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ABSTRACTS

List of Abstracts of the Seventeenth Annual Meeting of the European Chemoreception Research Organisation

Plenary Opening Lecture

1. Deconstructing Smell

L.B. Buck

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We have explored how mammals detect odorants and pheromones and how the brain translates those chemicals into diverse perceptions and instinctive behaviors. We found that odorants are detected in the nasal olfactory epithelium (OE) by ~1000 different odorant receptors (ORs), whereas pheromones are detected in the vomeronasal organ (VNO) by two smaller receptor families. Our studies showed that ORs are used combinatorially to encode odor identities. Exploring the patterning of OR inputs, we found that each sensory neuron in the OE expresses a single type of OR and that neurons with the same OR are scattered in one zone but synapse in a stereotyped fashion in OR-specific glomeruli in the olfactory bulb. At the next level, the olfactory cortex, we discovered another stereotyped map of OR inputs but here signals from different ORs are targeted to partially overlapping clusters of neurons and single neurons receive combinatorial OR inputs. By comparing responses to binary odorant mixtures versus their components, we found evidence that the cortical neurons act as coincidence detectors whose activation requires combinatorial OR inputs, thus providing an initial step in the reconstruction of an odor image from its deconstructed features. To explore how pheromones alter reproductive physiology and behavior, we made mice expressing a transneuronal tracer in gonadotropin releasing hormone (GNRH) neurons. These studies revealed that GNRH neurons receive pheromone signals from the OE as well as the VNO. We recently discovered a second class of chemosensory receptors in the OE, called TAARs, that recognizes at least one pheromone and may also be involved in the detection of other social cues by the OE.

Panel Discussión 1: Human Pheromones?

2. Human Pheromones?

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The question whether humans communicate chemosensorily is still under debate. While some authors argue that meaningful communication in humans is not mediated by chemical cues, others are convinced about the existence of such communication. In the round table discussion, these controversial points of view will be focused. Especially, it will be questioned weather the term ''pheromone'' is useful in investigating human chemosensory communication. If pheromones are considered to exist, they should bear some special features and these need to be specified. First, what is the chemical nature of pheromones: Are they expected to consist out of few or multiple substances, and are these substances olfactorily perceivable or not? Second, do humans have to have a special organ (e.g. the vomeronasal organ) in order to be able to respond to pheromones? Third, what kind of responses are pheromones considered to elicit? Does the perceiver respond behaviourally and/or physiologically, and is this response associated with cognitive changes? Fourth, does the communication have to contribute to the evolutionary fitness of the signal-sender and/or the perceiver? Finally, the assumed features of putative human pheromones form the basic methodological conditions for pheromone studies in humans: Namely, what kind of substances should be investigated, what kind of responses should be measured, and does the investigation need to be framed by a theory of evolutionary meaning?

3. A Putative Human Pheromone Induces Changes in Cortisol, Positive Mood, Physiology, and Sexual Arousal

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Pheromones are species-specific chemosignals. A hallmark of pheromonal communication is the ability of the chemosignal to modify endocrine state in conspecifics. While clear in many species, whether humans communicate with pheromones remains highly controversial. Here we found that merely a few sniffs of androstadienone, a molecule present in the saliva, semen, and sweat of men, significantly increased the sexual arousal, physiological reactivity, and critically, levels of the hormone cortisol in women. That a single molecule emitted by men is capable of stereotypically modifying mood, physiological arousal, and endocrine state in women qualifies this molecule as a human pheromone.

Invited Lecture

4. Gastronomy in the XXI Century: From Chemistry to Physics

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A review was undertaken of epistemology applied to gastronomy, defining the external sensorial stimulation by food and its cerebral

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perception as key to the gastronomic experience including emotions and establishing conceptual bases for the new science designated Molecular Gastronomy and Cuisine. In this context, one of the major challenges for basic scientists in the 21st century is to identify, as has been done for taste and smell, the molecular and physiological mechanisms that underlie oral perception of the texture of food and to determine how they influence gastronomic pleasure. This is of great importance because the progressive introduction into foods of complex polymer compounds, some in the form of unstable soft materials that may have a half-life of only minutes, has opened up new perspectives for the creation of an almost unlimited number of new textures, which currently form the basis of creative high cuisine. Finally, examples are presented of the arrival of the laboratory and scientists into the kitchen, describing developments in which our research group has played a major role: introduction of the preparation of foods with liquid nitrogen to modify their texture (cryocuisine), carried out with Dani García of the Calima de Marbella Restaurant (Malaga); study of the type of microscopic degeneration of hepatocytes in good and poor foie gras, performed with Andoni L. Adúriz of the Mugaritz Restaurant (Guipúzcoa); and a study in progress on the role of collagen gelatinization of the perimysium and endomysium in meat tenderness, in conjunction with Jordi Parra of Bodegas Campos Restaurant (Córdoba).

Poster Session 1

5. Olfactory Sensory Neuron-Derived Cell Line Odora as a Model for Studying Orexins Signal Transduction Pathways in the Rat Olfactory Epithelium

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Orexins A and B (OxA and OxB) are multifunctional neuropeptides implicated in a number of motivated behaviours but especially sleep–awake cycle and feeding regulation. Orexins act via two G-protein–coupled receptors Ox1R and Ox2R. We have demonstrated the presence of both orexins and their receptors at all levels of the rat olfactory system: epithelium, bulb, piriform cortex, however, their role and signalling pathways in olfactory mucosa (OM) remain unknown. OM is composed of multiple cell types including olfactory sensory neurons (OSN) and supporting cells, both expressing OxRs and orexins. To study orexin signalling in one OM cell type we choose OSN-derived Odora cell line as a model system. We compared main components of adenylyl cyclase (AC) and phospholipase C (PLC) pathways by Western blot and RT-PCR and demonstrated that the key components are well present in Odora cells as in OM. In Odora cells heterologously expressing Ox1R or Ox2R, both OxA and OxB induced increase in intracellular Ca++ concentration. Significant elevation of cAMP and IP3 were also observed, indicating activation of AC and PLC pathways. However, underlying activation mechanisms were different at Ox1R and Ox2R. Moreover, OxA and OxB induced a transient activation of MAP kinases (ERK42/44). To verify if these pathways were similarly activated in the tissue, we measured cAMP and IP3 level in dissected OM after OxA or OxB treatment. Slight increase in IP3 but no detectable effect on cAMP formation was observed in OM. The patched distribution of OxRs and the multitype cellular structure of OM could be the reason of this discrepancy.

6. Stimulation by Tastants of Non–Taste Receptors in Taste-Bud Cells

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Some bitter and sweet tastants were previously found to activate the inhibitory pathway of adenylyl cyclase (AC) in frog melanophores via melatonin and alpha-2-adrenergic receptors (Zubare-Samuelov et al. 2003. Am J Physiol 285:C1255–C1262). Since previous and present results suggest that such receptors are present in taste-bud cells, their responses to stimulation by some tastants were investigated in taste tissue. A 10-s stimulation of isolated intact circumvallate (CV) taste-bud sheets (TBS) by the bitter naringin and the sweetener saccharin reduced the isoproterenol-stimulated cAMP formation, whereas the bitter cyclo(Leu-Trp) peptide had no effect. The melatonin receptor antagonist, luzindole, or the alpha-2-adrenergic receptor antagonist, yohimbine, almost abolished the naringin-reduced cAMP but had no effect on the saccharin-reduced cAMP. On the other hand, the presence of alpha-1-adrenergic receptor antagonist, prazosin, almost abolished the saccharin-induced reduction of cAMP. As in the 10-s stimulation paradigm, subsecond stimulation by naringin of isolated broken foliate and CV taste papillae homogenate (BCH) reduced cAMP levels, and this effect was abolished in the presence of either luzindole or yohimbine. However, cAMP level was elevated following subsecond stimulation of BCH by either cyclo(Leu-Trp) or saccharin. It is concluded that naringin stimulates the inhibitory pathway of AC via melatonin and alpha-2-adrenergic receptors in taste tissue during both subsecond and 10-s stimulation paradigm. Saccharin stimulates the inhibitory pathway of AC via alpha-1-adrenergic receptors but it does so only in TBS during the 10-s stimulation paradigm.

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7. Taste Properties of Natural and Synthetic Avenanthramides

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Avenanthramides are a class of natural polyphenolic compounds isolated from oat and other plants. They have antioxidant properties and have been positively correlated to the fresh taste of such cereal. However, it is not reported whether such property is to be intended as cooling effect or as not rancid and, in any case, the sensory properties of the singular avenanthramides are unknown. We developed new structure–activity relationships (SAR) considering the chemical and physical properties of 75 known cooling compounds owing to different chemical classes (menthol and derivatives, icilin, cyclic alpha-ketoenamines, etc.) and a group

of avenanthramides. Through the SAR studies it emerged that the avenanthramides constitute a cluster with icilin, the compound with the maximum cooling activity actually known. We designed and set up a novel and short and efficient synthesis of the natural avenanthramides and of some analogues of the same family. Each one of the 12 synthesized avenanthramides was properly purified and submitted to sensory analysis. Three avenanthramides showed as expected a cooling activity, while one avenanthramide resulted active as taste modifier and it was able to mask the taste of ethanol.

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8. Extraction of Bitter Compounds from the bulbs of *Muscari comosum* (Lampascione)

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Nature is a rich source of sapid compounds, in particular of vegetal origin, many of which have a bitter taste. Even if this particular taste had always been associated with the concept of toxicity, as potentially dangerous substances have an unpleasant taste, at the same time a light bitter taste results to be appreciated as it confers a complex note to foods and beverages. The work presented here is set up in this context. The matrix studied is the bulb of ''Lampascione,'' the common term used to define the bulb of *Muscari comosum*, a spontaneous plant belonging to the family of the Liliacee, very common in southern Italy and used in the traditional cooking. This bulb is known to be very bitter and also some diuretic and antiinflammatory activities associated to its use had been reported. In literature, there are some works regarding the extraction of natural compounds from the bulbs of *M. comosum* but no relationships between the compounds identified and their taste are reported. The bulbs were extracted through a series of solvents with different polarity. The obtained fractions were submitted to preliminary sensory analysis and the most interesting fractions, characterized by a strong bitter taste, were submitted to further purification and structural analysis. From the ethereal extract, several and already known 3-benzyl-4-chromanones were isolated: 3,9-dihydroeucomnaline, 8-*O*-demethyl-7-*O*-methyl-3,9-dihydropunctatine, and muscomine. This last product, which was isolated in sufficient amount and purity to be submitted to a preliminary sensory analysis, resulted intensely bitter.

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9. Modulation of Amiloride-Sensitive Sodium Pathway by Nicotine in Rat Fungiform Taste Cells

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Several studies indicate that smoking affects salt taste perception in humans, leading to excess consumption of salty food. However, the mechanisms underlying the action of smoking on salt taste are currently unknown. We addressed this issue by studying the effect of nicotine, one the main constituent of tobacco smoke, on the taste system of the rat. We performed two-bottle preference test with NaCl solutions and found that nicotine, injected subcutaneously induced an increase in the preference for salty solutions over water. Addition of 50 μM amiloride to all solutions, however, made taste responsiveness of nicotine-treated rats indistinguishable from that of control littermates. These behavioural studies were consistent with nicotine acting on an amiloride-sensitive pathway. In taste cells, amiloride-sensitive sodium channels (ASSCs) are thought to be involved in the transduction mechanisms for sodium. Therefore, we performed patch-clamp recordings from taste cells of rat fungiform papillae to study the action of nicotine on ASSCs. In whole-cell, voltage-clamp conditions, open ASSCs produced a stationary inward current at a membrane potential of −80 mV. We found that bath-applied nicotine (1–50 μM) induced a reduction of such a current. In the presence of amiloride, however, nicotine was unable to produce any effect. Moreover, the reversal potential for the current inhibited by nicotine was close to the equilibrium potential for sodium ions. Taken together, our data suggest that nicotine may alter salt taste reception by affecting the activity of ASSCs in taste cells.

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10. The Trigeminal Nerve Projections in *Xenopus laevis* during Metamorphosis

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The trigeminal nerve is a cranial nerve, which functions to ensure the motor and sensory innervation of the face and oral and nasal cavities mainly. It comprises of three branches. We describe here the configuration and the development of the ophthalmic (V1) and maxillary (V2) branches projections labelled by a carbocyanine dye (DiI). The V2 branch innervates the tentacles and the lateral teeth. The V1 branch separates in two sub-branches: the superficial branch innervates the medial part of the teeth and structures located on the anterior part of the roof of the mouth that we assume to be gustatory buds clusters. The timing of development of the structures and of the V1 fibres projecting to it let suppose a possible induction of the trigeminal innervation on the formation of these structures. The deep branch of V1 splits into a lateral fascicule that projects to the lateral teeth and a medial one that projects into the olfactory cavity. Some fibres project in the ventral part of the olfactory organ but we did not manage to determine clearly the ventral targets. The main part of the fibres project dorsally to the posterior border of the principal cavity, stopping at the limit with the lateral cavity. As this innervation does not seem to be linked to the epithelium, we propose that it could be implicated in the process of closure of the principal cavity in regard to the detection of water at the near entrance of the nostril.

11. Information-Theoretic Analysis of Odor Intensity at the Olfactory Epithelium

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In this paper, we estimate the information capacity of a synthetically generated population of olfactory receptor neurons (ORN) expressing the same olfactory receptor protein (ORP). This study is aimed

at improving our understanding of odor intensity encoding at the olfactory epithelium. The ORNs of the olfactory epithelium belong to different types depending on the ORP that they express. Different ORPs allow binding to different odorant molecules or molecular features. Consequently, the quality of the odorants is captured at the olfactory epithelium as a population code across ORN of different type. At the same time, the intensity of the odorants is encoded by the firing frequency of the ORNs. Despite the apparent simplicity of this intensity-coding scheme, the diversity within ORN of the same type suggests a more complex encoding mechanism where odor intensity is also captured as a population code but across ORN of the same type. To study this coding mechanism, we have generated a population of ORN mimicking the existent diversity on a population of ORN of the same type. The population has been generated following the statistical distributions experimentally found by Rospars et al. for the different parameters of the ORN: firing frequency, firing threshold, maximum frequency, response duration, and latency. To understand the role that each one of this parameters play on the odor intensity coding process, we analyze the information conveyed by the population as the statistical distribution of these parameters is varied. This allows to determine the optimal distributions from an information-theoretic point of view and compare them with the empirically obtained distributions.

12. Unconventional Sensory Cells in the Nasal Epithelia of Rodents and Humans

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The conventional view of the nasal cavity of rodents and humans attributes ciliated olfactory neurons (OSNs) to the main olfactory epithelium (MOE), and in rodents, microvillous VSNs to the vomeronasal organ (vestigial in humans). Free nerve endings were described for the respiratory epithelium (RE). This notion was challenged by studies revealing presumed microvillous OSNs for the MOE. Similarly, the RE contains not only free nerve endings but also solitary chemosensory cells (SCCs) that previously had been described only for fish. In rodents, SCCs detect irritants and express components of the bitter taste transduction cascade. The present study investigates the structural features of the nasal epithelia to obtain a thorough knowledge of the cell types present in the rodent and the human nasal cavity. Standard techniques of immunocytochemistry and both SEM and TEM electron microscopy were used to characterize unconventional neurons in the MOE and the RE. Two different types of likely OSNs are present in the rodent MOE that bear microvilli at their apical endings. At least one of these types of microvillous cells expresses the transient receptor potential channel Trp M5. The human MOE revealed a cell type that strikingly resembles the crypt OSN of fishes. The RE of humans and rodents contains several types of cells with potential chemosensory character. These cells have the morphology of SCCs and express Trp M5. Subsets of these cells express gustducin, calbindin, and/or the vesicular acetylcholine transporter (VAChT). These findings suggest the existence of unconventional possible receptor cell types in the MOE and RE of rodents and humans.

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13. Pheromone Deficiency in C57BL/6 Mice Strain Associates with Low β-Glucuronidase Activity and Major Urinary Proteins (MUPs) Content

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There are rapidly growing evidence that the functional activity of androgen-dependent pheromones in mice is strongly associated with major urinary proteins (MUPs). MUPs are encoded by multigene family of about 35 genes, which are clustered on chromosome 4. Another interesting and well-documented pheromonal model deals with β-glucuronidase (GUS, EC 3.2.1.31), which is encoded by the gene Gus (Chr. 5) and involved in the transformation of the immobilized chemosignal (propheromone) in the form of glucuronides to physiologically active substance. The detailed analysis of plasma testosterone level, MUPs content, and GUS activity in the voided urine, kidney, preputial, and salivary glands in male mice of different genotypes revealed no significant correlation between GUS and MUPs only in C57BL/6JY strain with well-known low pheromone activity level. These data strongly suggest the importance of coordinated testosterone-dependent expression of Mup and Gus genes in pheromonally mediated social behaviors in *Mus musculus* and represent the first example of specific gene net basis designed by nature for effective chemical communication in this species. The obtained results may shed light on particular causes of interstrain differences in pheromone activity, present valuable approach for dissection of behavior phenotypes by using the pheromones as a fine natural tool, and can effectively trace the concrete biochemical pathways from gene(s) to social behavior.

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14. One Cell–Multiple Receptors: Identification of Novel Testicular Odorant Receptors that Mediate Distinct Sperm Motility Patterns

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In addition to the expression of odorant receptors (ORs) in sensory neurons of the olfactory epithelium, ORs are also present in other tissues (ectopic expression), for example, in human testicular tissue. Such testicular ORs have been attributed a potential function as molecular mediators of mammalian sperm behaviour. Recently, we identified and characterized a human testicular OR, OR1D2, that triggers chemotactic and chemokinetic responses in navigating sperm in vitro. Whether other putative sperm ORs control similar behavioural responses and whether the popular ''one cell–one receptor'' concept also applies to OR expression in male gametes are important questions in reproductive biology. Here, we report functional description of two novel human testicular ORs. Comparative analysis of the activation profiles of all three receptors

in a heterologous expression system revealed distinct nonoverlapping receptive fields. Our findings thus provide a tool to investigate individual OR-activated signaling mechanisms in mature sperm and determine their specific role in sperm motility and chemotaxis. Using a combination of calcium imaging and video motion analysis, our results show that individual human sperm cells express several functional Ors, which differentially trigger distinct signaling cascades and behavioural responses. These data provide new insight into the physiological principles that underlie OR-mediated sperm behaviours.

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15. The Role of Hormonal Status of Signal Recipient in Pheromone Reception in House Mouse

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It is well known that exposure of male mice to the urine from receptive females produce considerable increase of plasma testosterone. We studied the influence of steroid hormones, testosterone and corticosterone (in plasma and urine), of males on reception of female chemical cues in vomeronasal organ. Test subjects were adult male mice of different social status. Hormone assay was performed using ELISA technique. Plasma testosterone ranged from 0.1 to 12 ng/ml. Corticosterone ranged from 120 to 640 ng/ml. Patterns of c-fos expression were recorded in receptor tissue in response to stimulation with urine from receptive females. We observed activated cells in basal and apical zone of receptor tissue regardless of differences in plasma testosterone level of males. Another group of males was exposed to predator urine (*Felis catus*) for 3 days in environmental chamber. Monitoring of steroid hormones showed that testosterone ranged from 1 to 2 ng/ml, corticosterone from 840 to 1050 ng/ml. We did not observe pattern of activation in receptor tissue in stressed males with high corticosterone levels (over 800 ng/ml). The data obtained indicate that glucocorticoids may play an important role in pheromone reception in vomeronasal organ.

