative olfactory dysfunction (parosmia, phantosmia). A total of 868 patients were included; 160 of these patients (18%) complained of parosmic sensations, 59 (7%) mentioned odor phantoms. In patients without qualitative olfactory dysfunction, smell loss was most probably due to trauma (30%), infection of the upper respiratory tract (23%), sinonasal disease (17%) or it was idiopathic (22%). In patients with parosmia these figures were 7, 70, 8 and 12% respectively; in patients with odor phantoms they were 20, 54, 15 and 17% respectively. Thus, parosmias and phantoms were most frequently encountered in olfactory loss following URTI. Among hyposmic patients, those with parosmia exhibited a better discrimination of odorants but were less proficient in odor identification at the initial test. However, at the second test there was no significant difference between patients with ($n = 67$) or without parosmia ($n = 223$). In patients with parosmia decreased olfactory function was found in 22%, improvement in 39%. Similar figures were seen in patients without parosmia (21 and 40%). At their first test, patients with phantosmia were significantly worse in terms of odor identification compared to patients without phantosmia. At their second test, overall olfactory function was decreased in 38% of the phantoms patients; improvement was found in 41% of the patients. In conclusion, parosmias are found most frequently in olfactory dysfunction following URTI. Apparently, the presence of parosmia is not a predictor of the prognosis of olfactory dysfunction. In contrast, phantoms appear to indicate a higher likelihood for a further decrease of olfactory function.

192. Characteristics of odor perception in patients with mood disorders

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Strong emotional correlates are a well-known phenomenon in odor perception, substantiated by psychophysical, psychophysiological studies and data from cerebral imagery. But behind this evidence, questions are arising about differences between particular

populations. The present research aims to study the characteristics of odor perception in subjects who are concerned by emotional perturbations. More particularly, olfactory perception has been investigated in a sample of patients suffering from psychiatric disorders such as depression and addiction (drug addiction, eating disorders) and compared with healthy control subjects. The clinical population ($n = 75$) has been selected in patients admitted to the Psychiatry Department of Besançon Hospital. Olfactory perception has been evaluated with the 'Test Olfactif Clinique' (UMR CNRS 'Neurosciences et Systèmes Sensoriels', Lyon). This test evaluates sensitivity to two odors, and measures other aspects such as detection and recognition with a panel of 16 odors. Furthermore, the subjects have been asked to evaluate on linear scales the intensity and the hedonic valence of each of these 16 odors. Different scales of interest in psychiatry have also been completed to collect data on the mood and affective states of the patients. The preliminary results indicate a reduced olfactory sensitivity in depressive patients (and not in patients with addiction disorders) compared to control subjects ($P < 0.05$, two-tailed t -test). The abilities in detection and recognition are not significantly different. The patients with addiction disorders evaluate the intensity of the odors higher than depressive patients and than control subjects ($P < 0.05$). Depressive patients evaluate odors as more pleasant compared with the control subjects ($P < 0.05$). These results will be discussed according to the neurological processes involved in odor perception and in different psychiatric emotional disorders.

193. Prevalence and risk factors for self-reported odour intolerance: the Skövde population-based study

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The present study was performed to determine the prevalence of both self-reported general odour intolerance in adults and such sensitivity to odours as to have affective and behavioural consequences. Furthermore, we aimed to relate odour intolerance to explanatory variables and risk factors. We undertook a cross-sectional, population-based epidemiological study. A random sample of 1900 inhabitants over the age of 20, stratified for age and gender, were recruited. Subjects were invited for clinical examinations that included questions about general odour intolerance, respiratory symptoms and smoking habits, as well as a smell identification test. The Chemical Sensitivity Scale for Sensory Hyperreactivity (CSS-SHR) was used to quantify affective and behavioural consequences. In total 1387 volunteers (73% of the sample) were investigated. The overall prevalence of self-reported general odour intolerance was 33% [95% confidence interval (CI) 30–36%], with problems mainly from the upper respiratory tract. The prevalence of affective and behavioural consequences of odour intolerance (CSS-SHR score >43) was 19% (95% CI 15–22%). The risk for the latter condition was increased in women

compared with men [odds ratio (OR) = 2.3; 95% CI 1.5–3.6], but no increased risk related to current smoking or impaired sense of smell was found. This study demonstrates that intolerance to odours is a widespread problem in society, and that it is about twice as common in women as in men.