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16. Wound State Monitoring by Volatile Markers

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Wounds are injuries to body tissues caused by disease processes or events such as burns, punctures, chronic leg or decubitus ulcers, and results of surgery. Wounds become infected when microorganisms from the environment or from the patient's body enter the open wound and multiply. The symptoms related to an infection include abnormal flushing of the skin, heat, pain, and tenderness and abnormal odours, such as fruity odours that often indicate the presence of *Staphylococcus* or foul odours due to presence of gram-negative bacteria. Standard techniques for microbiological detection are surface swabbing and wound biopsy culture. Surface swabbing is the most used technique mainly because it is quite inexpensive and is not invasive but can give only a representation of the surface infected and is time consuming. Biopsies are invasive and are inconvenient. We are developing a fast reliable method for detection of microbial infection by monitoring the headspace from the infected wounds. In this paper we present preliminary results obtained by analysing the headspace volatiles emitted from *Streptococcus aureus*, *Pseudomonas aeruginosa*, and *Bacterium fragilis* in order to identify volatile markers of infection. The results obtained from this GC–MS study will allow us to build a noninvasive mobile system for noninvasive wound monitoring using an array of gas and odour sensors to be used for point of care monitoring of patients.

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17. Repeated Exposure to an Initially Disliked Vegetable Enhances its Acceptance by Infants at Weaning

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Young children are often "neophobic" with respect to foods and hence will frequently reject unfamiliar foods. It has been shown that repeated exposure to a new food increases both its acceptance and intake even in very young infants, although it may need 8–10 exposures to achieve these effects. There is little information as to how long mothers actually persist in offering initially refused foods. In a recent questionnaire study in two European countries we observed that, if the infant refused a new food, mothers rarely offered it at more than 2–3 meals before giving up and deciding that her child did not like it. In this study we evaluated and quantified the effects of persistent exposure (at least 8 times) to an initially disliked vegetable. Moreover, we compared this effect with intake of, and liking for, a liked vegetable. Acceptance was evaluated at home over a period of 16 days (with disliked and liked vegetables offered on alternate days). Forty-nine German infants (mean age 5.2 ± 0.1 [SEM]) were tested. Acceptance was measured by intake and by mothers' liking ratings (9-point category scale). The vegetables rejected were artichoke (by 18 infants), peas (by 10), green beans (6), and cauliflower (6). The (most) liked vegetable was usually carrot. Our results showed that for most of the infants, repeated exposure to an initially disliked vegetable increased intake to the same level as that of the liked vegetable but that more than 2–3 repeated exposures were needed. In contrast, intake and liking scores for the well-liked carrot purée was relatively constant over the exposure period. Based on these findings, it is worth encouraging mothers to persist for a longer period when trying to persuade their infants to consume a disliked food.

18. The Olfactory Sensitivity Depends on the Feeding States

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Body weight is precisely controlled through a fine adjustment of energy balance. Among the adjusting factors, olfaction plays a major role in food intake control since it mainly defines the palatability. The olfactory neural processes are in turn influenced by the metabolic and nutritional status of an organism. To our knowledge, the question of changes in olfactory detection performances in relationship to food intake has never been studied in animals. The question is addressed here in rats throughout a behavioral paradigm based on a conditioned olfactory aversion (COA). COA was obtained for water odorized with 10-5 isoamyl acetate (ISO). Once aversive, each rat, in hungry and in satiated states, was offered the choice between odorized and pure water. The pertinence of their choice objectifies their olfactory detection abilities. The results show that the hungry rats better detect ISO at low concentrations than the satiated ones. A second step consisted in mimicking hunger and satiety by icv injections of orexin (OX) and leptin, respectively. Using the same paradigm, the rats were tested when receiving either the peptide or NaCl. The rats were significantly more sensitive to ISO when they received OX than NaCl. By contrast, the rats treated with leptin were significantly less sensitive. The results that OX and leptin decreases and increases the olfactory sensitivity, respectively, are coherent with the expected role of olfaction in feeding behavior. These studies objectify that the nutritional status directly modulates the olfactory detection power of the animals (the hungry rats smell better) and furthermore that orexin and leptin play major roles to define the hungry and satiated states, respectively.

19. Neonatal Operant Responding Controlled by the Reinforcing Effects of Milk and Ethanol

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Newborn rats are able to obtain milk when suckling from a surrogate nipple. While attached to this nipple, they also display vigorous head and forepaws movements similar to those exhibited during breastfeeding. In this study, the probability of execution of these movements was analyzed as a function of the contingency existing between these behaviors and intraoral infusions of a natural reinforcer (milk), a relatively neutral stimulus (water), or ethanol (3% v/v). The main goal was to analyze whether ethanol's sensory cues act as a positive reinforcer leading towards self-administration of this psychopharmacological agent. These goals were examined using a new technique in which 5-day-old rat pups were positioned over a soft texture while having access to a touch-sensitive sensor. Forepaw or head movements leading to the activation of the sensor resulted in intraoral infusions of the above-described fluids (paired groups). Yoked controls were employed for each of these solutions. All pups were subjected to two training trials (15 min each) followed by a 9-min extinction trial. Paired pups were found to rapidly increase their rate of responsiveness when reinforced with milk. During extinction, these pups still exhibited higher levels of responding when compared with appropriate controls. When ethanol served as the reinforcer clear differences, similar to those observed in milk reinforced pups, emerged during extinction. Water was not found to modify performance patterns of Paired pups when compared with Yoked infants during training or extinction. These results indicate that newborns rapidly acquire operant responses when reinforced with milk. Furthermore, these results indicate that ethanol is highly reinforcing during early postnatal development.

20. Odorant-Induced Nuclear Translocation of beta-Arrestin2 in Human Spermatozoa

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A growing number of proteins originally found in endocytic structures of the plasma membrane appear to be able to traffic to the nucleus. Beta-arrestin1 and beta-arrestin2 play a key role in the regulation of G protein–coupled receptor-mediated signaling and act as scaffolding proteins for ERK1/2 and JNK3 cascades. Beta-arrestin2 undergoes constitutive nucleo-cytoplasmic shuttling, being imported into the nucleus by an unknown mechanism and exported through the classical NES-dependent pathway, but a direct nuclear function is not established. Besides their expression in the neurons of the olfactory epithelium, chemosensory olfactory receptors (ORs) reside in spermatozoa. Human testicular OR (hOR17-4) was recently identified, cloned, and functionally expressed in heterologous HEK 293 cells system. In addition, it was shown that it functions in human sperm chemotaxis. Here, we show that beta-arrestin2 is translocated to the nucleus upon stimulation of the hOR17-4 and does activate transcription in a GAL4 reporter assay. hOR17-4 is expressed in the midpiece of mature spermatozoa, where it also caused nuclear translocation of beta-arrestin2. hOR17-4 desensitization and odorant-induced translocation of beta-arrestin2 are dependent on PKA-mediated phosphorylation. Furthermore, activation of hOR17-4 in spermatozoa causes nuclear occurrence of the phosphorylated isoforms of members of the MAPK family. Analysis of the nuclear interaction partners of beta-arrestin2 indicates that it might be involved in the regulation of gene expression during particular processes in fertilization.

21. Taste Disorders in Patients with Free Intraoral Flaps

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Objectives: Free microvascular flaps are commonly used to reconstruct intraoral defects after tumour resection. Even though functional results are usually good, little is known about taste function in these patients. Methods: In 15 (age: 45–70 years) patients (postoperative range: 1–4.5 years) and 15 age-matched controls, taste function and subjective impairment were evaluated. Psychophysical threshold testing was done using taste strips. Moreover, olfactory function was psychophysically evaluated using the Sniffin' Sticks test battery. Nine out of 15 patients had additional radiotherapy postoperatively. Results: In contrast to normogeusic controls, in 11 patients taste was significantly reduced. One patient was hypogeusic, 10 ageusic, and only in 4 patients taste testing revealed normogeusia. Subjective impairment was low. Olfactory function—in contrast—was only slightly reduced. Conclusions: There is a high incidence of taste disorders in patients after

intraoral reconstructive surgery, which exists in patients both with and without additional radiotherapy, even though subjective impairment is low. The pathomechanism is most likely multifactorial and needs further investigation.

22. Basolateral Monosodium Glutamate Induce a Closure of Channels in Some Rat Taste Bud Cells

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Fungiform taste bud cells were recorded in an isolated rat tongue epithelium. Using the whole-cell configuration, voltage-clamp recordings indicated a diversity of voltage-gated currents upon depolarizing pulses or ramps. Some cells presented a large TTX-sensitive voltage-gated inward current. These cells were able to generate action potentials either spontaneously or upon depolarization. The duration of the action potential varied from one cell to another one, according to the kinetics of activation of the outward K+ voltage-gated current. Other cells presented only voltage-gated outward current largely suppressed by barium-TEA. The effects of monosodium glutamate (MSG, 100 μM) were examined when applied on the basolateral side of the recorded taste cells. Voltage-clamp recordings indicated no modification of voltage-gated currents. In some cells, depolarizing ionic channels (which were open at rest) were transiently closed and increased the cell input resistance. These observations suggest a resulting increase of the electrical sensitivity of the taste cells, which might be related to the ''taste enhancer effect'' of glutamate. This effect of MSG was observed in cells with various amplitudes of voltage-gated currents.

23. Gas Detecting Mechanism of New Ultrasonic Real-Time Gas Molecule Sensor

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The observation of odor and air exchange with high temporal accuracy is indispensable to obtain strict chemosensory event-related potentials (CSERPs) or magnetic fields as proposed by Evans. There have been no suitable methods, however, by using previously proposed gas detecting technique for real-time observation of gas stimuli. We have, therefore, developed new technique to realize accurate measurement of gas molecule concentrations with millisecond temporal resolution by utilizing ultrasound. Principles of this ultrasonic gas sensor, however, were still unclear. We, therefore, tried to clarify the principle of measurement. We changed the distance between sounder and receiver, with variation of mixture rate of oxygen and nitrogen. We concluded the key to this phenomenon is phase shift of the multiplex beam interference pattern, between the ultrasonic sounder and the receiver, which is affected by the molecular weight. We succeeded in detecting including 1% hydrogen (v/v) gas from pure nitrogen with high signal-to-noise ratio over 50 dB. This result shows that our gas sensor has enough potential to detect leak of hydrogen from container or pipe.

24. Insulin Reduces Several Types of Potassium Conductances in Mitral Cells

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Olfaction is a sensory dimension that is an integral part of food intake since many animals rely on odor cues for food seeking. The olfactory bulb (OB) is the main target of some feeding regulatory mechanisms, either through hypothalamic anatomo-chemical relationships or direct actions of peripheral regulatory peptides. We got first insights in interactions between OB processing and food intake by showing that orexin acts on mitral cells (MC) in rat OB slices. OB also contains very high concentration of one another peptide, which plays a major role in adjustments of energy balance and feeding behavior: insulin. It has been proposed that insulin could act on OB dissociated neurons by inhibiting Kv1.3 channel conductance. We presently addressed the question of both the action of insulin on MC in situ and localization of receptors in the OB. Immunohistochemical study showed that insulin receptors were localized on MC soma in Wistar rats (P15–P25). Patch-clamp recordings of MC in OB slices revealed that 30/51 cells (59%) react to insulin. To decipher the insulin action, the delayed rectifier or transient K currents (pharmacologically isolated) were measured at steady state or at peak, respectively, during insulin (1 μg/ml) perfusion. Insulin significantly reduced the delayed potassium current by 27.3 +/− 2.64% in 16/30 of tested cells, acting within a mean delay of 3.1 +/− 0.54 ms. A complete recovery was obtained in 7 MC. Insulin also significantly reduced the transient current by 32 +/− 9.27% in 9/12 of tested cells. A complete recovery was obtained in 3 MC. In current clamp recordings, insulin reduced latencies and delayed repolarisation of evoked spikes (5/9 cells). Altogether, insulin action on potassium conductances may result in a facilitating action on MC excitability.

25. Experts and Novices Olfactory Categorizations in Wine: Differences and Similarities

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This study explored olfactory categorizations in experts and novice wine consumers. We used wine from two different grape varieties: 10 Melon de Bourgogne (MB) and 10 Chardonnay (CH) wines. We choose these two varietal wines because previous results have shown a relative overlap of their aroma signatures, which prevent them from being distinguished too easily in sensorial evaluations. All participants performed 4 olfactory tasks: a CH and MB conceptual typicality rating task, a familiarity rating task, a liking task, and a perceptual free sorting task based on aroma similarity. All tasks were performed orthonasally. First, we observed a clear agreement between experts in typicality scores. Moreover, despite a slight overlap we found a clear differentiation between CH and MB for expert's typicality scores. For novices, no such agreement on typicality scores was observed and we found a complete overlap between both types of wines. Taken together, these results suggest that experts developed through successive wine tasting separate consensual sensory concepts for CH and MB wines. Second, significant correlations were found between

familiarity and Melon de Bourgogne typicality scores and between hedonic and CH typicality scores for experts. The result on familiarity could be explained by the fact that experts came from the area of MB production. Finally, Multidimensional Scaling analyses of the sorting tasks showed a clear separation between MB and CH for experts but also, to a certain extent, for novices. This last result indicates that no specific learning is required to distinguish CH from MB aromas exclusively on the basis of olfactory similarities.

26. Aliphatic Odorants with Two Oxygenic Functional Groups Stimulate Posterior Glomeruli in the Rat Olfactory Bulb

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We have been mapping 2-deoxyglucose uptake evoked by systematically different odorant chemicals across the entire glomerular layer of the rat olfactory bulb. We have found that most aliphatic odorants stimulate regions of the glomerular layer that are related to their particular oxygen-containing functional group. In the present study, we tested whether aliphatic odorants containing two such functional groups (esters, ketones, acids, alcohols, and ethers) would stimulate the combinations of glomerular regions that are associated with each of the functional groups separately. Our results indicate that these very highly water-soluble molecules only rarely evoked activity in the regions responding to the individual functional groups; instead, they activated posterior glomeruli located about halfway between the dorsal and ventral extremes in both the lateral and the medial aspects of the bulb. Other very highly water-soluble odorants, including small molecules with single oxygenic groups, also stimulated these posterior regions almost exclusively, suggesting a possible relationship between odorant water solubility and posterior glomeruli. Others have reported a ready absorption of water-soluble odorants by mucosa in the olfactory epithelium. Given the topography of the epithelium-to-bulb projection, absorption of water-soluble odorants in the central channel of the main olfactory epithelium would be expected to activate the dorsal aspect of the bulb rather than the posterior aspect. We therefore speculate that the activated posterior glomeruli may receive projections from sensory neurons located in the nasal cavity somewhere in the air path prior to the central channel of the main epithelium.

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27. Distribution of Tachykinin-Related Peptides in the Primary Olfactory Centre of the Heliothine Moth *Heliothis virescens*

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The antennal lobe of heliothine moths consists of 60–65 glomeruli. Olfactory coding mechanisms responsible for reproductive behaviour have been thoroughly investigated in these species. To gain further insight into the anatomical and neurochemical organization of the primary olfactory centre, we investigated, for the first time in an adult moth, the distribution of tachykinin-related peptides (TKRPs) in the antennal lobe of *Heliothis virescens*. Immunocytochemical experiments demonstrated that members of this neuropeptide family are present in local interneurons of the antennal lobe. In addition to local neurons, a pair of centrifugal neurons showed tachykinin immunostaining. This neuron type had cell bodies in the lateral subesophageal ganglion, arborizations in the antennal lobe, and projections in the inner antenno-cerebral tracts. Double-label immunofluorescence including three other antisera, antiallatostatin, allatotropin, and FMRFamide, showed that tachykinin immunostaining differed considerably from immunostaining with antisera against the three other peptides; whereas TKRPs were present in neurons with blebby processes distributed throughout each glomerulus, members of the three other peptide families were present in neural branches forming a dense and homogenous pattern confined to the glomerular core. Furthermore, total lack of colocalization of TKRPs with the three other peptide families in cell bodies of the lateral cell group suggests that TKRPs are present in a particular subtype of local interneurons of the antennal lobe.

28. Chemical Modulation of the Olfactory Properties of Natural Products: The Germacrone Case

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Essential oils (EO) are the main source of natural products in perfumery and other industries, as odor improvers or correctors. Germacrone, 1, is an odorless compound with a germacrane skeleton. It occurs in the EO from numerous plants widely distributed, being the major constituent in some of them. Thus, 1 constitutes a 52% and a 40% of the EO from *Geranium macrorrhizum* (1) and *Baccharis latifolia* (2), respectively. We have achieved the isolation of 1 through a simple crystallization from the EO, which simplifies its industrial use as raw material. Cyclizations of 1 using different electrophiles are found on bibliography (3), but all of them gave mainly eudesmane skeleton compounds, which are not reported to possess fragrant properties. The present work shows the results of cyclization of 1 using protic acids as electrophiles at low temperature. This cyclization gave three molecules (2–4) with new sesquiterpene skeletons involving bi- and tricyclic systems and intense odor properties. For example, the fragrance of tricyclic ketone 3 is of vanilla type, whereas that of spirane 2 is of wood type. Thus, only one chemical transformation affords compounds with unusual structures and aromatic properties, which make them interesting substances to be used in Perfumery as fixatives.

We thank the MEC (project BQ2002-03211) and the Alban Programme (scholarship E04D041998AR) for financial support. 1a) Ognyanov et al., Coll. Czech. Chem. Commun. 1958, 23, 2033. b) Ognyanov et al. 1958. Perfumery Essent. Oil Record 49:617. 2a) Loayza et al. 1995. Phytochemistry 38:381. 3a) Masatake et al., Bull. Chem. Soc. Japan 1976, 49, 3148. b) Hiroshi et al. Chem.

Pharm. Bull. 1977, 25, 6. c) Tsankova et al. 1980. Tetrahedron 36:669. d) Tsankova et al. 1987. Tetrahedron 43:4425.

29. Effect of Haemodialysis on Olfactory Function

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Chemosensory function has poorly been investigated in chronic renal failure patients. The effect of haemodialysis on the chemosensory functions remains largely unknown. Several authors showed poorer olfactory performances in chronic renal failure patients compared to controls. Recent works on chronic renal failure patients tested by the Sniffin' Sticks test battery evidenced a gap between the olfactory threshold performances and their ability to identificate and discriminate odors. Thus, poorer olfactory performance in these patients results rather from a central (identification) than from a peripherical (threshold) impairment. We have studied the effect of haemodialysis on both olfactory identification and threshold. Methods: Eighteen patients with chronic renal failure were included in the study. Olfactory tests were done 1 h before and after haemodialysis. Olfactory testing was done with the Sniffin' Sticks test battery, a well-established and validated olfactory test device. Thresholds were evaluated with an *n*-butanol and acetic acid step dilution test. Data were compared to published normative data. Results: Overall olfactory performance was slightly poorer compared to a normal population before haemodialysis. Olfactory identification and acetic acid threshold were impaired before haemodialysis. After dialysis, identification ($P = 0.0043$) and acetic acid threshold ($P = 0.0034$) improved significantly, while *n*-butanol threshold did not change ($P = 0.34$). Conclusion: This confirms previous studies on poorer olfactory overall performances in chronic renal failure patients. The improvement of olfactory identification performance after haemodialysis brought some further arguments for a central cause of olfactory impairment in chronic renal failure patients.