194. Olfactory bulb volumes in patients with idiopathic Parkinson's disease

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Olfactory loss is among early signs of idiopathic Parkinson's disease (IPD). The present study aimed to investigate whether this loss would be reflected in a decreased volume of the olfactory bulb (OB) established through magnetic resonance imaging. Eleven consecutive IPD patients were compared to nine healthy, age-matched controls. Results indicated that there is little or no difference between IPD patients and healthy controls in terms of OB volume. It may be hypothesized that olfactory loss in IPD is not a result of damage to the olfactory epithelium but rather results from changes in the central nervous system.

195. Dissociation between psychophysiological and verbal correlates of emotional processes in response to olfactory and visual stimuli following frontotemporal damages

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Contemporary neuropsychological studies have stressed the distributed and multicomponential nature of human affective processes. Here, we examined facial electromyographic (EMG) (zygomatic and corrugator activity), autonomic (skin conductance and heart rate) and verbal measures of affective valence and arousal in a patient (T.G.) with left periamygdalar and left orbitofrontal damages, when exposed to hedonically valenced visual and odour stimuli. Unlike control subjects, the facial EMG and autonomic activity of T.G. did not differentiate between pleasant, unpleasant and neutral pictures. Further, only the controls reacted with larger corrugator EMG activity and higher skin conductance to unpleasant odours. By contrast, the subjective feeling states (pleasure and arousal ratings) remained preserved in T.G. for hedonically valenced pictures and unpleasant odours. The covariation between facial and self-reported measures of negative valence was a function of the nature of the olfactory task only in T.G. (i.e. implicit versus explicit tasks). Taken together, the data suggest a functional dissociation between brain substrates underlying neuromuscular, autonomic and experiential processing of affective valence and arousal, with the amygdala and/or the orbitofrontal cortex mediating rapid physiological changes in response to emotionally salient stimuli, and other brain regions participating in the mental representation of emotional states.

196. Simultaneous application of matching and mismatching gustatory and olfactory stimuli investigated by functional magnetic resonance imaging

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Smell and taste interact. The simultaneous application of congruent stimuli, e.g. sweet taste and pleasant odors, has a different effect on processing of olfactory information than incongruent simultaneous stimulation examined by chemosensory event-related potentials. Our aim was to examine this phenomenon using functional magnetic resonance imaging (fMRI). Fourteen healthy, normosmic volunteers (7 male, 7 female, age range 22–40 years) participated. Olfactory stimulation with phenyl ethyl alcohol (PEA; stimulus duration 1 s, 30% v/v) was applied using an olfactometer OM2S (Burghart Instruments, Wedel, Germany). Gustatory stimuli of 0.1 ml each were sucrose (A, saturated solution), citric acid (B, 4% solution) or water (C). Intensity of sucrose and citric acid were matched. All subjects took part in three randomized sessions of 9 min each. In each session one tastant was applied. MRI experiments were performed on a 1.5 T Siemens Sonata system. Functional imaging was performed using a single-shot EPI sequence (FOV = 220 × 220 mm, slice thickness = 4 mm, matrix = 64 × 64, T_R = 2 s, T_E = 40 ms, flip angle = 90°). Analysis was performed in SPM2 (Wellcome Department, UK). Preprocessing included slice timing correction, realignment, spatial normalization and smoothing with a Gaussian kernel of 9 mm width. An intersubject analysis was performed using a random effects model. PEA and citric acid showed higher and more extended activation in posterior association areas than PEA and sucrose, or PEA and water. Moreover, taste conditions A and B showed activation in hippocampus and amygdala which was not present in condition C. All three conditions showed activation in the cerebellum, even though this was highest for condition C. The fusiform gyrus showed most activation in condition B. We conclude that matching and mismatching olfactory and gustatory stimuli activate different cerebral areas. Posterior association areas are more active in mismatching conditions.

197. From mouse bioassay to human eye exposure to reactive indoor chemistry—what have we learned about eye and airway irritation?

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Neither chemically unreactive volatile organic compounds (VOCs) nor particles can explain reported symptoms in indoor environments, because the concentrations are far below known estimates for eye/airway irritation. Oxidation products of chemically reactive VOCs (alkenes) have been proposed as an explanation for eye and airway complaints. We report results of a mouse bioassay and a human eye exposure model from reaction mixtures of limonene and ozone (O_3) products (LOPs), α -pinene and O_3 products (AOPs), isoprene and O_3 products (IOPs), the nitrate radical (NO_3), and

methacrolein (MAC). LOPs, IOPs and AOPs produced significant upper airway irritation in the ASTM mouse bioassay, in contrast to the residual reactants. The reduction of the respiratory rate (a measure of trigeminal irritation) was between 30 and 55% (lowest for LOPs and POPs, highest for IOPs). Chemical analysis by conventional methods showed that identified oxidation products and residual reactants at the measured concentrations could not account for the observed bioresponse, assuming normal addition of irritation effects. The results suggest that airway irritant(s) of unknown identities are formed in the reaction mixtures. They are possibly intermediate in nature, because extension of the age of the reaction mixture and increase of the relative humidity diminish the bioresponse. Male subjects were randomly single-blind eye exposed to the reactants LOPs, IOPs, NO_3 , MAC, as well as clean air, while viewing an educational film. The exposure time was 20 min, in addition to pre- and post-clean air baseline measurements (21°C, 20% RH) for 8 min. Eye blink frequencies (BFs) were recorded during the entire session of 36 min, and eye irritation was reported qualitatively. LOPs and MAC resulted in significant BF increases compared with that of clean air; and the findings coincided with qualitative reporting of weak eye irritation. BF increase was borderline significant for IOPs and NO_3 . Lowest observed effect levels of BF increases were 18% during exposure to 89 p.p.b. MAC, and 13% for a 10 min old LOPs mixture of initially 92 p.p.b. limonene and 101 p.p.b. O_3 . The BF tended to decrease with an increase in the RH from 20 to 50% for mixtures of initially ~350 p.p.b. limonene and ~300 p.p.b. O_3 . LOPs cause trigeminal stimulation of the eye and possibly eye irritation at reactant concentrations close to the highest concentrations measured indoors.

198. Olfactory deficits in patients affected by hepatic encephalopathy

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The olfactory system is impaired in several neurological diseases such as Parkinson's, Alzheimer's, Huntington's and Korsakov's (see Talamo *et al.*, 1989; Moberg and Doty, 1997; Doty, 2001; Zucco *et al.*, 2001), and also in Down's syndrome subjects and HIV-infected patients (see Hornung *et al.*, 1998; Zucco and Ingegnieri, 2004). However, no data are available on a possible olfactory pathology in patients affected by hepatic encephalopathy (HE). Such a syndrome may occur in both acute and chronic liver disease and may range from subtle mental impairment to coma (Butterworth, 1996). The patients exhibit significant alterations in monoaminergic and glutamatergic mechanisms in the brain; and also in the GABA and in the endogenous opioid neurotransmitter systems. Among these, glutamate and dopamine are also involved in the transmission of olfactory information. The present research is aimed at exploring the presence of olfactory deficits in HE patients. On these grounds, two groups of cirrhotic patients (with alcoholic and viral aetiology respectively) and two groups of HE patients were examined on identification and recognition olfactory tasks. Preliminary data show the presence of olfactory deficits within all the groups of patients according to the severity of the disease. The role of chronic alcohol consumption and the role of some neurotransmitters on olfaction is discussed.