30. Effects of Dopaminergic and Opioidergic Drugs on Female Mice Chemoinvestigation of Rewarding Male Pheromones and Neutral Odorants

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In mice, male-derived nonvolatile chemicals are not just innately attractive but also reinforcing (capable of induce place preference) to adult females. With the aim of studying the neurochemical basis of the primary rewarding properties of sexual pheromones, we tested the effect of i.p. injections of dopamine (SCH 23390, Sulpiride) and opiates (Naloxone) antagonists on the chemoinvestigation displayed by female mice in male versus female-soiled bedding two-choice tests. Both treatments failed to suppress the preference displayed by female mice towards male chemosignals. We also investigated the effects of i.p. injections of dopamine agonists (Amphetamine, SKF 38393, Quinpirole) on male-soiled bedding preference, as well as their effects on olfactory sensitivity to neutral odorants, by means of habituation–dishabituation tests. Dopamine agonists had differential effects on preference for male chemosignals and olfactory function. Thus, at some doses, amphetamine suppressed male-chemosignals preference but increased olfactory sensitivity, whereas some quinpirole-treated mice appeared functionally anosmic but maintained their preference for male-derived stimuli. These results suggest that neither dopamine nor opiate receptors are of major importance for the primary reward of male pheromones. In addition, our findings provide indirect support to the idea that, in female mice, neutral odorants and male sexual pheromones are processed in distinct neural systems that are differently sensitive to modulation by dopaminergic drugs. This might reflect differences in the neural mechanisms and circuits of the olfactory and vomeronasal systems.

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Session 1

Symposium 1: Differentiation of Novel Cells in the Olfactory System

31. Cues Underlying Neurogenesis in the SVZ-OB System

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Continuous structural plasticity and neurogenesis in restricted areas of the adult mammalian CNS have been clearly established. The olfactory bulb (OB) of rodents is the brain region most enriched throughout life by new neurons, whose precursors are generated by neural stem cells residing into the forebrain subventricular zone (SVZ). SVZ neuroblasts undergo long distance migration to the OB where they differentiate into interneurons. In the adult, migrating neuroblasts are organized as chains, enwrapped by an astrocytic glial meshwork (glial tubes). Chain formation and glial tubes assembly occur during the first 3 weeks of postnatal life, a developmental period in which the SVZ-OB system is characterized by deep structural and molecular changes. SVZ-glial compartment directly contributes to the neurogenic niche, a complex molecular milieu of localized and overlapping pathways of hormones, neurotransmitters, and growth factors, including members of the BMPs and regulators. An intracellular regulator of several steps of SVZ neurogenesis is the cAMP response element–binding protein (CREB). CREB activation, via phosphorylation, correlates with defined steps of OB interneuron differentiation, and transgenic mice lacking CREB show reduced survival of newborn neurons in the OB, indicating a role in differentiation/survival of SVZ newly formed neurons. Moreover, experimentally induced loss of functional connections from the periphery results in downregulation of CREB phosphorylation, accompanied by reduced neuroblasts migration and survival, suggesting that CREB is also involved in SVZ cell migration. Thus, integration of new neurons in the OB is the result of multiple cues regulated by a complex interplay between genetic and epigenetic influences.

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32. Olfactory-Like Cells in the Cribriform Mesenchyme: A Possible Role in Axonal Wiring

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Olfactory receptors (ORs) are supposed to render chemosensory neurons in nasal epithelia responsive to odorants and pheromones. During prenatal development, some OR subtypes are also found to be expressed in cells located in the cribriform mesenchyme between the olfactory epithelium and the telencephalon. Monitoring the onset and time course of expression revealed that the ôextraepithelialô expression of olfactory receptors in the cribriform mesenchyme begins very early and is transient: in a time window between embryonic stages E10.25 and E14.0. In situ hybridization experiments have shown that most of the receptor subtypes were expressed in rather small cell populations, except receptor subtype mOR256-17, which was found in a significantly larger portion of extraepithelial cells. These cells in the cribriform mesenchyme also expressed key elements of olfactory neurons, including the olfactory marker protein OMP, the G protein Golf and adenylyl cyclase III. Upon visualization by specific antibodies, these cells turned out to have long protrusions extending along the surface of nerve fascicles. They are often located at bifurcations where two small axon fascicles merge to form a stronger bundle. In this region, olfactory nerve fascicles coalesce forming a coherent nerve. Within the compact nerve bundle, a population of axons visualized by OR-specific antibodies was no longer distributed evenly but rather was segregated within the nerve. These findings suggest that OR proteins in the membrane of olfactory cells in the cribriform mesenchyme and of axonal processes may be intimately involved in critical processes, such as sorting and fasciculation of outgrowing axons, which are fundamental for initiating and establishing the precise wiring of the olfactory system.

33. Functional Properties of Immature Dopaminergic Neurons in the Adult Mammalian Olfactory Bulb

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A significant fraction of the cells generated in the subventricular zone (SVZ) and added in adulthood to the olfactory bulb (OB) is constituted by dopaminergic (DA) neurons. In the mammalian OB, besides mature DA neurons, confined to glomerular layer (GL), cells in which the transcription of the tyrosine hydroxylase (TH) gene occurs in the absence of significant translational activity are present in the mitral cell and in the external plexiform layers (ML and EPL). It has been proposed that these are cells recently migrated to the OB from the SVZ, and committed to become DA but not yet entirely differentiated. Using a transgenic animal model expressing GFP under TH promoter we have studied the functional properties of these cells (TH-GFP) with the patch-clamp technique in thin slices. TH-GFP cells in the EPL are autorhythmic, as are mature DA neurons, whereas TH-GFP cells in the ML are not. The pacemakers are a T-type Ca-current and a persistent Na current, the same as for mature DA neurons. The cells in the EPL are synaptically connected to the olfactory nerve, whereas those in the ML are not. Finally, the progressive maturation of the voltage-dependent currents has been described. Our interpretation of these observatio ns is that TH-GFP cells outside the GL are immature DA neurons recently migrated to the OB from the SVZ, which disrupt their migration at the level of the ML, and send a dendritic process towards the GL trying to establish a synaptic contact. Only when/if this attempt succeeds, the TH-GFP cells receive some kind of consensus signal from the GL allowing them to fulfill their differentiation towards the DA phenotype and to complete their migration on the way to their final destination crossing the EPL.

34. Matrix Metalloproteinases (MMPs) in Primary Olfactory Pathway Formation

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The topography of olfactory sensory neuron (OSN) axonal projections from olfactory epithelium (OE) to olfactory bulb (OB) is an essential determinant of odor coding. The mechanisms subserving the sorting and targeting of axons are complex but it is widely accepted that ORs are necessary, but not sufficient to account for the specificity of targeting. During the earliest stages of olfactory pathway formation, OSN axons extend through the mesenchyme and associated ECM and contact the presumptive OB where they enter the CNS through fenestrations in the basement membrane and form a presumptive nerve layer. The MMPs are proteolytic enzymes that participate in ECM degradation and are implicated in the formation of connections in the developing CNS. I hypothesized that MMPs may be influencing OSN–ECM interactions by sculpting the ECM environment in the olfactory system (OS). There are at least 25 members of the MMP family and, collectively, these proteases can degrade all constituents of the ECM. To investigate whether MMPs are acting in the OS during development, I employed 2 approaches. First, an RT-PCR screen of MMP family members to profile gene expression at different developmental stages of olfactory development, beginning at E9 when OSN axons are first exiting the olfactory placode through early postnatal development. Second, in situ zymography is being used to detect and localize specific protease activities. Preliminary results demonstrate that a) select members of this family are active in the OS; b) there is differential gene expression between OE and OB; and c) protease activity is spatially localized within the OS. Thus, MMPs appear to have a role in olfactory pathway formation.

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35. Expression and Role of Complex Carbohydrates in Axon Guidance in the Olfactory System

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Primary sensory neurons in the vertebrate olfactory systems are characterised by the differential expression of distinct cell surface carbohydrates. We show here that the histo-blood groups Sda (or CT1 antigen) and H are expressed by primary sensory neurons in the olfactory system, while the blood group A carbohydrate is expressed by a subset of vomeronasal neurons only in the developing accessory olfactory system. We have used both loss-of-function and gain-of-function approaches to manipulate expression of these carbohydrates in the olfactory system. In null mutant mice lacking the alpha(1,2)fucosyltransferase FUT1, the blood group H and A carbohydrates were not expressed in the olfactory systems which

caused delayed development of the nerve fibre and glomerular layers in the main olfactory bulb. In contrast, ubiquitous expression of blood group A on olfactory axons in gain-of-function transgenic mice perturbed the ability of vomeronasal axons to terminate in the accessory olfactory bulb and affected the selective targeting of axons in the main olfactory bulb. During regeneration following bulbectomy, vomeronasal axons were unable to effectively sort out from the main olfactory axons when blood group A was misexpressed. These results provide in vivo evidence for a role of specific cell surface carbohydrates during development and regeneration of the olfactory nerve pathways.

36. Embryonic Geniculate and Trigeminal Neuron Survival and Differentiation: Effects of BMP4 and Noggin In Vitro

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At rat embryonic day 13 (E13), nerve fibers from geniculate and trigeminal ganglia are within the tongue. By E16, these ganglion cells innervate fungiform papillae and surrounding tongue epithelium, and thus are exposed to target-derived signaling factors. Bone morphogenetic protein 4 (BMP4), known to be involved in neuron survival and differentiation and its antagonist, noggin, are expressed in tongue by E13, are intense in papillae at E15–16, and can dramatically influence taste papilla development. To determine if these proteins affect neurons that innervate tongue and papillae, we compared survival and neurite outgrowth in E13 and E16 geniculate and trigeminal ganglia explanted and cultured with exogenous BMP4, noggin, or brain-derived neurotrophic factor (BDNF), a standard growth factor. Compared to geniculate ganglia exposed to BDNF, at E13 and E16, either BMP4 or noggin resulted in a substantial decrease in neurons and neurite extension. The reduction was especially pronounced at E13. Survival and neurite extension were also decreased in E13 trigeminal neurons exposed to either BMP4 or noggin. In contrast, at E16, low concentration of BMP4 or noggin was able to sustain a large population of trigeminal neurons but exposure to high concentrations of either BMP4 or noggin resulted in reduced neuron survival and neurite extension; this reduction was not as pronounced as at E13. Thus, for the trigeminal ganglion effects of these factors are both age and concentration dependent. Geniculate and trigeminal ganglia display unique requirements for survival and differentiation factors during different developmental stages that perhaps relate to the heterogeneity of their respective neuronal populations.

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Symposium 2: The Effects of Drug Exposure on Aversion Learning

37. The US-Preexposure Effect in Lithium-Induced Flavor-Aversion Conditioning: Underlying Mechanisms

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In several procedures, it has been shown that Pavlovian conditioning is retarded by prior exposure to the event to be used as the unconditioned stimulus (US); this is referred to as the US-preexposure effect. This effect is well established in flavour-aversion conditioning with lithium-induced nausea as the US. Two (not necessarily mutually exclusive) explanations have been offered for this effect. One possibility is that habituation occurs during preexposure and that this influences not only the ability of the US to evoke an unconditioned response (UR) but also its effectiveness as a reinforcer. The second possible interpretation is that US preexposure allows the acquisition of associative strength by contextual cues and that these then act to block the acquisition of strength by the experimenter's conditioned stimulus (CS). We report a series of studies using rats designed to evaluate these explanations for the US preexposure effect on flavour aversion conditioning, where LiCl is used as US. The results support the interpretation of the US preexposure effect in terms of blocking, suggesting that conditioning to injection cues is a fundamental factor in producing the attenuated aversion commonly observed and gave no support to the habituation hypothesis. Preexposure to the US, consisting of injections of LiCl given in the home cage, appears to establish an association between injections cues and the US, which is responsible for the retardation of subsequent conditioning with LiCl as the reinforcer. Preexposure may also result in habituation but the effect is independent of this association and it plays no part in the retardation of conditioning. This dissociation presents an explanatory challenge for current learning theories.

38. Context-Induced Conditioned Nausea: Effects of Prior Exposure to the Context

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A context in which rats have experienced the effects of an injection of lithium chloride will come to evoke behaviour that has been interpreted as reflecting a state of conditioned nausea. This effect has been put forward as an animal model of the anticipatory nausea that patients undergoing chemotherapy sometimes develop as a reaction to the cues of the clinic. We report a series of studies using rat subjects which suggest that prior exposure to the context before its pairing with nausea can produce a robust latent inhibition effect. We also investigate a procedure (the presentation of a novel flavour during preexposure) that appears to potentiate latent inhibition of the context. The theoretical basis for this potentiation remains obscure, but its occurrence encourages the possibility of using latent inhibition as a clinical intervention for the alleviation of both anticipatory and posttreatment nausea in patients.

39. Ontogeny, Hippocampus and Contextual Specificity of Taste Memory

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Current knowledge on the anatomic and functional organization of the learning and memory support a hippocampal role on context-dependent learning phenomena. The study of this specific hippocampal function may benefit from a developmental approach,

because the context modulation of learning is modified both during early development and aging. Previous studies in our lab using a task which involves safe taste preexposure and taste aversion conditioning have shown that both, retrieval of safe (Manrique et al. 2004. Neurobiol. Learn. Mem. 82: 77–80) and aversive (Morón et al., Learn Mem. 2002, 9:218–223) taste memories are bound to the time of day. The modulation of safe but not that of aversive taste memories requires an intact hippocampus in adult rats (Gallo. 2005. Chem. Senses. 30:i160–i161). Results will be presented showing different behavioural patterns according to the developmental stage. Peri- and adolescent rats show enhanced contextual specificity of aversive but not of the safe memories. Aged intact rats show no modulation of neither aversive nor safe memories. Interestingly, lesions of the dorsal hippocampus in aged rats unveiled the contextual specificity of taste aversion. Therefore, the study of the developmental changes in the performance of a hippocampal-dependent task, suggest dynamic and plastic adaptations of the brain function throughout the life.

40. Plasticity of Odour-Evoked Activity in the Honeybee Antennal Lobe: Coupled Aversive Conditioning and Calcium Imaging

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Intense previous work has studied the neurobiological bases of olfactory learning in two insect models, the fruit fly *Drosophila melanogaster* and the honeybee *Apis mellifera*. Until now, however, direct comparison between results in the two species has been difficult because most of the fruitfly work was based on aversive conditioning (reinforcer: electric shock), while the bee work was based on appetitive conditioning (reinforcer: sucrose solution). To allow more direct comparisons between the two models, we have developed a novel aversive conditioning paradigm in the honeybee. The Sting Extension Response (SER) is the reflex extension of the bee sting in response to a mild electric shock. By presenting an odour (CS) in temporal association with the electric shock (US), we show that bees are able to form odourûshock associations. This conditioning is clearly associative, as shown in differential conditioning experiments, in which bees respond to the odour associated to the electric shock (CS+) but not to another nonreinforced odour (CS−). Using a pharmacological approach, we show that SER conditioning depends on the dopamine system in honeybees, as in fruit flies. To study plasticity in the first relay of the insect olfactory pathway - the antennal lobe - related to aversive conditioning, we have developed a coupled SER conditioning/calcium imaging preparation. We can at the same time monitor the behavioural responses of a live bee learning to differentiate between two odours (CS+ and CS−), and record calcium signals evoked by these odours during conditioning. We thus image the honeybee brain while it learns aversive associations, and relate brain activity changes directly to learning success.

41. Effect of Two Bitter Substances on Olfactory Learning in the Moth *Heliothis virescens*: Acquisition and Extinction

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Sucrose stimulation of the gustatory receptors on the antennae of the moth *Heliothis virescens* elicits the proboscis extension reflex, a response utilized in appetitive learning experiments. By pairing an odour stimulation (conditioned stimulus, CS) with a sucrose stimulation (unconditioned stimulus, US), the moths learn to associate the odour with the sucrose reward and will subsequently extend the proboscis when stimulated with the odour alone. A previous study of appetitive conditioning in *H. virescens* was aimed at optimizing the acquisition success. In our study we first wanted to further increase acquisition in order to study acquisition and extinction combined with putative aversive stimuli. The effects of a stronger US on acquisition, retention and extinction were tested. Increasing the US from 1 to 2 M improved acquisition, but an increase to 3 M had no further effect. Retention was not affected by the US but by the time elapsed after training. As expected memory declined with time, being strongest after 15 min and 2 h and weaker when tested at 8, 24 or 48 h. Interestingly, extinction was slower in the 48 h group, suggesting that the memory had consolidated. Most taste sensilla on the *H. virescens* antennae house gustatory receptor neurons responding to sucrose. There are also responses to other substances like quinine and sinigrin, both bitter tasting to humans. Spike analysis indicated two different neurons detecting these substances. In ongoing studies we use inhibitory learning (preexposure to CS or CS paired with sinigrin or quinine) and facilitated extinction experiments (CS paired with sinigrin or quinine during extinction) to find out whether the two tastants mediate different degrees of aversive behaviour.

Symposium 3: Dendritic Differentiation and Development in the Olfactory Bulb

42. Olfactory Receptors Signal Identity

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The large family of ORs are now implicated in at least two processes which effectively determine the phenotypic identity of OSNs. First they are responsible for selective ligand recognition and therefore determine the molecular receptive range of a neuron. Secondly they are critical determinants in targeting axons to a particular glomerulus, thereby providing a topographic identity to OSNs. In the ligand binding process there is a well-defined transduction cascade to which the receptor is coupled and which results finally in signal generation and synapse activation. There is no indication that the ORs on OSN cilia are able to have any effect on cell behavior absent this transduction process. On the other hand, the ORs expressed at axon terminals have been proposed to have a direct effect, possibly through homotypic interactions, on directing axons into the proper glomerular relationships. However, there is evidence that at least the maintenance of the glomerular map requires activity in OSNs, although the source and nature of this activity are not yet defined. Is there a role for activity-dependent, receptor-mediated, signaling in

axon guidance as well? Does glomerular formation and maturation require the activation of a second messenger cascade, as well as expression of the proper OR? Using embryonic retroviral injection technology we have altered gene function in select early developing OSNs. Manipulation of the various molecules known to function in the transduction pathway causes large changes in axonal targeting and glomerular formation, suggesting a critical role for activity generated biochemical processes in OE-OB development.