Symposium 7: Development of olfaction

199. Regulation of expression of zebrafish odorant receptors during development

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During differentiation, olfactory receptor neurons select a single odorant receptor (OR) gene for expression out of a large gene family. The mechanisms of this extreme selectivity are largely unknown. We have performed a developmental analysis of expressivity and penetrance of expression for 10 zebrafish OR genes to obtain a dataset of sufficient quality for testing different hypotheses about OR gene regulation. Statistical analysis of the data supports a model in which the final choice of an individual OR gene occurs stochastically from within a group of genes sharing a deterministically defined onset of expression. During a protracted juvenile developmental period individual regulatory influences seem to modify the expression of OR genes, a notable example being a transient decrease in expressivity of two OR genes. In a search for OR gene promoter elements we have generated transient transgenes in zebrafish using segments of 5'-flanking regions of OR genes and the reporter gene GFP. We report here that short 5'-flanking regions of two zebrafish OR genes suffice for OR-like expression and moreover restrict expression of a reporter gene to subgroups of olfactory receptor neurons. Olfactory receptor neurons expressing a ZOR6A promoter construct target the mediodorsal olfactory bulb, a small subregion of the total termination area. Our results show the autonomous function of proximal, *cis*-acting elements on different levels of odorant receptor gene regulation.

200. Formation and refinement of olfactory sensory neuron projections during development

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Olfactory information is conveyed from the nasal neuroepithelium to the main olfactory bulb, the first relay station in the brain, via axons of the olfactory sensory neurons. Each neuron extends a single axon to the bulb where it synapses onto second-order neurons in a spherical region of neuropil, called a glomerulus. A glomerulus represents the site of convergence for neurons expressing the same olfactory receptor. The glomeruli from neurons expressing the highly homologous receptors of the mOR37 subfamily are located close together, nevertheless each glomerulus receives input with very high precision from only one receptor-subtype-specific population of neurons. To monitor the unfolding of this precise olfactory map during development, the onset of receptor expression, the outgrowth of axons as well as glomerulus formation for two neuron populations expressing different mOR37 subtypes was investigated on transgenic mouse lines. The data indicate a synchronous onset of receptor expression at about embryonic day 10 (E10). From E15, axons of both populations terminate in a common, small area of the presumptive olfactory bulb. During a short postnatal phase, the two axon populations segregate into distinct, protoglomerular structures. Between E11 and E16, populations of cells expressing distinct

olfactory receptor types are located in the cribriform mesenchyme, between the prospective olfactory epithelium and the developing telencephalon. Molecular phenotyping demonstrated that these 'extraepithelial' cells coexpress key elements characteristic of neurons in the nasal epithelium. Studies on transgenic mice showed that they are positioned along the axon tracts, and each population expressing a given receptor gene is specifically associated with the axons of those olfactory sensory neurons with the same receptor type. The data suggest that they either might be guide posts for the outgrowing axons or migrate along the axons into the brain.

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201. Spatial gene expression and cell specification in the mouse olfactory epithelium

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Progenitor cells in the mouse olfactory epithelium generate >1000 subpopulations of neurons, each expressing a unique odorant receptor (OR) gene. This event is under the control of spatial cues since neurons in different epithelial zones are restricted to expressing region-specific subsets of OR genes. We show here that mature neurons in one defined zone selectively express NADPH:quinone oxidoreductase (NQO1), an enzyme that catalyses reduction of quinones. Immunohistochemistry and *in situ* hybridization analyses show non-overlapping expression of NQO1 and the neural cell adhesion molecule 2 (NCAM2) in OE and axon terminals within glomeruli of the olfactory bulb. Analyses of mice target-deleted in the Lhx2 transcription factor reveal that neurons do not diversify into subpopulations expressing different OR genes, NQO1 and NCAM2. Lhx2^{-/-} embryos have, however, a normal distribution of neuronal progenitors that leave the cell cycle, acquire pan-neuronal traits and form axon bundles. Increased cell death in combination with increased expression of the early differentiation marker NeuroD, as well as reduced expression of late differentiation markers (Gαolf and OMP), suggest that neuronal differentiation in the absence of Lhx2 is primarily inhibited at, or immediate prior to, onset of OR expression. Aberrant regional expression of early and late differentiation markers, taken together with unaltered zone-specific expression of genes in the progenitor and supporting cell layer of Lhx2^{-/-} embryos, shows that Lhx2 function is not required for all aspects of regional specification. Collectively these results indicate that a cell-autonomous function of Lhx2 is required for differentiation of progenitors into a heterogeneous population of individually and regionally specified mature olfactory sensory neurons.