43. Molecular Regulation of Mitral Cell Dendritic Development

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It has become increasingly clear that the development of the neuronal circuitry uses the following strategy: a blueprint of the neuronal network is laid down during embryonic stages; subsequently, during early postnatal development, precise functional circuits are achieved after remodeling and refine the existing connections. To study the development of the olfactory connectivity, we focused on the cellular and molecular regulations of the mitral cell dendritic morphogenesis. As the first relay neurons in olfactory system, mitral cells receive information from the peripheral sensory neurons, and integrate and relay olfactory information to olfactory cortical regions. To achieve precise wiring, mitral cell grows an elaborate dendritic tree during embryonic stage (E15–E18) that spans 20–30 glomerular space, which we term the ôbloomingö phase. This dendritic tree was dramatically pruned to achieve single apical dendrite morphology during early postnatal stages (P0–P10), which we term the ôpruningö phase. A genome-wide search was conducted to compare transcription differences within the olfactory bulb between the ôbloomingö and the ôpruningö phases. A novel gene, twc11, was identified to be exclusively expressed in mitral/tufted cells. Its expression is upregulated within the ôpruningö stages and subsequently downregulated. The expression pattern and the transcription regulation imply that it plays a role in the remodeling of the olfactory connections. I will report in vitro and in vivo evidence of twc11 function in the regulation of the olfactory circuitry.

44. Nature and Nurture in Adult Neurogenesis

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The olfactory bulb represents the only brain area where local GABAergic neurons are continuously replaced. How the newborn neurons integrate into a preexisting neural network and how basic functions are maintained when a large percentage of neurons are subjected to continuous renewal, are important questions that have attracted our attention. We shall see how the production of GABAergic interneurons is specifically adapted to experience-dependent regulation of neural networks and what are the programmed mechanisms molded by experience. In particular, we shall report the degree of sensitivity of the bulbar neurogenesis to the level of sensory inputs and, in turn, how the adult neurogenesis adjusts the neural network functioning to optimize sensory information processing. We will bring together recently described properties and emerging principles of interneuron function in the olfactory bulb that support a much more complex role for these cells than just providers of inhibition.

45. Differentiation and Targeting of Mitral Cell Dendrites in the Olfactory Bulb

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During early embryonic development, cells in the olfactory bulb and the olfactory epithelium follow simultaneous but independent intrinsic developmental processes. However, we must also consider the relationship between the arrival of axons from the olfactory sensory axons and the dendritic refinement of mitral cells at progressively later stages of development. We investigated the distinct morphological features displayed by mitral cells during their development, analyzing the relationship between the changes undertaken by these neurons and the arrival of the olfactory sensory axons. Immunostaining for specific markers of developing axons and dendrites, was coupled with localized fluorescent tracer injections to reveal the morphological changes, the continuous reorientation and the final refinement that these cells undergo. We identified three main chronological events: 1) newly generated neurons become established in the intermediate zone and project to the lateral olfactory tract; 2) reorientation and dendrite spreading while olfactory sensory axons penetrate into the inner layers of the olfactory bulb. This is a sensitive period, which is dependent on the arrival of afferents that establish a spatial and temporal gradient, facilitating protoglomerulus and glomerulus formation between E15–E16; and 3) final refinement of the radially orientated cells to adopt the mature morphology. These results suggest that sequential development and maturation of mitral cells may have important implications for the initial wiring of the olfactory bulb and the establishment of the olfactory epithelium to olfactory bulb topography.

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46. A Mutational Study of Olfactory Receptor Function in *Drosophila* Larvae

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Drosophila larvae have only 21 olfactory receptor neurons (ORN), expressing 25 olfactory receptors (OR), each of which projects to a separate glomerulus in the larval brain. As such, the maggot is the simplest possible model of olfactory processing in an animal with a brain. We have used the genetic flexibility of *Drosophila* to study the function of two olfactory receptors (Or42a and Or49a) in a variety of conditions, using both behavioural and electrophysiological measures of the larval olfactory response. The responses of null mutants for each gene and of double mutants are compared with the responses of wild-type larvae and with larvae expressing only Or42a or Or49a. We will also present data from mutants we have created by deleting part of the Or sequence, providing a rich image of receptor-ligand function in both isolation (single Or larvae) and larvae with 21 functional ORNs. This approach enables us to study potential interactions between ORNs during peripheral coding and the contribution of individual receptors to the olfactory percept. We also present data showing the

ability of these mutants to show peripheral adaptation, and the effect of this on the larval olfactory response.

47. A Role for MHC Class I Molecules in the Main Olfactory Bulb of Mice

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The mechanisms involved in establishing and maintaining the exquisite organization of synaptic connections found at the glomerular layer of the main olfactory bulb (MOB) remain to be resolved. Recent findings have linked major histocompatibility class I (MHCI) molecules and the MHCI signaling pathway to neuronal restructuring and refinement. In this study, we have demonstrated histochemically that MHCI molecules are expressed in the MOB of mice. Additionally, we show that mice deficient in the expression of MHCI molecules display defects in the targeting of olfactory sensory neurons to the MOB. TAP1 gene-targeted mice lack a critical component for the expression of MHCI molecules on cell surfaces. In these mice, we find an increased number of P2 labeled glomeruli per bulb as compared to the control littermates. Additionally, the location of these glomeruli is shifted along the rostral–caudal axis of the bulb as compared to the control mice. Although severely deficient in the expression of MHCI molecules, TAP mice do express MHCI molecules. Therefore, we characterized mice that lacked both TAP and beta-2 microglobulin (B2m). In these animals, we found severe defects in the formation of P2 glomeruli including a loss of P2 glomeruli in some cases. We also found the bulbs of these animals to be smaller and to have smaller glomeruli on average.

Oral Session 1: Taste and Smell Psychophysics

48. Simultaneous Gustatory Stimuli Modulate Ortho- and Retronasal Olfaction

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Objectives: Olfactory processing is influenced by simultaneous gustatory stimulation. The aim of this study was to examine, whether ortho- and retronasal olfactory stimuli are processed differently in the presence of a simultaneous gustatory stimulus. Methods: Two test sessions were performed with 32 young, healthy subjects (16 men, 16 women). Psychophysical testing was performed using the ''Sniffin' Sticks'' test battery; olfactory event-related potentials using a computer-controlled air-dilution olfactometer were recorded. Phenylethylalcohol (PEA) and vanillin were used as olfactory stimuli; sweet and sour taste stimuli were simultaneously applied intraorally. Olfactory stimuli were presented through the ortho- and retronasal routes. Results: During orthonasal stimulation latencies P2 of olfactory event-related potentials were found to be shorter compared to retronasal stimulation. Moreover, application of a sweet taste produced shorter latencies when olfactory stimuli were applied through the retronasal route. In contrast to that a taste, not contextually related to the odorous stimulus, shortened latencies in response to orthonasal stimuli. Conclusions: Olfactory stimuli presented through the orthonasal or retronasal routes are differentially affected by concomitant application of gustatory stimuli.

49. Predicting Subjective Ratings of Intensity, Pleasantness and Familiarity in Binary Odor Mixtures Based on Their Constituent Parts

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Most studies in human olfaction have been limited to investigation of single odors. However, outside the laboratory we usually encounter mixtures of different smells, not single odors, though the mixtures are usually perceived as a unified whole. Indeed, olfactory mixtures may be processed quite differently from single compounds, highlighting a need for studying these two phenomena in conjunction. Olsson and Cain proposed a model that relates the perceived intensity of a given binary mixture to the original intensity ratings for each of its constituent compounds. Our goal was to determine whether the predictions made by the model with respect to intensity could be generalized to other aspects of odor quality such as pleasantness and familiarity. Our stimuli consisted of nine unfamiliar and ten familiar isointense odors with similar hedonic ratings, mixed in 50–50 proportions to create 41 familiar and unfamiliar mixtures. Twenty right-handed subjects (mean age = 22.45, SD = 3.98) rated the intensity, pleasantness and familiarity of these mixtures and each constituent single compound. The ratings for perceived intensity, pleasantness, and familiarity of the binary mixtures were within the expected range for 40 of the 41 mixtures. Thus, although the model was initially proposed as a means of predicting odor intensity, our findings suggest that it can be extended to the perception of ''higher-order,'' and perhaps more subjective, odor qualities such as pleasantness and familiarity.

50. Thermally Dependent Sweet Water-Taste

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Understanding the impact of temperature on sweet taste is complicated by the diverse properties of sweeteners and the overlapping transduction mechanisms of thermal and gustatory senses. Neohesperidin dihydrochalcone (NHDC) is a dihydrochalcone glucoside sweetener that is obtained by extracting bitter citrus flavonones from Seville oranges. It is estimated to be eighteen hundred times sweeter than sucrose, but unlike sucrose, NHDC elicits a more prolonged time temporal profile of sweetness, and leaves a lingering mouth feel, which mimics the aftertaste of licorice. Interestingly, NHDC also curiously produces a thermally dependent sweet water-taste. Sweet water-tastes are after effects from tasting a stimulus and subsequently perceiving taste qualities when plain water is rinsed. Water-tastes are similar to color after images in which a white piece of paper appears to have color after a subject fixates

actual colored paper. The objective of this study was to determine the effect that different physical temperatures and menthol prerinses have on the sweet water-taste of NHDC. We found that sweet water taste is elicited when cold water is rinsed after NHDC but not when warm water is rinsed. We further found that this effect is enhanced by menthol prerinses. Sweet water-taste has been shown to be a sweetener receptor, TAS1R2-TAS1R3, determined phenomenon. Thus, we believe there are elements of the sweet transduction sequence that are modified by NHDC to become cool sensitive.

51. Comparing Stimulus Localization Ability for Four Sensory Modalities

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One of the ways in which people develop a cognitive representation of an unknown environment is through their knowledge on a number of landmarks. Although vision seems to be the most sensitive modality in discriminating between spatial locations close to and far from the human body, landmark information may be perceived through other senses as well. In the present study, we compared the performance for four sensory modalities in a spatial memory task. For each modality, we selected 10 stimuli that rated low in recognizability and average in complexity, intensity, and pleasantness. We used two-dimensional, single-line, black-and-white drawings of free forms (vision), 1 s manipulated environmental sounds (audition), surface textures of natural and artificial materials (touch), and unfamiliar smells (olfaction). Stimuli were presented in 10 cubes that allowed them to be perceived through a single modality only. Participants were instructed to walk a specific route in a room, during which they opened the 10 cubes and perceived their content. In a computer task, they indicated where in the route they had perceived each stimulus. The proportion of correct answers was highest for vision and touch, and lower for olfaction and audition. Response speeds did not differ between modalities. These outcomes tentatively suggest that people are better in remembering the location of stimuli for those modalities that are preferably used for spatial localization under natural conditions.

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52. Cross Modality of Texture and Aroma Perception Does Not Depend on Orthonasal or Retronasal Stimulation

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A series of experiments has been conducted in which healthy human subjects were exposed to sequences of strawberry aroma pulses which were presented through a computer-controlled olfactometer. Just prior to exposure to the aroma the subjects consumed either water, a custard, or protein gels with different textures. The time between oral consumption of the food, including swallowing, and the exposure to the aroma varied between 0.5 and 6.5 s. The aroma pulses were administered either in an orthonasal or retronasal fashion using a computer-controlled stimulator based on air-dilution olfactometry. The subjects rated the intensity of the strawberry aroma. It was observed that the intensity of aroma decreased with increasing firmness of the food that was consumed. Aroma pulses delivered 6.5 s after swallowing were perceived as being more intense compared to aroma pulses delivered immediately after swallowing. Significantly higher odor intensities were reported for the aroma stimuli supplied orthonasally in comparison to retronasal administration. The observed effect of texture on aroma intensity was not significantly altered by the mode of aroma delivery, i.e. orthonasal or retronasal stimulus administration.

Poster Session 2

53. Olfactory Abilities and Behaviour in 6–12 Year Old French Children

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The present study investigated children's olfactory function through self- and parent-reports coupled with lab tests of olfactory abilities. A cohort of 215 children answered the Children's Olfactory Behaviour in Everyday Life (COBEL) questionnaire. Among those, 67 were selected in the highest and lowest quartiles of COBEL score, considered as odour-oriented (OO) and poorly odour-oriented (pOO), respectively. The participants (girls/boys: 31/36; aged 6–11) rated 2 pleasant (strawberry, phenyl-ethanol) and 2 unpleasant odorants (isovaleric, butyric acids) on a 7-point hedonic scale. The Sniffin' Sticks test yielded threshold, discrimination and free and forced-choice identification. Parents reported their perception of their child's olfactory behaviour, information about home odours and social data. No gender differences were found for any olfactory variable, but discrimination and cued identification increased with age. OO and pOO children differed only on hedonic reactivity, OO rating odours more contrastedly. Olfactory sensitivity was negatively correlated with the hedonic ratings of pleasant/unpleasant odorants, suggesting that more sensitive children are less tolerant to odours. Parents were not reliable reporters of their children's olfactory competence, as no link appeared between their own and their children's reports. Parents' awareness of their offspring smell was restricted to the child's negative reactivity to odours. Finally, the children's ability to identify certain odours improved with increasing diversity of the olfactory environment and with increasing number of female siblings. Overall, these results provide new insights on children's responses to aspects of their olfactory ecology.

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54. Olfactory Reception is Mediated by the cAMP and IP3 Transduction Cascades in *Drosophila melanogaster*

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Two main transduction pathways depending on the second messengers IP3 and cAMP, respectively, appear to be involved in olfactory reception in vertebrates as well as invertebrates. The presence in the olfactory neurons of two main transduction cascades has been interpreted as a coding mechanism to generate differential messages. Studies in vertebrates have proposed that both second messenger cascades coexist at least in some olfactory receptor neurons and cross-talk. In this report we directly address the question as to whether modifying one or the other transduction pathway at the reception level affects olfactory perception—deduced from behavioral data in complete living animals—using *Drosophila melanogaster* as a model system. Transduction cascade changes were achieved in different olfactory neuron subsets by overexpressing intermediate genes such as dnc (encoding the cAMP phosphodiesterase) or the IP3k1 gene (encoding for a IP3kinase) in 8 Gal-4 transgenic lines with the Gal-4/UAS method. Abnormal olfactory behavior in response to ethanol, acetone, ethyl acetate or propionaldehyde was found by dnc as well as IP3k1 overexpression in 75% of the lines (not always the same lines in both cases), depending on the affected neuronal subset. Since enhancer-trap expression of these Gal-4 lines is restricted to olfactory receptor neurons, these results confirm previous reports showing that the cAMP transduction cascade affects olfactory reception in *Drosophila melanogaster*. Moreover, mutations in both genes, dnc and IP3k1, have been related to changes in the electroantennogram. The high ratio of affected lines suggests that cAMP as well as IP3 transduction cascades mediation of olfactory reception occurs in an important extent of the olfactory receptor neurons.

55. Description of Women's Fragrances and Polysemy

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This research focuses on the description of complex women's fragrances. The difficulty related to the description of odors is a known fact. One of the methods usually used when one wishes to describe any type of object is the sensory profiling method. This method has limitations related to the use of sensory properties isolated from their context, i.e. from the sentence. However, the use of isolated terms raises the issue of the polysemy of the terms employed. On the one hand, a sensory term can have several meanings; on the other hand, the same perception can be expressed by several terms. That is what we wanted to demonstrate in this study. The experimental material consisted of nine women's fragrances. The participants were 28 women aged 18 to 30. The experimental protocol involves (1) classifying fragrances and describing the groups thus formed, (2) a free association task. The data analysis focused on the entire set of verbalizations. The analysis was conducted with the help of Alceste software. The results show that certain descriptors (fresh, mild, sweet, flowery, fruity, etc.) are associated with several contexts. For example, the descriptor fresh evokes 4 contexts and different definitions, including the contexts of nature (the freshness of spring and flowers), the seashore (the word fresh is associated with the wind, the breeze and moisture), food (freshness evokes fruits and vegetables) and cleanliness (the word fresh is associated with soap and shower gel). This study demonstrates the polysemy of descriptors commonly used to describe odors and fragrances as well as many other objects. It shows that one must be cautious about using isolated terms. These conclusions call for the development of other methodologies for assessing sensory perceptions.

56. Brainstem c-fos Expression after Nondelayed TAL Induced by NaCl

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Two different TAL modalities have been hypothesised:concurrent and sequential TAL. In the former, a contiguous interstimulus interval is required and therefore a rapid vagal processing of visceral noxious substances. Thus, the aim of this study was to identify brainstem structures involved in nondelayed TAL induced by hypertonic NaCl, comparing structures participating in the processing of gustatory or aversive stimuli. A further aim of the study was to analyse anatomical differences in gene expression resulting from the processing of gustatory and visceral sensory information according to whether stimuli are offered in isolation or after a learning process. For this purpose, a learning and control group were established, intake of the gustatory stimulus was associated with administration of hypertonic NaCl in the learning group, whereas the flavour was associated with saline in the control group. This acquisition process was repeated during four sessions, after which the experimental group learned to avoid the gustatory stimulus, consuming a significantly smaller amount compared with the control group. Once learning was established, half of the animals in each group were offered the flavour and the other half were administered with the visceral stimulus (hypertonic NaCl or saline). In this final session and in parallel with the above process, a further 16 animals were randomly divided among four groups that received a single administration either of the gustatory stimulus or water or of the visceral stimulus. The pattern of gene activation in different brainstem regions of the vagal–parabrachial axis is described and results are interpreted according to the proposed TAL modalities.

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57. Role of Early Experience in the Development of Taste Preferences in Infants

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Early flavor experiences can differ dramatically among infants since the types of milk fed (e.g., human milk, milk-based formula, hydrolysate formula) differ in their content of certain compounds that have specific taste qualities. Of specific interest is the pronounced bitter, sour and savory flavor of protein hydrolysate formulas. To determine whether early exposure alters the acceptance of specific tastes during weaning, the present study examined the infants' acceptance of the five basic tastes. To this end, we tested three groups of infants who were between the ages of 4 and 10 months and had recently started feeding infant cereal. One group of infants was breastfed and had no experience with formulas; the second group was feeding a milk-based formula; whereas the third group was feeding a protein hydrolysate formula. Each infant was tested on six separate occasions. In counterbalanced order, we videotaped them as they were feeding a sweet (lactose)-, salty (NaCl)-, bitter (urea)-, sour

(citric acid)-, savory (glutamate)-, or plain (water)-tasting cereal. In addition to recording intake and rate of feeding, we analyzed each videotape to record a variety of facial expressions which are indicative of liking or rejection. The data are forthcoming.

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58. Identification of Olfactory Signal Transduction Genes in *Drosophila melanogaster*

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The fly *Drosophila melanogaster* has emerged as a premier model organism for the study of the chemical senses because of the availability of a sequenced genome, powerful molecular genetic techniques, simple behavioral assays and electrophysiological tools. In *Drosophila* odor signals are detected by a large family of 62 seven-transmembrane receptor proteins, the odorant receptor (Or) family. The signal transduction pathway(s) activated by the *Drosophila* Or proteins are largely unknown. We are taking a number of approaches to identify genes involved in these pathways, and results from two approaches will be presented. Firstly we have screened a large number of EMS-generated homozygous viable mutant strains for electro-antennogram defects, and identified a mutant which has reduced responses to all tested odors. Progress towards mapping and cloning the gene involved will be discussed. Secondly we have examined the expression patterns of many genes which act in the inositol-trisphosphate and cyclic-nucleotide pathways, candidate pathways for olfactory signal transduction, and the results of our expression studies and the phenotypic characterisation of several mutations will be discussed.

59. Age-Related Expression of Fos in Response to Olfactory Cue after Taste-Potentiated Odor Aversion Retrieval in the Rat: A Behavioural and Immunohistochemical Study

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Rats develop strong aversion to an olfactory cue paired with delayed illness if it is 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 due to its robustness and rapid acquisition. The memory of olfacto-gustatory cues linked to possible toxic food is essential for animal survival. On the other hand, it is well-known that aging have deleterious effects on learning and memory. Thus, our aim was to examine whether the expression of TPOA could be also subject to deleterious influence of aging. Experiments were performed in Sprague Dawley rats aged of 1.5 months (young), 12 months (adult) and 24 months (old). Since animals do not all show similar age-related impairments, rats were also submitted to two other learning paradigms besides TPOA (rapidly acquired olfactory discrimination task and spatial homing board task). We used Fos immunochemistry in order to map the patterns of activation of forebrain areas known to participate in aversive memory such as the basolateral amygdala nucleus (BLA), the entorhinal (EC) and the insular (IC) cortices and structures involved in olfactory memory: piriform cortex (PCx), the dorsal hippocampus (Hipp) and the orbito-frontal cortex (VLO). Both the acquisition and the retrieval of the olfactory discrimination task and the spatial task were impaired in old rats. In contrast, the retrieval of TPOA was increased in old rats compared to the young and adult ones. We found that the number of structures showing a significant increase in Fos expression was reduced with aging. Fos immunochemistry was significantly increase only in the BLA, IC and EC regions whatever the age studied.

60. PN64 Wistar Rats Behave as Adolescents Concerning the Temporal Modulation of Taste Learning

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We previously reported that the time of day acts as a context disrupting the latent inhibition (LI) phenomenon in Wistar rats, using a behavioural procedure that included five habituation days and free intake during conditioning (Manrique et al. 2004. Neurobiol. Learn. Mem. 82:77–80). It has also been demonstrated that the hippocampus plays a role in the temporal context dependency of LI in adult rats (Gallo. 2005. Chem. Senses 30:i160–i161). Unpublished results in our lab suggest that PN64 rats use the change between preexposure and conditioning to modulate the aversive instead of the safe taste memories, in contrast of the results observed by adult rats. However, when the same animals became adults (PN100) they expressed the temporal specificity of LI consistently with previous reports (Manrique et al. 2004. Neurobiol. Learn. Mem. 82:77–80). The results may be explained according to the reported hippocampal dependency of the phenomenon in adult rats by protracted maturational changes of the hippocampal system in late adolescence and early youth.

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61. Expression of the G Protein α-Subtype Gustducin in Mammalian Spermatozoa

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Sperm chemotaxis is a general guidance mechanism of spermatozoa to eggs throughout the animal kingdom. Since the G protein subunit α-gustducin is accepted as a marker of chemosensitive cells, attempts were made to explore whether α-gustducin is also expressed in spermatozoa of different mammalian species. RT-PCR experiments performed with specific primers revealed PCR products which sequences were fully consistent with the reported α-gustducin sequences. To identify the testicular developing cell type in which α-gustducin is expressed, immunohistochemical experiments were performed with an anti-α-gustducin–specific antibody. The most intense immunoreactivity was visible in differentiating spermatids

lining the lumen of the seminiferous tubules whereas no staining was detectable in spermatogonia and primary spermatocytes. To verify whether α-gustducin is also expressed in mature spermatozoa, epididymal mouse and rat sperm were subjected to immunocytochemistry as well as immunogold electron microscopy. An intense staining was obtained within the circumference of the midpiece-localized mitochondria, on the axoneme and the outer dense fibers surrounding the microtubules of this region, whereas no labeling was detectable in the end piece regions. Analyzing ejaculated human sperm for α-gustducin labeling revealed a comparable segmental distribution pattern of α-gustducin expression within the tail. Although a possible function for α-gustducin has yet to be determined, the axonemal-associated localization within the midpiece and principle piece of different mammalian spermatozoa raises the possibility that this G protein α-subunit may process intracellular signals controlling microtubule dynamics and thus sperm motility.

62. Age-Related Changes in the Processing of the Rabbit Mammary Pheromone: Morphological Transformation in the Responses

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Rabbit newborns are governed by olfaction in their daily quest for nipples and milk. One signal carried in milk, the mammary pheromone (MP), turns on a sequence of searching and grasping responses in an ''all-or-none,'' apparently stereotyped mode. Between birth and day (d) 10, over 75% of the pups tested display such responses when exposed to the MP. After d10, the rate and shape of the response sequence changes, to apparently decline around weaning (cf. twin poster by Coureaud et al., this meeting). Here, we focused on the morphological patterning of the responses in growing pups. MP-released responses were systematically videotaped in 64 pups varying in chronological age and in related maturational steps on: d2 (n = 22), when they are exclusively milk-fed and rely mainly on olfaction; d10–11 (n = 25), when still feeding milk and vision sets on; d29 (n = 17), when independence from milk-feeding becomes complete. The behavioural testing consisted in videotaping pup 15 s before/during/after exposure to the MP on a glass-stick. The variables were frequency, latency and duration of sniffing, searching, oral grasping and other related items. The results revealed that 95, 80 and 0% pups searched/grasped the stimulus on d2, 10–11 and 29, respectively. On d2, the mean occurrences per pup of searching and grasping responses to the MP were 3.3 and 16.0. They remained similar on d10–11 for searching (2.5; P = 0.07), but decreased significantly for grasping (10.0; $P <$ 0.01). These results, which analysis will be completed at the time of the meeting, suggest that not only the behavioural activity of the MP to young rabbits declines between birth and weaning, but also that the response morphology changes as a function of sensory maturation, metabolic needs, and social development.

63. Early Expression of Fz Receptors during Mouse Olfactory System Development

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The olfactory system is an attractive model for studying general principles of axon extension and regeneration. Olfactory sensory neurons (OSNs) are broadly distributed in the olfactory epithelium (OE) yet their axons target-specific glomeruli, restricted areas of olfactory bulb (OB) neuropil, with exceptional precision. Despite the fact that OSNs expressing the same olfactory receptor (OR) are intermingled with others, all that express the same OR target only a few glomeruli on each OB. The ORs have been strongly implicated in the targeting of these axons and a variety of trophic and repulsive molecules are found in the olfactory pathway. Nevertheless, a cohesive mechanism of targeting by OSN axons either during embryogenesis or in the adulthood has yet to emerge. Formerly known as morphogens, there is increasing evidence that Wingless-Int (Wnt) molecules, signaling through their Frizzled receptors (Fz) contribute in a variety of processes, such as cell proliferation, migration and the development of neuronal circuits. Using RT-PCR and immunohistochemistry techniques we have previously shown that several Wnt and Fz are expressed at embryonic day 13 (E13), E17 and at early postnatal stages. The aim of the present work was to establish when these molecules start to be expressed during olfactory system development. Fz-1 showed expression as early as E10 (Theiler stage 18) on OSNs cell bodies and, on growing axons and dendrites. By E13 it could be clearly distinguish on the apical knob and nascent cilia. Also Fz-3 expression could be seen from E10.5 (TS19) onwards. These data point towards an important role of Fz receptor molecules during olfactory epithelium development.

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64. Investigation on the Role of Nitric Oxide in Murine Peripheral Olfaction

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The small gaseous signalling molecule nitric oxide (NO) is involved in various physiological processes including regulation of blood pressure, immunocytotoxicity and neurotransmission. In the peripheral olfactory system, NO generated by the neuronal isoform of NO synthase (nNOS) and expressed solely during development and regeneration, seems to regulate neurogenesis in the olfactory epithelium as well as axonal outgrowth of the olfactory receptor neurons. However, an implication of NO in olfactory signal transduction has not been demonstrated yet. Here we show for the first time the expression of the endothelial isoform of NO synthase (eNOS) in mature olfactory sensory neurons (OSNs) of adult mice. We report that in these cells eNOS is able to produce NO in a stimulus-dependent way, thereby affecting the desensitization of odor responses. Immunocytochemistry using eNOS-specific antibodies revealed developmentally regulated expression of the endothelial NOS isoform in adult OSNs. We found that NO was liberated from OSNs in response to odor or depolarization in wild type, but not in eNOS deficient mice, pointing to eNOS being the enzyme responsible for activity-dependent NO production in OSN. Analyzing EOG recordings from wild type and eNOS-deficient mice revealed a significant role for NO in modulation of temporal aspects of olfactory signal processing and desensitization

of odorant-induced signals. In summary, we found evidence for presence and function of eNOS in mammalian olfactory sensory neurons and propose NO as a novel player in olfactory signal transduction. These findings may contribute to the understanding of mechanisms involved in odor adaptation.

65. Transsynaptic Tracing from Dual Injections in the Olfactory Bulb Shows Convergent Connectivity in Granule Cell Columns

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We demonstrated in previous transsynaptic tracing work that the modular organization of the olfactory bulb (OB) glomerular information processing unit extends in a well defined column structure from the glomerulus to the deep granule cell layer. Further, OB injection of the Bartha strain of the pseudorabies virus (PRV) bearing the green fluorescent protein (GFP) showed that the intrabulbar network arising from projection neuron lateral dendrites maintained a medial–lateral preference in a distributed manner. Single label tracing, however, could not show the degree to which the lateral network information converges between glomeruli. Here, we use the GFP expressing strain coinjected with a PRV strain bearing a variant of a red fluorescent protein (mRed1) to show information convergence and divergence in the OB. Distal as well as proximal glomeruli show convergence at the granule cell level. Preliminary results are discussed.

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66. The Physiological Processes of Peripheral Olfactory Coding in *Drosophila melanogaster* Larvae

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The peripheral olfactory system of *Drosophila* larvae consists of just 21 olfactory receptor neurons, each expressing one or two types of functional olfactory receptor. This does not appear to limit the ability of larvae to detect a large number of odours. Indeed electrophysiological studies have yet to find an odour that cannot be detected by these animals. There exists therefore a mechanism of coding at the peripheral level whereby receptors may have affinity for different odorant molecules and odorant molecules may act at a number of different receptor types. Using multiunit electrophysiology it is possible to record from a large subpopulation of the larval olfactory neurons at the same time. Examining the activity of individual neurons within these recording provides information on their specificities, relative specificities and their temporal dynamics. A second approach, using a Gal4-UAS technique, is to conduct the same recordings from larvae in which only 1 of the 21 neurons express functional receptors in it membrane. We therefore have two approaches to examine the neuronal activity of the entire peripheral olfactory system of *Drosophila* larvae. They allow us to demonstrate the specificities and dynamics in activity of individual olfactory receptor neurons, and provide insight into the nature of how combinatorial coding at the peripheral level contributes to the identification of different odours.

67. Olfactory Clefts Obstruction Is Equivalent to Absence of Olfactory Bulbs

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We previously demonstrated that the mucus sampled at the level of the olfactory clefts of normosmic subjects contains specialized proteins to trap, bind and convey odorant molecules through the olfactory mucus. Many physiological aspects of the olfactory clefts remain to be more precisely studied to understand the physiology of this domain and its pathologies. We performed endoscopic, CT scans and MRI observations, in addition to an olfactory test using 5 odorants, on normal subjects, on patients presenting a pathological bilateral obstruction specifically localized in the olfactory clefts, and on patients suffering from congenital anosmia since birth. Sensory data from normal subjects indicated rather large interindividual differences and MRI observations allowed to observe the olfactory bulbs and tracts. Patients with obstructed clefts, but no sign of obstruction in the other parts of the nasal cavities, revealed a sensory deficit similar to the deficit observed with anosmic patients due to absence, or aplasia, of the olfactory bulbs and modifications in the olfactory sulcus and gyrus rectus regions. This study shows that the alteration of the olfactory clefts patency is equivalent to the absence of olfactory bulbs.

68. Mitral and Tufted Cells Have Similar Spontaneous Firing Activities and Odor Response Properties

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In the mammalian olfactory bulb (OB), mitral (MC) and tufted cells (TC) form two morphologically different cell populations. They receive a same input from the olfactory mucosa, but have distinct projection patterns. They differ in the laminar position of their cell bodies, in the projection pattern of their secondary dendrites and axon collaterals within the OB, and in their axonal projection patterns to the olfactory cortex. These morphological differences suggest that MC and TC may be functionally distinct and process different aspects of olfactory information. To address this question, we recorded the spontaneous firing activity and odor-evoked single-unit responses from MC and TC in OB of freely breathing rats. All cells in this study could be antidromically activated from the lateral olfactory tract. Three cell classes were identified based on depth, dye deposits at successful recording sites, the form of the field potential and by electrode track reconstructions: mitral cells (MC), tufted cells situated at the limit of the glomerular layer (TC_GB) and tufted cells recorded in the core of the external plexiform layer (TC_notGB). The three cell types were not found to be different

regarding their impulse conduction velocity, their mean spontaneous firing rate, and their pattern of spontaneous activity with respect to the respiratory cycle. Their responsiveness to 8 odors also were not fundamentally different. Subtle differences were found, however, between TC_GB and the two other cell types. The spontaneous firing discharge of the former tended to be more synchronized with the expiratory phase of the respiratory cycle whereas the latter were more often synchronized with inspiration. TC_notGB cells can be considered electrophysiologically as displaced mitral cells.

69. Learning and Training in Descriptive Analysis of Gin

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The aim of this work was the selection and training of university students to perform the descriptive analysis of sensorial profile of gin. The stages of the study were: selection of the panel, training of the panellists and quantitative calibration of the gin attributes. Fourteen healthy individuals were selected (7 men and 7 women between 24 and 35 years old) by means of a questionnaire which captured the interest and motivation, physiologic situation and dietetic aspects and of data about the consumption of the product. During the training stage, different sessions were realized to identify and memorize the characteristic aroma of gin. Chemical substances and hydroalcoholic extracts of different botanical species used for gin aromatization were used as reference standards with different odour intensities. A vocabulary was developed, whose 5 attributes were statistically selected: juniper, citric, aniseed, liquorice and spices. Six gins were analyzed through an unstructured line scale to define the intensity of the 5 selected attributes. The analysis was performed in wine tasting transparent glasses of characteristics NFV09-110 (AFNOR) of standardized measure. 30 ml of gin were used, normalizing the alcoholic grade to a final concentration of 27% (v/v: ethanol/water) with natural mineral water. The samples were presented and numbered in a randomised order. The 5 reference standards and a card with 5 scales for orthonasal and 5 scales for retronasal perceptions were provided to the subjects. Also, spring water and soda biscuits were supplied for rinsing the mouth between samples. The data produced from panellist evaluation have been investigated by the multivariate analytical techniques and ANOVA. The results obtained showed as the attributes were discriminating and no redundant terms exist in the developed vocabulary.

70. The Proteome of Human Saliva Affected by Taste

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Saliva flow and its inorganic composition are affected by taste. However, very little is known about any effects of tastant on the organic composition of saliva. Any such effects are important in chemosensory science, as they would identify the responses of tastant with the oral tissues and fluids that can influence taste perception. The aim of our work was to investigate effects of tastant concentration and nature on proteome pattern of saliva. Whole and parotid salivas, after tasting weak and strong solutions of each of glucose, inositol monophosphate, calcium nitrate, nitric acid, were obtained from 4 healthy subjects. Salivation, following the stronger solutions, increased from 0.81 g/min after glucose to 1.23 g/min after acid. Salivas were analysed by 2 Dimensional-Electrophoresis MALDI-TOF-Mass Spectrometry. From whole saliva, characteristically different protein patterns were obtained from the different individuals. About 120 protein spots were compared for the 2 solutions of each tastant. The number of spots induced by the stronger tastants varied significantly and was 16, 10, 9 and 2 for the sour, bitter, umami, and sweet tastes, respectively. The types of protein expressed were similar for the different tastants and were, most commonly, annexins and calgranulin. Using a similar procedure, whole and parotid salivas were compared after bitterness stimulation. Ten spots were overexpressed in parotid saliva of which PRH2 protein and carbonic anhydrase VI were identified. Forty-two spots were underexpressed, of which cystatin1, annexin A1, enolase1, IgG1 were identified. As annexins and calgranulin were not overexpressed in parotid saliva, they are thought to originate from stimulation of other oral glands, mucosa or tissues.

71. Interaction of tas1rs with Filamin an Actin Binding Protein

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Taste receptors are considered to be the cornerstones of tastant detection in the oral cavity. In order to study the proteins that interact with these receptors and which could therefore be potential organizers of protein networks at the apical pole of taste receptor cells, we conducted a yeast two-hybrid screen using the carboxy-terminal tails of mTas1Rs and hTas2Rs. Filamin, a protein linking plasma membrane proteins with the actin cytoskeleton and potentially involved in downstream signaling, was found to interact with mTas1R1 and mTas1R3. The interaction was confirmed by affinity pull down assay. Analysis of the residues involved in the interaction showed that mTas1R3 and filamin interact at regions containing Ig-like domain repeats 20–22 of filamin and amino acids 821–826 of mTas1R3 which display a predicted beta-strand secondary structure. These results suggest that in taste receptor cells, taste receptor proteins are organized by scaffolding proteins which may be involved in bringing together receptors and signaling complexes.

72. Peculiar Time of Day Modulation of Taste Learning During Ontogeny

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It has been proposed that the time of day can act as a temporal context in taste learning. Thus, previous results have demonstrated that

a time-of-day change between preexposure and conditioning in a taste aversion task disrupt the latent inhibition (LI) effect in Wistar rats (Manrique et al. 2004. Neurobiol. Learn. Mem. 82:77–80). Those groups preexposed and tested during the evening session but conditioned during the morning one, exhibited strong aversions been similar to nonpreexposed groups, while those preexposed, conditioned and tested at the same time of day showed weaker aversion than nonpreexposed groups, i.e. LI was evident. As it has been reported that the temporal context dependency of LI requires an intact hippocampus (Gallo. 2005. Chem. Senses. 30:i160–i161) and the hippocampal maturation is a protracted process during early development, we have explored the ontogeny of this phenomenon. In three experiments we compared rats with different ages: PN32, PN48, PN64. The evidence obtained showed that a time-of-day change between preexposure and conditioning did not disrupt LI. In contrast, all the groups exhibited a time-of-day modulation of the aversion: those groups tested at the same time of conditioning showed stronger aversion than those tested at different time of day. This phenomenon has been reported in adult rats using a different procedure including shorter habituation and restricted intake during conditioning (Morón et al. 2002. Learn. Mem. 9:218–223) and it does not depend on the hippocampal integrity. Therefore, the evidence seems to support a late onset of the temporal context dependency of LI, which is consistent with the hippocampal system role in the temporal context specificity of LI.