202. A comparative study exploring perinatal versus adult neurogenesis

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In mammals, the olfactory bulb constitutes one of the two regions of the brain where there is continuous neurogenesis throughout the entire life of animal. Interestingly, while bulbar interneurons born

in the adult brain play an important function in odor discrimination and olfactory learning, the functions as well as the target of local interneurons born perinatally remain unknown. This question is particularly pertinent since the majority of the local interneurons are born during early postnatal life when olfaction plays a primordial role in the growth and survival of rodent. During the first postnatal weeks, olfactory cues from mother, siblings and nest-associated odors are crucial for nipple localization and attachment, home orientation, arousal and huddling. In line with this, the present study was conducted to investigate whether early olfactory experience affects early postnatal (primary) neurogenesis and to analyze the consequences of this anatomical modifications on the olfactory performance and on adult secondary neurogenesis. Rearing in a citral-rich environment, during the first postnatal week induces preferences for citral not only in young (1 week old) animals but also in adults. Interestingly, while preference for citral in pups was associated with increased number of newly generated cells, neurogenesis in adult animals showing citral preference remained unchanged. Note that the distribution pattern of newly generated cells is different between early postnatal and adult animals, suggesting distinct functions of neurogenesis according to the birth date of newborn neurons. These results suggest that the early olfactory neurogenesis might be implicated in the induction and/or the maintenance of long-term olfactory memory, and that regulation of neurogenesis may be different.

203. Developmental plasticity of the olfactory pathway in the insect brain

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In insects, the antennae represent the main olfactory organs, and olfactory information is relayed to olfactory glomeruli in the antennal lobes of the brain. After processing in the glomeruli within primary olfactory centers, projection neurons carry the information to higher olfactory centers in the mushroom bodies and lateral protocerebrum. In previous investigations on the pheromone-specific system in the sphinx moth *Manduca sexta* we were able to show that axonal projections of olfactory receptor neurons and timing of neuron–glia interactions in the antennal lobe play a critical role for the establishment of a chemotopic arrangement of olfactory glomeruli. Our present investigations focus on effects of environmentally induced neuronal plasticity within the olfactory pathway in the brain of social insects (honey-bee and various species of ants). These studies revealed a striking developmental plasticity during the establishment and synaptic organization of primary and secondary olfactory centers in the brain. Nutritional factors during the larval period as well as rearing temperature during pupal development significantly affect postembryonic formation of olfactory glomeruli in the antennal lobes and the establishment of synaptic complexes in olfactory input regions of the mushroom bodies. Behavior tests indicate that changes in the synaptic organization within primary and secondary olfactory centers do affect olfactory behavior. We conclude that environmentally induced postembryonic neuronal plasticity within the olfactory pathway may play an important role in regulating diversity of olfactory behavior, as it can be observed in social insects among different castes and groups within castes.

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Symposium 8: Imaging and olfaction

204. Odors evoke dynamic glomerular activity patterns in the mammalian olfactory bulb

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On the network level of the olfactory bulb (OB) voltage-sensitive dye imaging revealed odor-specific sequences of glomerular activation and distributed OB activity locked to the nasal respiration cycle. The spatial distribution of this activity amplitude and phase was heterogeneous and was changed by sensory input in an odor-specific manner (Spors and Grinvald, 2002). To analyze the dynamics of these patterns at the level of input to the OB, we selectively loaded olfactory receptor neurons with Calcium Green dextran and imaged afferent glomerular calcium dynamics in freely breathing or artificially sniffing, anesthetized mice (Wachowiak and Cohen, 2001). Glomerular odor responses differed in response latency, rise time, decay time and modulation by sniffing. In response to esters and hydrocarbons, caudolateral glomeruli generally exhibited faster responses and more pronounced respiratory modulation. However, neighboring glomeruli could also exhibit different temporal response characteristics. Temporal response characteristics of individual glomeruli depended on glomerulus identity, odor identity, odor concentration, sniffing frequency and flow rate. Hence the odor-evoked spatial patterns can change significantly over time in a stimulus-specific manner already at the level of the input to the OB. The spatiotemporal dynamics of afferent activity patterns therefore need to be considered in models of olfactory coding.