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73. Early Neuronal Migratory Routes throughout the Olfactory Cortex

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During the initial telencephalic development, diverse cell populations migrate tangentially along different pathways to reach their adult final destination. Among those cell populations, some of the earliest migratory routes lead to different parts of the olfactory system, including the prospective olfactory bulb and piriform cortex. To analyze the origin and the different migratory routes using by those cells, we performed different tracer injections, combined with immunohistochemistry, into different parts of the telencephalic germinative ventricular zone in early mouse embryos (E10–12) that were cultured in toto for 24 h. We have found diverse telencephalic germinative areas that give rise to early neurons related with the olfactory system. First, cells originated in the rostral portion of the lateral ganglionic eminence migrate tangentially into the most anterior region of the telencephalon, at the level of the prospective olfactory bulb, coursing superficially through the rostral piriform cortex. Second, some neurons migrated towards the piriform cortex from both the rostral telencephalic area and the rostral telencephalic medial wall, using both directions of the rostro-caudal axis and through the medio-lateral axis of the telencephalon. In summary, we suggest a multiple origin of the first generated olfactory-related neurons in the telencephalon. These cell populations reach their final location using tangential migration and different migratory routes throughout the neocortical preplate.

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74. Dissociation between the Conditions of Flavour–Flavour and Flavour–Calories Learning in Conditioned Flavour Preference

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Conditioned flavour preference occurs after pairing an initially neutral—nonpreferred flavour—with a second flavour which is itself preferred. Two basic contents of learning have been proposed to give account of it. The first is referred to as flavour–flavour learning, and includes the sensory properties of the second flavour which produce hedonic reactions (i.e. flavour–sweetness association). The second is based on the motivational properties of the second flavour, and could be independent of its initial sensory–hedonic properties. When sucrose is used as second flavour, this learning relays on the post consumption consequences (i.e. flavour–calories). No such learning takes place using sodium saccharine. The conditions underpinning both learning contents (flavour–sweetness and flavour–calories) can differ in a number of ways. These experiments using female Wistar rats suggest that for flavour–sweetness learning both moderate solution-concentrations and relatively low number of pairings are optimal conditions, whereas higher concentrations, a larger number of pairings, and the relevant motivational state (i.e. hunger) are important for flavour–calories learning. The results suggest a dissociation between the conditions of the two kinds of learning depending on the amount of training, the intensity of the sensory experience (sweetness), and the motivational state. The results are discussed in terms of the role of the sensory experience and motivation.

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75. Intrabulbar Infusion of Sodium Butyrate, a HDAC Inhibitor Facilitates Olfactory Learning in Young Rats

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It has been reported that epigenetic mechanisms have an important role in synaptic plasticity and memory formation. Particularly as cellular mechanisms, chromatin structure is remodeled by modification of histone acetylation during the early stages of long-term memory formation. In the hippocampus decrease in acetylation of histone is induced by inhibition of the MEK-MAPK/ERK cascade. Preweanling young rats prior to eye opening depend on somatosensory and olfactory function for survival, as they can learn their dam's odor and approach her without visual information. In order to establish olfactory learning, the pairing of odor and somatosensory stimulation is crucial. We have previously shown that synaptic plasticity in the OB underlies aversive olfactory learning. Behavioral pharmacology shows that long term, but not short term, olfactory memory required activation of the transcription factor, CREB. Western blot analyses reveal that expression of P-MAPK/ERK was increased for 1 h after odor-shock training, followed by increase of P-CREB lasting for 6 h. Therefore we examined if intrabulbar infusion of the histone deacetylase (HDAC) inhibitor, sodium butyrate (SB) has a facilitatory effect on aversive olfactory learning in young rats. Animals infused with SB 1 or 2 h

after odor-shock training show aversion at the odor preference test 2 days later, but control rats no longer show aversive responses. These results show that HDAC inhibition consolidates aversive olfactory learning in young rats.

76. Age-Related Changes in the Processing of the Rabbit Mammary Pheromone: Comparison in Domestic and Wild Pups

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In wild as well as in breeding colonies, rabbit females nurse their litter only once/day during the first 2 weeks. The pups' orientation to the nipples depends then on common odorants emitted on the abdomen and potentially on the mammary pheromone (MP) released in milk. Here, we assessed the behavioural activity of the MP from birth through weaning in pups from domestic (D; n = 494) and wild type rabbits (W; n = 142). Independent groups of pups were tested for their searching responses to the MP on days 0, 2, 5 and 8 (period of exclusive milk feeding and reliance on olfaction/somesthesis), 10 (eyes opening), 16 and 22 (initiation of non milk food ingestion), and 28 (weaning onset). It resulted that pup responsiveness was maximal (> 75%) between birth and d8 in both types. From d10, type differences became apparent, searching rate remaining high on d10–16 in D pups (> 60%) but declining more sharply in W pups (40 then 11%). On d22, the response rate was still significant in D pups (40%) but vanished in W pups (0%). Finally, D pups also ceased responding by d28 (3%). Thus, the behavioural activity of the MP peaks during d0–10, i.e. when milk is the only resource of energy/immunity, and when pups are olfactorily driven to reach it. Thereafter, the protective effect of weight gain and the onset of vision tend to relax pup dependence on the MP, which appears completely turned off by d22 in W and d28 in D pups. This difference in responsiveness may correlate with variations in the selective pressures young rabbits of both types have to face: W pups may have to become autonomous feeders earlier than D pups, and they have to disperse. Thus, the weakening of the pheromonal bond may warrant earlier attraction to solid food substrates in the nest, and then around the burrow's entrance.

77. Differential Reaction of Olfactory Axons to Target Tissue: In Vitro Cultures

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The main olfactory system is characterised by a unique topographical organisation; thousands of olfactory sensory neurons (OSN) scattered within the olfactory epithelium (OE) project into a few glomeruli in the olfactory bulb (OB). The mechanisms for establishing the precise wiring pattern, notably for the guidance of axons and for the fine targeting within distinct glomeruli are still elusive. Recent findings suggest that the odorant receptor (OR) protein itself may contribute to targeting processes, but nothing is known about its functional role. To study the outgrowth of olfactory axons we have established in vitro cultures of explants from the OE of prenatal OMP-GFP mice, which allows visualising axons by intrinsic fluorescence. Monitoring the outgrowing fibers in a coculture of explants from the OE and the OB revealed that most of the GFP-positive axons showed a repulsive reaction when contacting neurites originating from the bulb. However, some of the axons fasciculated on their way towards and into the OB explant, where loose neuropil structures and/or dense bundles were formed. In the presence of explants from mesenchyme—located directly beneath the OE—the axons were attracted without making direct contact; as they reached the explant, the axons grew along the tissue border. The use of transgenic mice, which express GFP in distinct receptor-specific OSN, allows to monitor specific axon populations and its interaction with other cells, especially cells with OR proteins in the plasma membrane. For this purpose, several cell lines were used to express various OR-EGFP chimeras in combination with proteins, such as ß2AR, RTP1/2 and Reep1, which are supposed to improve membrane targeting of heterologous expressed OR.

78. Effect of Avermectins on the Olfactory Responses of the Midge *Culicoides imicola*

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The aim of this study was to examine the role of the olfactory system of the midge *Culicoides imicola* as the site of repellency of the antihelminthic avermectins. Incidental observations indicate that treatment with Dectomax or Ivomec (commercial formula of the avermectins doramectin and ivermectin, respectively) protects sheep from infection of bluetongue (BT) viruses. Our electrophysiological data obtained by means of the electroantennogram (EAG) recording technique support the hypothesis that sheep treated with avermectins may be protected from BT virus. In fact, the stimulating effectiveness of both lactic acid and butanone, commonly attracting stimuli for hematophagous insects—and also components of sheep odour—are decreased after addition of avermectins. This hypothesis is confirmed by results obtained after stimulation with the sheep fleece odour. The great decrease registrated on the first day after the treatment is in accord with the chemical characteristics of the avermectins commercial formula (Pfizer, product monograph of dectomax). In fact, these pharmacological compounds show a peak of effect within the first 24 h. Behavioural tests performed according to the procedure detailed by Bhasin show that insects in two choice situations are always more attracted by lactic acid than dectomax. In conclusion, the results reported here show that these antihelminthics have an effect on the olfactory sensitivity of the insect toward the animal host, by reducing the response to those compounds that generally attract the insect, and consequently reduce the possibility to bite the sheep thus transferring the BT virus.

79. Taste Aversion Learning in the Fetal Rat

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A pregnant rat was subjected to vinegar as the conditioned stimulus and 5 min later to oral LiCl as the unconditioned stimulus on embryonic Day 18 (E18). Progenies (12) were tested on vinegar (2%),

coffee (0.5%), saline (0.9%) and saccharin (0.1%) drinking 28 days after the exposure to paired vinegar-LiCl via the mother. On the initial single-choice test there were no significant differences in consumption between vinegar and coffee. Neither there were differences between them on a first double choice test. After a reinstatement treatment (a vinegar, low dose LiCl pairing), a second double choice test was given and the pups, on this occasion, expressed a significant aversion for vinegar. The results of this preliminary study suggest that neonatal associative learning can be acquired and retained to be expressed after birth.

Session 2

Symposium 4: Olfactory Roles of the Insect Mushroom Bodies and its Possible Mammalian Homologue, the Hippocampus

80. Synapse Number and Frequenin Levels in *Drosophila*

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Previous studies show that the number of synapses (N) is a very dynamic feature. Here, we have analyzed the effects of a Ca^{2+} binding protein, Frequenin, on N and its consequences in behavior. Frequenin is represented by two proteins, FRQ1 and FRQ2, encoded in independent genes. Locomotion is a behavior elicited by the optomotor response in the adult and it can be quantified in the so called Buridan's arena. Using the Gal4 > UAS procedure, the levels of FRQs can be modified in the central nervous system either up- or downwards. We find that increased levels of either FRQ 1 or 2 increase the probability of neurotransmitter release as measured in the larval neuromuscular junction (NMJ). In turn, this functional change evokes a more efficient locomotion in the adult as measured in the Buridan's arena. Surprisingly, this behavioral effect results from a reduction of N as measured in the larval NMJ. The same adult behavioral effect was detected when the FRQ1 and 2 levels were reduced by driving the expression of RNAi constructs yielding a 90% reduction of transcription of both genes. Under these attenuated FRQ expression, however, N is not modified, nor the probability of release. We conclude that the FRQ levels, rather than the number of N, are the cause of the behavior effect. These FRQ levels, however, yield the same behavioral effect when they are abnormally high or low. That is, the normal condition must be a window range of FRQ concentrations rather than the FRQ absolute levels. Current experiments try to identify more specific effects of FRQ levels in olfaction.

81. Mushroom Bodies in the Brains of Insects: Intrinsic Circuits and their Modulation by Olfactory Afferents

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The internal architecture of the mushroom bodies is similar across insects and suggests comparison with hippocampal formations in vertebrates. In the mushroom bodies, systems of many thousands of parallel fibers provide local circuits between multisensory afferents and efferent neurons and provide synapses onto systems of feedback neurons. These arrangements are organized within two parallel divisions, called the gamma lobe and the alpha-beta lobe complex. A number of laboratories have shown that these differ with respect to their developmental history and their probable participation in short and long-term memory. Recordings from the mushroom bodies from several different species reveal neurons that respond in a context-dependent manner to multisensory cues, others that anticipate motor actions or register directional change, and others that learn. These data support the notion that mushroom bodies are involved in much more than olfactory processing—a role conventionally ascribed to them—but that in some species olfaction is likely to play the dominant role in context-specific modulation of computations involving other sensory modalities.

82. Spatial Representations in the Brain of the Cockroach

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Spatial memory systems of the cockroach are sophisticated, interacting with multiple sensory modalities and other memory systems. The cockroach is capable of creating spatial maps based on egocentric and allocentric spatial representations using olfactory and visual information. There is an independence of, as well as interaction between, olfactory and visual spatial maps. Evidence suggests allocentric mapping is dominant over egocentric mapping as is visual over olfactory spatial maps. Lesions have identified that spatial memory involves bilateral and/or central processing and must interact with unilateral associative memory systems. A component of this complex memory system has been identified in the mushroom bodies using antibodies raised against phosphorylated calmodulin kinase. Changes in microglomerular synaptic complexes in the calyces correspond to key unilateral associative memory processing.

83. Evolution of Sensory Processing Circuitry in the Mushroom Body Calyx

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Insect mushroom bodies, while all sharing a general groundplan, display evolutionary malleability that reflects both phylogeny and behavioral ecology. The mushroom body calyx, a dedicated input neuropil, is an example. My research suggests that in terrestrial insects, olfactory input to the calyx is highly conserved and represents the largest proportion of sensory innervation, supporting the hypothesis that mushroom bodies function largely in higher olfactory functions. Sensory input from gustatory and visual centers, in contrast, is more variable and reflective of lineage-specific behavioral ecologies. Closer inspection of olfactory circuitry in the mushroom bodies of scarab beetles with generalist vs. specialist feeding ecologies, however, suggests that the processing of olfactory information differs substantially in these two contexts. Functional

synaptic units called microglomeruli are small and spherical in generalist plant-feeding scarabs, being organized around the punctate terminals of olfactory afferents. In contrast, microglomeruli of specialist dung-feeders are broadly ovoid and contain relatively giant afferent synapses. It is proposed that this latter organization facilitates the rapid and reliable transmission of a few salient ôsign stimuliö relevant to the restricted feeding range of dung beetles. In contrast, the former organization may permit finer resolution and integration of multiple stimuli representing the variety of food sources available to the generalist feeder.

84. Spatial Navigation and Entorhinal Grid Cells

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Spatial navigation depends on algorithms that integrate information about position and direction. These algorithms are implemented in a large brain network including both hippocampal and parahippocampal cortices, as indicated by the existence of place cells and head-direction cells in the hippocampus and dorsal presubiculum, respectively. The hub of the network is the medial entorhinal cortex (MEC). Layer II of the MEC contains position-sensitive neurons—grid cells—whose firing fields form a periodic triangular pattern covering the entire local environment. Grid cells were observed in all principal layers of MEC, but intermingled with head direction cells in layers III, V and VI. The two cell types formed a continuous population in which a subset of the neurons, predominantly in layer III and V, had conjunctive grid and head-direction properties. The majority of the cells were modulated by velocity. These results suggest that, despite the differential hippocampal and neocortical connections of the different layers, the area operates as an integrated unit, with significant interaction between grid cells in all principal layers and head-direction cells in layers III to VI. As the animal moves, activity may be translated across the sheet of grid cells in layer II by convergence of direction and velocity information in an afferent population of integrator cells in the deeper layers of the structure.

Symposium 5: Aromas in Spain (Foundational Symposium of the Spanish Network on Olfaction)

85. Neural Plasticity in the Adult Olfactory System: Experimental Models and Adaptative Responses

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The adult olfactory system is a convenient model to study neural plasticity, since it presents a continuous housing of new-generation neurons, involving both reconnection and reorganization phenomena. Moreover, the adult olfactory bulb is a well-known structure where neuronal typology, synaptology and neurochemistry are further detailed. We analyze the genomic, neurochemical and cytoarchitectonic modifications appeared after experimental modifications in the adult olfactory pathway, when all elements are already in place. Some experimental models are mutant mice, the pcd and the SeyDey mice, where mitral cells degenerate at P90 and most of dopaminergic interneurones are gone, respectively. We also surgically occlude the naris impeding the normal information flux through both mitral and tufted cells, the system being sensory deprived. Our results indicate that all models employed give in clear effects on gene expression, neurochemistry and connections, as well as on proliferation, migration and apoptosis rates. Moreover, these variations in the adult olfactory scenario make adaptations in the centrifugal inputs. Some of these changes are variations on the final fate of migrating neuroblasts and an centrifugal afferences reinforcement when mitral cells are lost, or a clear compensation of both serotonergic and glutamatergic receptors after sensory deprivation, aside from a wide neurochemical alteration on the interneuronal network. These data suggest that after elimination or functional inactivation of intrinsic elements within the rodent olfactory system, a large response including plastic changes at different organization levels is generated, probably directed to preserve the sensory functionality.

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86. What Can We Learn from Theoretical Models of the Olfactory System?

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The main objectives of developing theoretical models of the brain are twofold: 1) To explore the functional role of the properties observed by experimentalists; 2) To obtain compact theories able to account for different data, which can then be used to predict the results that might be obtained from new experiments, which can then be used to guide new experimental hypotheses and designs. Here we present two main findings that we have obtained by studying theoretically the olfactory receptor neurons (ORNs). Firstly we show that a population of ORNs with broad receptive fields extracts more information from a highly multidimensional stimulus than an equivalent sized neural population with specific responses, thus providing an explanation for why ORNs have broadly tuned receptive fields. Secondly, we show that simple biophysical arguments related to how odour ligands interact with olfactory receptors lead us naturally to a compact model of multiple ligand–receptor interaction that is able to explain different heterogeneous data published in the literature at the neurophysiological and psychophysical levels. Moreover, the same model predicts a new mode of neural operation where ORNs are able to extract qualitative features of the stimuli independently from their concentration. Finally, we claim that an integrated approach that effectively combines both theoretical and experimental work is crucial for understanding a complex system such as the olfactory pathway.

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87. The Formation of the Rostral Migratory Stream: New Insights in its Molecular Basis

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The subventricular zone of the telencephalon (SVZ) is one of the regions of the brain in which the neurogenesis takes longer. In the embryonic development, the neural precursors generated in the SVZ migrate towards the olfactory bulb (OB), where they will differentiate into interneurons. Neurogenesis in this region is also very important in the first postnatal weeks and it is conserved in adult, always living place to interneurons. This migratory process, from the SVZ towards the OB, forms the rostral migratory stream (RMS). Once RMS is mature, neuronal precursors migrate forming chains and traverse modified astrocytes organised as channels. Some of the molecules that modulate and determine this type of neuronal migration have been studied in the last years: the majority are molecules that act by contact, like the PSA-NCAM or Laminin; another ones are the diffusible molecules, like Slit-2 or Netrin-1. But, molecules involved in the early formation of the RMS (before the chains/channels are formed) remain largely unknown. Recently, one molecule like Anosmin-1, which is expressed in the RMS, has been shown to be involved in the migration of GnRH neurons and oligodendrocytes precursors during development. Among other aspects, we will study the relevance of the FGF-2/Anosmin-1 system in the early formation of the RMS and in the migration process of the neuronal precursors.

88. Projection of the Gr[3]neberg Ganglion to the Mouse Olfactory Bulb

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In mammals, sensory neurons from the main olfactory and vomeronasal systems project their axons to the olfactory bulbs, in the brain. A cluster of 300 neurons, distinct from these two systems, located at the very tip of the mouse nose and called the Gr[3]neberg ganglion, expresses the mature olfactory-sensory neuron-specific marker OMP. The ganglion is already present at birth and is maintained during adult life. Tracing experiments indicate that these neurons target ipsilaterally to a specific set of glomeruli located on the caudal part of the olfactory bulb, and that this connection is necessary for the survival of the ganglion. The location of the glomerular targets correspond to an area previously associated with suckling behaviour. These observations strongly suggest that this peculiar olfactory neuronal population plays a sensory role, possibly linked to chemoperception.

89. Generation and Differentiation of Excitatory and Inhibitory Neurons in the Olfactory Bulb. The Role of IGF-I and BDNF

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Whereas the olfactory bulb (OB) mitral/tufted neurons are generated from local neuroepithelial cells, many OB interneurons arise in the lateral ganglionic eminence (LGE) from precursor cells that express the transcription factors Dlx, Gsh2, and Er81; newborn neurons then migrate to the OB. Whether GABAergic and dopaminergic interneurons are also generated within the embryonic OB has not been studied thoroughly. In contrast to abundant Dlx2 and Gsh2 expression in ganglionic eminences (GE), Dlx2 and Gsh2 proteins are not expressed in the E12.5–E13.5 mouse OB in vivo. We found GABAergic and dopaminergic neurons in the E13.5 OB, in E13.5 OB slices transplanted with EGFP+ −E13.5 OB precursor cells, and in short-term dissociated cultures prepared from the rostral half of E13.5 OB. In OB slices, 31.7% of EGFP+ cells differentiated to GABAergic neurons. In OB cultures, 22% of neurons were GAD+. When transplanted into P5–P7 OB, embryonic OB precursors gave rise to interneurons possessing dendritic spines in close proximity to synaptophysin-positive boutons. Interneurons were also abundant in differentiating OB neural stem cell cultures; the neurons responded to brain-derived neurotrophic factor (BDNF) and expressed presynaptic proteins. In vivo, insulin-like growth factor-I (IGF-I) was a critical factor to regulate neuronal allocation and differentiation in the mitral and periglomerular layers. These findings suggest that, in addition to receiving interneurons from the LGE, the embryonic OB contains molecularly distinct local precursor cells that generate mature GABAergic and dopaminergic neurons. They suggest that IGF-I and BDNF may regulate layer formation and synaptogenesis in the OB.

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90. Clinical Study of Olfaction with CCCRC

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Objectives: To know CCCRC test to measure the sense of smell. To show our experience with CCCRC test in postviral, postraumatic and inflammatory anosmias. Materials y methods: Two olfactory tests, the Connecticut Chemosensory Clinical Research Center (CCCRC) test and University of Pennsylvania Smell Identification Test (UPSIT), have been used throughout the world. We used the CCCRC olfactory test made up of a threshold and suprathreshold test while CC-SIT relies solely upon suprathreshold measurement. We have 300 patients with chronic inflammatory disease that were assessed with CCCRC. Nasal polyposis is the most frequent disease that caused olfactory impairment. Postviral, postraumatic and toxic anosmia are less frequent. Results: For patients with nasal polyposis, the CCCRC: the sensibility was 86%; the specificity was 94%; the positive predictive value was 93% and the negative predictive value was 88%. The reliability was 92%. The unit cost of the CCCRC was 3.2 Ç. Ninety-eight percent of the patients thought that the test was easy to perform. There were not relationship between the extension of nasal polyposis and the total score of the CCCRC. For postviral anosmia, 60% of the patients recovered the sense of smell. The deadline to wait any recovery was 18 months. Conclusions: CCCRC test is a valid test in comparison with UPSIT. CCCRC is inexpensive and can be used in routine clinical settings

because it takes a short time and is easy to perform it. The olfactory impairment in nasal polyposis and postviral anosmia has aspects that need to be clarify.

Symposium 6: Taste and Olfaction: A Systems Approach

91. The Fundamental Role of Ingestive Experience in Determining Brain Response to Chemosensory Stimuli

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Unlike vision, audition and somatosensation, the chemical senses are represented in heteromodal paralimbic cortex that is specialized not only for sensory representation but also for the 1) formation of memories, 2) encoding of internal state and 3) representation of the biological significance of all sensory and motor events. As such, these factors may be expected to influence neural responses to chemosensory stimuli. In this lecture data will be presented from neuroimaging studies conducted in healthy human volunteers, which demonstrate the fundamental role of ingestive experience in determining 1) taste-odor neural integration and 2) brain response to odors presented either via the orthonasal or the retronasal route.

92. The Sense of Smell: Creating a Multidisciplinary Model for Systems Biology

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Systems biology aims at mapping genes onto behavior. For example, the decline in functional olfactory receptor (OR) genes through the primate series is believed to correlate with a weak sense of smell in humans. However, behavioral studies have shown that in primates, including humans, smell perception is surprisingly good. We hypothesize that in human evolution the decline in functional ORs has been offset by an elaboration of brain circuits. Critical to an assessment of this hypothesis is the evidence that odor stimuli are represented by odor maps (''odor images'') as the first step toward odor perception. We review recent studies of the maps, and of the steps in microcircuit processing of the maps in the olfactory bulb, olfactory cortex and orbitofrontal cortex. We next situate the maps as elicited by retronasal smell, and assess their impact on systems throughout the brain involved in assessment of flavor, including perception, emotion, memory and language systems. Finally, if smell has this strong an impact on contemporary human behavior, it must have been a major factor in human evolution. We consider the probable importance of retronasal smell/flavor brain images to ancestral humans in relation to the unique human trait—the shared prepared cooked meal—which is recognized to have played a key role in family bonding; planning for the future; diet selection; storing and sharing foodstuffs and deferring their consumption; and the development of language. These new perspectives should encourage better incorporation of basic research on smell and taste into diet recommendations by nutritionists, and on the role of smell and taste in diet selection in human evolution by anthropologists.

Supported by NIDCD and Human Brain Project.

93. Neural Ensemble Representation of Taste and Appetitive States

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The gustatory system enables animals to detect and discriminate among foods, to select nutritious diets, and to initiate, sustain, and terminate ingestion for the purpose of maintaining an energy balance. The decision to ingest food depends on many factors including its hedonic nature, its familiarity and context, and on the extent of one's hunger or internal state. To understand how this may occur in an awake, behaving animal we have simultaneously measured the activity of ensembles of neurons from different brain areas across the taste feeding pathway, including gustatory and orbitofrontal cortices, the amygdala, hypothalamus and nucleus accumbens. Recordings were performed while an animal is licking for food either transiently to distinguish among tastants and concentrations, or continuously until it is sated. We have found that ensembles of neurons better predict various states (e.g. satiety) than do individual neurons.

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94. Identification of Characteristic Odors of Components in Mixtures after Selective Adaptation: Specific and Dynamic Olfactory Coding

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Humans cannot reliably identify odors of components in mixtures containing more than three constituents, an effect known as mixture suppression. Furthermore, everyday experience has identifiable odors appearing and disappearing from complex ambient mixtures. We studied the effect of selective adaptation on mixture suppression in human subjects (N = 14, mean age 21 years). Characteristic odors of moderately intense, pleasant-smelling compounds (labels): 5 mM vanillin (vanilla), 1 M isopropyl alcohol (alcohol), 0.5 mM phenethyl alcohol (rose) and 1 mM l-menthol (mint), hidden in mixtures, were more readily identified after presentation of the other mixture components for a few seconds ($P < 0.0001$). This selective adaptation unmasked characteristic odors of each individual compound in 2, 3, or 4 component mixtures. The correct identification of individual mixture components was 56% without adaptation, but decreased to 38% for adapted components and increased to 74% for unadapted components. Cross-adaptation between shared functional groups or other features of the stimulus molecules was not evident. We suggest that these results relate directly to how olfactory qualities are coded. Rather than acting as molecular feature detectors, olfactory receptors may be tuned to recognize materials closely linked with perceived olfactory ônotesö. Rapid, focused activation of a few olfactory receptor types may dominate most odor percepts; adaptation and mixture suppression may promote identification of characteristic odors of chemicals introduced above variable backgrounds.

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95. The Enigmas of Olfaction

F. Montejo Torrell

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This speech begins with an introduction to the new scientific discoveries on olfaction made by Americans Richard Axel and Linda Buck. The introduction is followed by a reviewed vision of the theories that describe the Primary Olfactive Reception, which studies, from the chemoreception point of view, the bonding mechanism of odorous molecules on olfactive receptor proteins. Afterwards both the shape theory and the vibrational theory are explained. The former says that molecules selectively bind themselves to the receptor, like a key fits in its corresponding lock. And the latter, proposed by the biophysicist Luca Turin, claims that olfactive receptors behave like spectroscopes of inelastic electron tunnel effect. Then a series of questions related to olfactive perception and transduction mechanisms are made and possible answers suggested. Finally, the future of the olfactive perception model is discussed, highlighting both the need of going further into the study of the Primary Olfactive Reception with the aim of achieving a definitive description and the importance of the elucidation of the olfactive algorithm, which would enable us to predict the smell of a molecule given its molecular structure.

96. Odor Mixtures: From Receptors to Perception

M.J. Olsson

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How odor mixtures are perceived has been one of the key questions in human olfactory psychophysics for decades. The research has arrived to a number of principles of odorant interaction. With the emerging knowledge of receptor codes, neural pathways and cortical firing patterns in different species, it is increasingly possible to see how perception and neural processing fit together. In the case of mixture perception studies, the intensity and quality resulting from mixing different odorants have been of particular interest. In the case of odorant receptors, the effects of antagonists on specific odorant receptors have been discussed as a possible cause of hypoadditivity phenomena typically observed in odor mixtures. Recently, Zou and Buck showed that merging of the receptor codes of two odorants provides novel combinations of receptor inputs that stimulate cortical neurons beyond those activated by the single odorants, possibly explaining why a new percept can arise from mixing two odorants. These and other phenomena are reviewed and compared with human psychophysical mixture data.

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Oral Session 2: Smell, Life Cycle, and Diseases

97. Olfactory Dysfunction in Welders

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The sense of smell is vulnerable to damage from xenobiotic agents, reflecting, in part, the direct exposure of its receptors to the outside environment. Industrial vapors and dusts, particularly those of heavy metals, are known to adversely alter smell function. In this study, we evaluated the olfactory function of 43 professional welders who worked in poorly ventilated confined spaces for 1–2 years on the San Francisco/Oakland Bay Bridge. Nearly half reported having problems smelling and tasting. Relative to matched controls, the welders exhibited significantly lower scores on the University of Pennsylvania Smell Identification Test (UPSIT); respective means (SEMs) = 29.51 (0.90) and 36.55 (0.88). Eighty-eight percent scored below their individually matched controls. As in idiopathic Parkinson's disease, the olfactory test scores of the welders were unrelated to a broad spectrum of neurological and neuropsychological measures. Although blood levels of Mn were correlated with the time spent working on the bridge, workers with the highest Mn blood levels exhibited better olfactory function than those with the lowest Mn blood levels. The basis of this paradoxical phenomenon, which has been observed previously by others studies, is not clear.

98. Smell Identification and Taste Threshold are Normal in Essential Tremor

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Tremulous Parkinson's disease (PD) is sometimes confused with Essential Tremor (ET). An incorrect diagnosis may result in erroneous therapy, undue patient anxiety and potential legal consequences. Smell and taste are abnormal in PD but may be normal in ET. We wished to determine whether chemosensory measurement is normal in ET and if so this might help to distinguish it from PD. Methods: Three procedures were used: 1) University of Pennsylvania Smell Identification test (UPSIT) 2) Smell Threshold Test (Sensonics, Inc.) using phenyl ethyl alcohol in: i) 50 healthy controls, mean age 49 y (17–93 y). ii) 50 ET patients mean age 62 y (17–82 y). 3) taste threshold measurement with Rion TR-06 electrogustometer applied to a) tip of tongue (chorda tympani; CT) b) base of tongue over the most lateral vallate papilla (VP; IX). All participants scored at least 27/30 on the Mini-Mental Status Test. Results: There were no significant differences in any control vs. ET comparisons as follows (t-test, $P > 0.05$): 1) Mean UPSIT scores between controls and ET (32.8/40 vs. 32.3/40. 2) mean control smell thresholds: −6.7 v/v compared to −6.3 v/v for ET. 3) Mean taste threshold: controls CT: 11.2 dB; VP: 13.5 dB. ET CT: 13.5 dB and VP 14.6 dB. Conclusions: Smell identification and taste threshold are normal in ET compared to healthy controls. This information may be of value in distinguishing ET from tremulous PD patients.

99. Impaired Smell and Taste in Parkinson's Disease

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Objective: To evaluate the degree of smell and taste impairment in PD and to see whether taste measurement might be a useful

diagnostic test. Background: The earliest changes in Parkinson's Disease (PD) occur in the olfactory bulb and dorsal vagal/glossopharyngeal nuclear complex. We wished to measure the prevalence of dysgeusia compared to healthy controls. Methods: 1) University of Pennsylvania Smell Identification test (UPSIT) 2) taste threshold with a Rion TR-06 electrogustometer using a sterile stainless steel electrode applied to either side of a) tongue tip, chorda tympani (CT;VII), b) over the lateral-most circumvallate papilla (VP; IX) in the following groups: i) 44 Controls, mean age 50 y (17–93 y). ii) 68 PD patients, mean age 65 y (48–84 y) of whom 15 (22%) were taking levodopa preparations which might impair taste. All participants scored 27/30 or more on the Mini-Mental State Examination. Results: a) UPSIT: mean scores out of 40 were significantly worse (paired *t*-tests) in the PD group: Controls 33 (normal); PD 19 b) Taste: mean thresholds were significantly worse (paired *t*-tests) for anterior but not posterior tongue regions: Control mean threshold for CT: 10.7 dB and for VP 13 dB. Patients' mean threshold for CT: 17.2 dB and VP 15.5 dB. Six patients had no recordable response from the vallate papillae. Conclusion: Smell identification and anterior & posterior tongue taste threshold measurements were significantly abnormal in PD. Assessment of smell and taste may be of value in confirming a diagnosis of PD particularly in its early phase and in identifying relatives at risk particularly in familial form of PD.

100. Does Pregnancy Affect Olfaction?

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Although there is considerable anecdotal evidence to suggest that pregnancy affects olfactory sensitivity, scientific evidence is inconsistent. Whereas hedonic ratings appear to be affected by pregnancy, odor identification on a restricted range of odors does not. We tested 100 nonsmoking women 18 to 44 years of age. Sixty pregnant women (20 in each trimester), 20 recently postpartum women and 20 aged matched controls completed the 40-item UPSIT. Intensity ratings and number of scratches were collected as indicators of sensitivity, and participants rated the pleasantness of each odor. Participants also rated their own sense of smell. Mean UPSIT scores did not differ significantly across groups indicating no difference in odor identification for a large range of odors, although item-by-item analyses identified menthol and watermelon as identified more accurately by pregnant women. Trends of planned comparisons suggested that women in their 1st trimester of pregnancy rated odors as being more intense and less pleasant ($P = 0.06$), and that they scratched odor strips less often ($P = 0.02$). Consistent with previous reports, 90% of pregnant women reported that specific odors smell less pleasant (e.g., meat, smoke and body odor) and 60% reported that some odors smell more pleasant (e.g., fruit). Although two thirds of pregnant women rated their olfactory sensitivity to be enhanced during pregnancy and overall pregnant women's self-rated olfactory sensitivity was higher than control's, self-ratings were not correlated with behavioral measures of olfaction. These results suggest that previous findings may reflect the fact that pregnancy effects are small and variable. Moreover, they raise interesting questions about the relationship between self-report and behavioral measures.

101. Identification of Odors as Affected by Aging: Normative Data and Priming Effects

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The first study investigated cued-odor identification performance of a set of 32 edible and 32 nonedible natural common odors in a group of young adults and two groups of older healthy Spanish participants. The results showed that 49 out of 64 odors were correctly identified by over 70% of the participants in all groups. Young and elderly young perceivers odor identification performance did not differ. However, the oldest group showed a significant decline in odor identification performance. The normative data obtained in this study were used in selecting the stimuli for an olfactory priming study in which participated two different young and older adults groups. The results of this study showed two main findings: 1) olfactory priming was preserved with age; and 2) olfactory priming is resistant to the passage of time as it was still was present after a month from the first study episode.

102. A Familiar Smell Can Modulate Emotions in Menopausal Women

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Physical and physiological changes that accompany menopause affect the welfare and mood of women. In the present study, we familiarized with a pleasant smell, menopausal women who had difficulties in experiencing pleasure and we assessed whether that familiarization may improve their mood and emotions using behavioral and physiological tools. Forty-eight 55–65 years old women were involved in a between-subjects design whereby 24 of them applied a skin care product containing a pleasant smell (test group) and 24 applied the same product but unscented (control group). We selected women having difficulties in experiencing pleasure (anhedonia scores between 9 and 30). Both groups used the skin care product at home daily for 5 days and filled in mood and emotions questionnaires before and after the daily care. After 1 week familiarization with the product, subjects came to the laboratory for another mood evaluation and for physiological recordings while exposed to the pleasant smell (S1) contained in the scented product and another pleasant smell (S2) used as control. The use of scented skin care product for 1 week induced long lasting effects on mood: decrease in annoyance ($P < 0.04$), disgust ($P < 0.05$), interest ($P < 0.01$) and stress ($P < 0.03$) in the test group vs. control group. Short-term effects (daily evaluation during the week of application) were as well observed in the test group (decrease in anxiety ($P < 0.02$) and fear ($P < 0.03$)). These effects on mood were associated with an increase in facial zygomatic activity specifically in response to S1 in the test group ($P < 0.03$). Taken together, these results suggest that the pleasant smell of a cosmetic product contributes to the well-being in a population of menopausal women who are less prone to experience pleasure.

Panel Discussion 3: Gas Sensor, Odour Sensor and Biosensor

103. DNA-Coated Nanotubes as Versatile Chemical Sensors for Artificial Olfaction and Taste

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Artificial olfaction and taste are poised to make important contributions to medical diagnosis, military ordinance detection and industrial process control. Much recent work has focused on the development of sensor arrays comprised of nano-scale, low-power devices that provide sensitive and diverse chemical sensing cheaply and robustly. Field-effect transistors with organic semiconductors have many attractive properties for these applications. We have recently shown that DNA-decorated carbon nanotube field-effect transistors also have selective odor responses that depend on the base sequence of the single-stranded DNA used to decorate the carbon nanotube and the chemical nature of the applied odor. If arrays of hundreds of sensors having diverse sensitivities to many classes of odorants can be developed, then implementation of new algorithms for pattern recognition able to detect weak known odors in a background of strong unknown odors becomes possible. The ability to readily engineer DNA polymers in conjunction with the development of fundamental understanding of odor-elicited structural changes in DNA bound to the graphene surface of a carbon nanotube will contribute to development of sensor arrays with requisite size, sensitivity and diversity to test this idea. Molecular modeling of odorant–DNA interactions on the nanotube surface are beginning to make testable predictions regarding charge redistributions underlying odorant-elicited transconductance changes. Since carbon nanotube based field-effect transistors continue to function in aqueous environments, these devices are also suitable for development of electronic tongues or more narrowly focused sensors for medically important aqueous analytes.