205. Mapping odorant chemistry across the glomerular layer of the rat olfactory bulb

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To explore relationships between odorant chemistry and spatial activity patterns in the olfactory bulb, we have embarked on a research program in which we expose rats to pure odorants differing systematically in chemical structure. We map odorant-evoked uptake of [¹⁴C]2-deoxyglucose across the entire glomerular layer into anatomically standardized data matrices that can be compared quantitatively across different animals. We find that each odorant stimulates a combination of glomerular clusters, or modules, distributed across the layer. Odorants sharing functional groups or hydrocarbon structural features typically overlap in their stimulation of particular modules, and changing an important feature of the odorant molecule such as the functional group causes loss of activity in certain modules but leads to activation of others. Functional group position, cyclic structures, the presence and location of triple and double bonds, and the stereochemistry at double bonds can also serve as important molecular features that influence which modules respond to the odorants. More subtle changes in molecular structure, such as small changes in length, are often represented as a change in the particular glomeruli activated within a module, so that steady increments in length generally result in orderly progressions of activity across modules. Odorant representations can be

amplified by prior experience that results in the formation of early odor preferences, and differences in activity patterns across concentrations or across different odorants can predict differences in the perceived odor. The systematic nature of the changes in bulbar activity patterns with changes in odorant chemistry should help to identify the salient characteristics of odorant molecules that must be recombined at subsequent stages of olfactory processing to generate perceptions and to confer significance on particular odors.

206. A model of taste–odor integration in the human brain

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Gustatory and olfactory stimuli interact perceptually and neurophysiologically to produce a unitary perception of flavor. A model of taste–odor integration will be presented in which it is argued that (i) the neural representation of taste provides the fundamental substrate with which olfactory representations come to be associated; (ii) that taste–odor integration is dependent upon experience, intensity of the components and mode of olfactory stimulation (orthonasal versus retronasal olfaction); and (iii) that the core network includes the anterior ventral insula, dorsal insula/operculum, orbitofrontal cortex, amygdala and anterior cingulate cortex. Psychophysical and neurophysiological evidence from my and other laboratories will be presented to support these arguments and predictions for future experiments will be made.

207. Emotion, memory and the human piriform cortex

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Animal studies of the olfactory system implicate piriform cortex in critical aspects of odor learning and memory. To examine whether human piriform cortex is similarly engaged in more complex operations, or acts as a mere chemosensory relay, we have combined functional magnetic resonance imaging (fMRI) techniques with olfactory paradigms of emotional learning in healthy human subjects. One important finding to emerge is the observation that human piriform cortex contains functionally dissociable subregions along its rostral–caudal axis. Posterior areas participate in basic aspects of odor processing, whereas more anterior regions are preferentially responsive to emotionally salient odors. We have also found that piriform cortex is significantly activated during classical conditioning paradigms of visual–olfactory associative learning. Responses in this region are sensitive to reinforcer devaluation of a pleasant food odor, implying that piriform cortex may encode current, and updated, representations of food-based reward. Finally, in a cross-modal (odor–object) episodic memory task, piriform cortex was activated during successful recognition of old (versus new) objects, even in the absence of odor, suggesting that sensory traces of the original engram are preserved in this structure. Taken together, our data indicate that human piriform cortex is not simply a sensory intermediary, but rather that this region of ‘primary’ olfactory cortex is functionally comparable to higher-order ‘association’ cortex.

208. Lack of piriform activation in schizophrenia during olfactory processing

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Impairments of olfactory processing in patients with schizophrenia (SZ) have been reported in various olfactory tasks such as odor detection, discrimination, recognition memory, identification and naming. The purpose of our study was to determine if impairments in odor familiarity and hedonic judgments in SZ patients observed in a previous behavioral study are associated with lack of activation in the temporolimbic and frontotemporal olfactory areas, commonly observed in schizophrenia. Twelve SZ patients, and 12 healthy control (HC) subjects, all right-handed male, were tested using the H₂¹⁵O positron emission tomography. Both groups were paired in age and school years, and underwent a total of eight scans. In addition to an odorless baseline condition, they had either to judge odor familiarity or hedonicity, or to detect odor, giving behavioral responses by pressing a button. Eighty-four different odorants were used. Using SPM99, and focusing on olfactory areas, regional cerebral blood flows (rCBFs) acquired during olfactory conditions were compared with those obtained for baseline condition. Contrast images of both groups were then compared to highlight between-group differences. Behavioral data showed that both groups had equivalent olfactory acuity, but that SZ patients experienced odors as less familiar and pleasant odors as less pleasant than HC subjects. SZ patients exhibited lack of rCBF in the left frontal piriform cortex in the three olfactory tasks when compared with HC subjects. More specific lack of activation was found in the right gyrus rectus, the left medial orbital gyrus and the left orbital part of the inferior frontal gyrus during familiarity judgments, and the left anterior insular gyrus and the left orbital part of the inferior frontal gyrus during hedonicity judgments. SZ patients did not show any greater olfactory-related activation than HC subjects. rCBF differences and their lateralization in the left hemisphere were explained in terms of anhedonia of SZ patients.

Posters: late submission

209. Pheromones, hormones, brain and behaviour

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Responses to pheromones and olfactory cues are key elements of behaviour in animals from every part of the animal kingdom. Hormones mediate the action of many pheromones in vertebrates and invertebrates (reviewed in T.D. Wyatt, 2003, *Pheromones and Animal Behaviour: Communication by Smell and Taste*. Cambridge University Press, Cambridge). Studies of pheromones, hormones and the brain reveal numerous mutual influences and feedback loops between them. By influencing either the olfactory sensory cells (peripheral effects) or the neurons in the central nervous system involved in processing olfactory information, hormones also help to orchestrate appropriate responses to pheromone signals, according to social status, reproductive maturity, sex and environmental variables such as day length or temperature. Indeed in some aquatic species, leaking hormones have evolved into pheromones. Classically pheromones have

been divided into releasers (which 'release' a specific reaction or behaviour) and primers (which cause a developmental process by signals to the brain to release the hormones of the endocrine system). However, reality may be more complex: many pheromones have both actions and some hormone-mediated reactions can be rapid. In social insects and social mammals, such as honey-bees and the common marmoset (a New World primate), only one female in a group reproduces. Queen pheromone signals, mediated by hormones in the receiving subordinates, may be honest co-operative signals, not control. Mechanisms of olfactory memory in vertebrates have been clarified by studies of how the mother sheep (ewe) learns the odour of her lamb, via the main olfactory system. The female mouse's learning of her mate's odour, which is the basis of the Bruce effect of pregnancy block, is similar but occurs via the vomeronasal olfactory system. Both model systems require simultaneous appropriate odour stimulation and noradrenaline release in the olfactory bulbs by nerves stimulated by birth or mating respectively.

210. Genotype dependence of *Gus* and *Mup* gene coexpression: relevance to functional activity of androgen-dependent pheromones in laboratory mice

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The physiological activity of androgen-dependent pheromones in *Mus musculus* L. is strongly associated with major urinary proteins (MUPs) (Bacchini *et al.*, 1992; Böcskei *et al.*, 1992; Keverne, 1999; Cavaggioni and Mucignat-Caretta, 2000; Marie *et al.*, 2001; Novotny, 2003). MUPs are coded by the *Mup* gene(s) cluster which is located on chromosome 4 (Bishop *et al.*, 1982). The detailed studies of structural basis of ligand binding properties to MUPs can shed light on the functional role of this protein in individual pheromonal 'fingerprinting' (Churakov *et al.*, 1992; Evershed *et al.*, 1993; Robertson *et al.*, 1996; Churakov and Novikov, 2000; Hurst *et al.*, 2001; Timm *et al.*, 2001; Sharrow *et al.*, 2002, 2003). Another well-developed genetic model deals with β -glucuronidase (GUS; EC 3.2.1.31) coded by the gene *Gus* (Ch. 5). The enzymatic activity of this protein is also connected with pheromonally mediated social behaviors (Ingersoll *et al.*, 1982; Bush, Paigen, 1992; Novikov, 1993). The detailed quantitative analysis of seven MUP electrophoretic bands, GUS activity in the voided urine, kidney, preputial and salivary glands, and plasma testosterone level in male mice of CBA/LacY, C57BL/6JY inbred strains and their F1 hybrids revealed significant positive correlation between these parameters only in CBA/LacY genotype with high pheromone activity level. These data suggested the existence of a gene-net basis for pheromonally mediated social behavior in *M. musculus* L. and stress the importance of coordinated testosterone-dependent expression of *Mup* and *Gus* genes. The results obtained may shed light on particular causes of interstrain differences in pheromone activity, present a valuable approach for dissection of social behavior phenotypes by using the pheromones as a fine natural tool, and can trace the concrete biochemical pathways from gene(s) to behavior (Novikov, 1993, 2003).

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211. Chemosensory proteins of insects: from discovery to function

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In their screening for antennal specific proteins in *Drosophila*, McKenna *et al.* (M.P. McKenna, D.S. Hekmat-Scafe, P. Gaines and J.R. Carlson, 1994, *J. Biol. Chem.*, 269: 16340–16347) and Pikielny *et al.* (C.W. Pikielny, G. Hasan, F. Rouyer and M. Rosbach, 1994, *Neuron*, 12: 35–49) reported the first dipteran odor-binding proteins and a non-odorant binding protein (OBP) named OS-D (*olfactory sensilla-type D*). Since then, ~50 OS-D homologs have been identified, primarily in insect species. This new protein family has been given the name 'chemosensory protein' (CSP) after finding specific expression in contact chemosensory sensilla (S. Angeli, F. Ceron, A. Scaloni, M. Monti, G. Monteforti, A. Minnocci, R. Petacchi and P. Pelosi, 1999, *Eur. J. Biochem.*, 262: 745–754). CSPs share no sequence similarity to other carrier proteins of sensory molecules; they have ~110 amino acids, four conserved cysteines, two disulfide bridges, six α -helices and a hydrophilic channel that passes through the protein. Their tissue distribution is very intriguing. They are found throughout the whole insect body, but mainly in chemosensory organs such as the mouthparts, the antennae and legs (J.-F. Picimbon, 2003, in: G.J. Blomquist and R.G. Vogt, eds, *Pheromone Biochemistry and Molecular Biology*. Academic Press, New York, pp. 539–566). In this communication, I will review our understanding of CSP with a particular focus on our results about evolution, diversity, ontogeny, tissue distribution, expression pattern, regulation, genomics and protein structure.

212. Desensitization of odorant receptors

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Olfactory receptors (ORs) comprise the largest single subfamily of G-protein-coupled receptors (GPCRs), numbering nearly 1000 in many mammals. It has been shown that ORs are expressed not only in olfactory epithelium of the nose, but also in other tissues. ORs are expressed in testis of some species (human, dog, rat, mouse). There they can play an important role in sperm development, chemotaxis and oocyte-spermatozoa interaction. However, investigation of OR trafficking steps have been severely impeded by poor OR expression in heterologous systems and by the lack of specific antibodies. The main purpose of our studies is the investigation of OR endocytosis after ligand exposure. Endocytosis of GPCRs involves phosphorylation of the receptor by G-protein receptor kinases, PKA or PKC, followed by binding of arrestin and clathrin and accumulation of receptors in clathrin-coated pits. We showed β -2-arrestin redistribution in response to activation of olfactory receptors in transiently transfected HEK293 and odora cells. This finding allows us to use this β -arrestin-GFP translocation assay to follow olfactory receptor desensitization after ligand treatment. With this assay system, in combination with Ca-imaging, we have investigated the role of olfactory receptor phosphorylation in the desensitization process by creating mutated receptor constructs and by employing pharmacological approaches.