104. Environmental and Industrial Applications for Tailored-Array Electronic Noses

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E-noses deliver real time, continuous monitoring of odours, specific to an industrial application. We have tailored e-noses for two large industries in Australia: sewage treatment and meat processing. The devices have sufficient sensitivity for offensive odours detectable by the neighbouring community and can discriminate odours from various sources on the industrial sites. The metal oxide sensors are selected after conventional chemical analysis of air samples from the site. The electronic architecture allows rapid development of site-specific e-noses. The devices are robust over a wide range of temperatures and humidity in high indoor and low outdoor odour conditions. A predictive alarm system was developed, as well as an automatic calibration and cleaning system for the sensors. The devices can record in two ''modes'': a continuous stream of data (''monitor mode'') or in ''sniff mode'': at programmed intervals, alternating between ambient air and carbon filtered air. Sniffs allow finer analysis and discrimination of odours and their duration. Data from known sources on site, feed into a statistical model of the odour identity. These tailored-array devices can be deployed as independent units or as multiunit systems to report, say, odour status along a boundary or to map the dispersal of odour over a wide area. Their value to site owners is the timely action that can be taken to avert complaints from neighbours and minimise risks of litigation and enforced costs. Efficient control of odour abatement equipment, with associated cost savings adds further value. The devices are now being used in research for ''flock management'' of sheep in Australia (see The Australian Sheep CRC E-Sheep Project, http://www.sheep.crc.org.au and ChemoSense, 2006, 8(2):10–11).

105. Olfactory Receptors in Immobilized Nanosomes: Molecular Mechanisms of Odorant Detection and Novel Concept for Bioelectronic Nose

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The perception and discrimination of odorants are essential for the survival of living species and are thoroughly investigated by biologists and chemists in order to understand the molecular mechanisms of olfactory signalization. An OR and an appropriate G protein were co-expressed in S. cerevisiae cells under optimized experimental conditions. OR functional response was tested in whole yeast cells using luciferase as a bioluminescent reporter. Membrane nanosomes were prepared by mechanical disruption and sonication of the membrane fraction. By Surface Plasmon Resonance, we were able to quantitatively evaluate OR stimulation by an odorant through Golf protein uncoupling, in nanosomes immobilized on a sensor chip. We demonstrate that ORs in nanosomes retain their specificity and selectivity towards odorants, as in whole cells. This assay also provides the possibility for quantitative assessment of the coupling efficiency of the OR with different G subunits, without the interference of the cellular transduction pathway. Our findings will be useful to develop a new generation of electronic noses for rapid and noninvasive detection and discrimination of volatile compounds, particularly amenable to micro- and nano-sensors formats.

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106. 1986–2006: State of the Art of 20 Years of Research on Electronic Noses Carried Out under European Union Programmes

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In 1986, was first published in Nature the concept of ''electronic nose,'' i.e. multichemical gas sensors devices coupled to pattern recognition techniques. In the following years, a rapid commercial development of e-noses was observed to the extend to count in 2000 more than 20 companies marketing this kind of apparatus. Metal Oxide Sensors (MOS), organic Conducting Polymers gas Sensors (CPS), Bulk Acoustic Wave sensors (BAW) or Quartz crystal MicroBalance sensors (QMB), Surface Acoustic Waves sensors (SAW) and more recently Mass Spectrometry (MS) were the sensing technologies developed or adapted for electronic noses devices and majorically patented. If classic algorithms (PCA, FDA, PLS,···) are generally integrated in commercial systems for off-line data statistic processing, specially designed Artificial Neural Networks (ANN) or Fuzzy Logic (FL) algorithms were also developed for on-line applications. A large part of theses developments were carried out in the framework of national or international programmes associating industrials and universitary researchers. Since the 1st RTD Framework Programme in 1986, a lot of projects were financially supported by the European Union (EU) with the objectives to develop and conceive new sensors or novel devices for generic or dedicated applications. The present paper reports under a ''reader digest'' form the results of a technological monitoring survey on R&D projects on electronic noses and smart gas sensors carried in the framework of European projects since the last 20 years.

Firmenich Award Lecture

107. Functional MRI of Taste and Smell Across the Lifespan: Towards the Big Picture

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After more than a decade spent investigating the senses of taste and smell with functional Magnetic Resonance Imaging (fMRI), what picture of the human flavor perception can we draw? The first experiments on human taste perception with functional MRI showed the existence of functionally distinct areas in the insular lobe, in agreement with the notion of the dorsal insula as receiving primary gustatory projections, and of the ventral insula as a higher order cortical area. Studies combining taste and lingual somatosensory stimuli further suggested a specific role of the Rolandic operculum in coding the somatosensory aspect of flavor, and of the left ventral insula for pure taste. Understanding the modification with age of the smell component of flavor is critical to our understanding of feeding behavior across the lifespan. Another dimension of flavor was investigated through studies performed with retronasally presented odors on young and elderly populations. These studies showed a significant reduction of activation in the primary olfactory regions of elderly subjects. Finally, new protocols are developed to investigate areas of interest, such as event-related fMRI paradigms that allow the presentation of multiple tastants to the subject in a random fashion.

Poster Session 3

108. Speed–Accuracy Tradeoff in Olfaction

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Does a mouse use more time to solve harder odor discrimination problems? To resolve a controversy between results obtained in the reaction-time experiments by Uchida and Mainen (2003. Nat. Neurosci. 6:1224–1229), and Abraham, Spors et al. (2004. Neuron 44:865–876), we developed a new behavioral paradigm, in which we control the time of the mouse's odor exposure. The mouse is trained using a two-alternative choice odor discrimination paradigm, in which timing of odor exposure is controlled by latency of an auditory signal. The mouse is trained to keep its nose in the odor sampling port during odor delivery until an auditory signal indicates the availability of water reward in one of two water ports, while odor stimulus indicates whether reward is available in left or right water port. The complexity of the task is varied by presenting pairs of odorant mixtures with different similarities between the members of each pair. We measured the discrimination accuracy as a function of the time of odor exposure and the difficulty of the discrimination task. We found that longer enforced odor exposure (from 200–1200 ms) leads to more accurate odor discrimination (from 60% to 95%), even beyond the level at which mice perform voluntarily, in the reaction-time paradigm developed for rats by Uchida and Mainen. Mouse performance when the animal controls odor sampling duration is therefore not optimal and may be determined by other behavioral factors. Longer odor exposures are needed to obtain the same level of odor discrimination accuracy for harder tasks. We conclude that, as in other sensory modalities, the accuracy of odor processing crucially depends on both duration of stimulus exposure and difficulty of the task.

109. Olfactory Hypersensitivity in Migraine Patients: A H2(15)O-PET Study

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Olfaction has been poorly explored in migraine disease. Olfactory hypersensitivity (OHS) may occur during attacks and seems to be specific of migraine. OHS is also observed between attacks and is associated with the presence of odor-triggered migraine and an impaired hedonicity judgment of odors. The physiopathology of OHS remains unknown. The aim of our study was to investigate whether activation patterns specifically associated with and without olfactory stimulation are modified in migraine patients with OHS when compared with control subjects (CS). Eleven migraine patients and twelve CS participated in a H2(15)O-PET study. Six scans were

performed including 3 scans in which odors were delivered, and 3 scans without odors. Three different sets of 20 odorants each were used. Regional cerebral blood flow measured during olfactory condition was compared with that obtained for odorless baseline condition. Random-effect analyses were performed to compare differences between groups (patients vs. controls) and olfactory conditions (odor vs. no odor). As in CS, olfactory stimulation significantly induced more activation in the piriform cortex (PC) in migraine patients, suggesting that odors were well perceived by all subjects. However, patients further showed a hyperactivation in the left PC in both olfactory conditions when compared with CS. This result seemed specific, since no similar hyperactivation was observed in the right PC and in both amygdalae. We suggest that hyperactivation found in the left PC in patients could be associated with OHS. Furthermore, areas as the anterior insula and the inferior frontal gyrus were not activated in patients during olfactory stimulation, also suggesting an alteration of olfactory processing in secondary areas in these patients.

110. Labelling, Identification, and Recognition of Wine-Relevant Odorants in Expert Sommeliers, Intermediates, and Untrained Wine Drinkers

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This study examines verbal description, identification and recognition of wine-relevant odorants and identification and recognition of common odorants as a function of domain-specific expertise. Four groups participated: 12 untrained wine drinkers, 12 second and 12 third level students attending sommelier classes, 12 professional sommeliers. Participants were required: 1) to smell 10 wine-relevant odorants and to try to describe each of them; 2) to recognize and to identify each of them, and 3) to recognize and to identify 10 common odorants. Results showed superior olfactory identification of wine-relevant odorants by Sommelier and untrained controls compared to second and third level of intermediates. No difference among groups were observed for the identification of the common odorants and for the recognition of both wine-relevant and common odorants. Sommelier exhibited superior linguistic ability, related to wine-relevant odorants. Our data do not fit the concept of verbal overshadowing. Accordingly whether a domain-specific perceptual expertise, but not a verbal expertise, is well developed this has a detrimental effect on recognition tasks if people are forced to name the stimuli. At odds our intermediates (with a perceptual expertise exceeding their verbal expertise) exhibited good recognition memory as well as the other groups. Probably, an intratask interference effect took place, since the not well developed verbal expertise of intermediates interfered only with the identification of wine-relevant odorants. On the contrary expert Sommeliers (with well developed verbal and perceptual expertise) and untrained control (with absent or modest verbal and perceptual expertise) were protected by interference.

111. Effect of Wine-Tasting Course on Odorant Identification Ability

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In wine-tasting courses the students are assumed to learn to recognize odorant notes related either to the specificity of wines or ''off-odours''. Most of the defects are represented by simple chemicals (like chloroanisoles for the characteristic musty taint), and during the training, aqueous solution of a given molecule was presented no more than 3 times. In addition, they can appear in wines from different origins, with uncontrolled frequencies. In order to measure the effect of the training on olfactory abilities of the students, we tested the changes of performances of the subjects to identify a series of learned odorants and control odorants not introduced in the training course. The procedure can be used to evaluate the training or to measure the individual performances. They can also be regarded as a test of the perceptual learning generalization.

112. NMDA Receptors and Neuronal Nitric Oxide Synthase (NNOS) in the Rat Amygdala Modulate Conditioned Odor Aversion

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Organisms are able to distinguish between nutritive and noxious edibles based on chemosensory information, namely odor and taste (i.e., flavor). Animals readily learn to avoid a novel flavor if they experience visceral malaise after its ingestion. While conditioned taste aversion (CTA) has been extensively studied, the mechanisms underlying conditioned odor aversion (COA) are less understood. The glutamatergic and nitrergic neurotransmission systems play a critical role in neuronal plasticity as well as in a wide variety of learning tasks. The present study was aimed at exploring the role of the NMDA receptor–dependent neuronal nitric oxide synthase (nNOS) activation on COA. Adult male Wistar rats were implanted with permanent guide cannulae. After recovery, rats were deprived of water for 24 h. The next 2 days rats were habituated to drink water in a conditioning box for 10 min; on Day 3, they were exposed to vanilla scent while drinking; one day later (acquisition) rats were exposed to almond scent and i.p. injected with LiCl (0.15 M). On Day 5, rats received water again to reestablish baseline drinking, and on Day 6, retention was tested by comparing the preference between vanilla and almond. In the first experiment we evaluated the effect of NMDA receptor antagonist AP4 on COA during the acquisition, consolidation and retrieval phases. In the second, the effects of L-NAME or 7-NINA (a selective nNOS inhibitor) during the same learning phases were tested. Both, NMDA and nNOS blockade disrupted acquisition and early memory consolidation (first 15 min) of COA, without affecting its retrieval. We concluded that the NMDA-dependent nNOS activation mechanism plays a key role in olfactory memory formation.

113. The Recognition Threshold of Taste is Increased with Anosmics

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Some anosmics report on tasting sweet, sour, salty or bitter worse than in former healthy days. Is that self-estimation correct or a confusion of terminology? We tested 39 anosmics (24 male, 15 female;

age range 18–82 years, average: 51 years) with help of the 3-drop technique according to Henkin. The subject had to recognize twice the drop containing the stimulus in ascending manner. The same procedure was done with smell-healthy persons matched by age and sex. The results were compared. All anosmics recognized each taste quality only in higher concentration. Male and female anosmics didn't taste differently, the same did younger compared with older anosmics. But younger anosmics (n = 20) had in opposite to the older ones a poorer recognition threshold than their healthy control group. The different duration of anosmia seems to have no influence on the gustatory function. All anosmics had an increased recognition threshold for the four taste qualities. The cause is unknown. The acquired taste odour memory trained permanently during eating and drinking can not be recalled by anosmics. One might suggest that pure gustatory stimuli have to be stronger to become aware.

114. Is the Composition of Aroma Compounds in Human Milk More Diverse than in Milk Formulas?

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A new-born infant is exposed to different flavours by feeding practices and differences in flavour exposure between breast milk and instant milk may be expected. Flavour exposures probably influence the infant's acceptance pattern of novel foods at weaning. Therefore, differences in milk feeding practices may to some extend explain why breast-fed infants apparently accept novel foods more readily than formula-fed infants. Differences in early infant feeding may also contribute to the understanding of why formula-fed infants in some studies have a higher prevalence of childhood obesity compared to breast-fed infants. The present study aims to compare the odorant composition and variation of odorants in infant formula and breast milk. Breast milk samples are obtained from 5 women, who express approximately 20 ml of breast milk before lunch and 2, 4, 6 and 8 h after. A weighted diet recording is performed over the test day. All women complete three test days allowing for intra- and interindividual comparisons of the aroma volatiles. Formula milk brands available on the Danish market are analysed and compared with breast milk. The volatile aroma compounds in milk samples are determined by dynamic headspace sampling. The temperature of milk samples is equilibrated for 30 min in a 35 ˚C water bath and a 10 ml sample is purged with nitrogen (100 ml/min) for 45 min under magnetic stirring (200 rpm). Volatiles are collected by Tenax traps and thermally desorbed to a GC column with subsequent separation, identification and quantification by GC–MS. Comparison of odorant composition of breast milk and formulas will explore if breast milk contains a more diverse and variable range of volatiles than formula milk. Results will be presented at the conference.

115. Olfactory Learning and Homeostatic Plasticity

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N-methyl-D-aspartate (NMDA) receptors play a key role in the synaptic plasticity that underlies learning and memory. The plasticity exhibited by NMDA receptors can also lead to the specific cell death referred to as excitotoxicity. Amidst the conflicting perspectives, adaptive mechanisms presumably have evolved to help promote beneficial synaptic changes and reduce runaway excitation and excitotoxicity. Indeed, in neuronal circuits, when synaptic activity is enhanced beyond a normal level of activity, several forms of feedback mechanisms are engaged to return the system to its functional range. However, little is known about a feed-forward mechanism to achieve synaptic potentiation while preventing hyperexcitability. We have studied a simple memory system, in which female mice learn to recognize the urinary pheromones of the male with which they mate. The acquisition of pheromonal learning requires the association of the pheromonal signal and the mating signal, which is conveyed by noradrenergic projections, at the accessory olfactory bulb, the first relay in the vomeronasal system. It is associated with neural changes at the reciprocal dendrodendritic synapses between mitral and granule cells. In this study, we show a strategy employed by noradrenaline (NA) to gate NMDA receptor-dependent long-term potentiation (LTP) while counting the bad process. NA facilitates the induction of LTP at the mitral to granule cell synapse by enforcing high-fidelity synaptic transmission. This ability of NA stems from a decrease in glutamate release via activation of presynaptic alpha2-adrenergic receptors and a subsequent reduction in a postsynaptic depolarizing plateau during theta frequency stimulation.

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116. Localization of TRPV 2 in the Axon of Mouse Olfactory Cells

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TRPV2, a member of the transient receptor potential family, has been isolated as capsaicin-receptor homolog and is thought to respond to noxious heat. Here, we show that TRPV2 mRNA is expressed in GAP43-positive immature and OMP-positive mature olfactory sensory neurons. Intensive TRPV2 immunostaining was observed at the olfactory axon bundles in olfactory mucosa. TRPV2-positive labeling was preferentially found in the olfactory nerve layer in the olfactory bulb. Furthermore, we demonstrated that cell bodies of olfactory sensory neurons settled in cell layer expressing TRPV2 mRNA are insulin-like growth factor (IGF)-I receptor immunopositive. The increase in intracellular calcium levels in olfactory neurons isolated from adult olfactory mucosa was found to be induced by the application of IGF-I, and was not observed in the presence of SKF96365, an inhibitor of TRPV2. In embryonic stages, TRPV2 immunoreactivity was observed on axon bundles of developing olfactory neurons in the nasal region starting from 12.5 days of gestation and through fetal development. Observations in this study indicate that TRPV2 localizes to growing olfactory axons and contributes to the elevation of intracellular calcium levels in olfactory neurons in response to IGF-I.

117. Repetitive Exposure Could Elevate Odor Pleasantness

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Odor pleasantness is affected by various factors such as appearance, culture or experience. In this study, we investigated mere exposure effect in olfaction, especially influence of the number of exposure to odors on pleasantness judgments. The materials were 12 spice odors that were unfamiliar and neutral for Japanese students. In the exposure session, half of the 12 odors were presented in pairs to healthy 22 participants. Two of the 6 odors were presented 10 times, other 2 were 5 times and another 2 were 1 time. The participants were asked to smell each pair and to select stronger odor. In the judgment session, 6 exposed odors and 6 novel odors were presented in a random order. The participants were asked to smell the odors and to judge their pleasantness, familiarity, and perceived intensity. In the recognition session, the participants smelled those 12 odors again, and judged whether each odor had been presented at the exposure session or not. As the results, only the odors smelled 5 times were significantly preferred to the novel odors. Ten times or 1 time presentations could not affect odor pleasantness. Intensity and familiarity judgments showed no significant differences among 4 conditions (10, 5, 1 time exposures and no exposure). The recognition performance of 10 times and 5 times presented odors were above the chance level. These results suggested that appropriate number of exposure to odors could elevate their pleasantness.

118. FMRI of Gustatory Processing—Analysis of Individual Patterns of Activation

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Aim of the present FMRI-based study was to investigate individual differences in the central nervous processing of gustatory stimuli. The perspective was to use this technique in the clinical evaluation of patients with gustatory loss. A total of 12 volunteers participated (6 male, 6 female; age range 21–51 years; all right handed). Normal gustatory/olfactory function was ascertained in all subjects. Gustatory stimulation was performed using dedicated Teflon® tubing ending in a delivery device placed on the subject's tongue, held in place by the lips and front teeth. Within the context of a block design (gustatory stimulation, rinse, stimulation with water, rinse) subjects received sweet (sucrose) and salty (NaCl) liquid gustatory stimuli. Analysis was performed by means of SPM2. While all subjects clearly perceived the different gustatory stimuli during the course of the sessions, FMRI activation exhibited considerable intraindividual differences. Contrary to expectations only 2 of the 12 subjects exhibited activation in the insular cortex. In a much more consistent manner, however, 8 of the 12 subjects exhibited activation in the postcentral area, possibly indicating interactions between gustatory and somatosensory sensations. While more research is needed, the present data indicate that gustatory FMRI produces inconsistent patterns of activation which may be difficult to interpret in terms of the individual evaluation of patients with gustatory dysfunction. Apart from these preliminary conclusions, the present data also suggest an interaction between the somatosensory and gustatory modalities.

119. Chemosensory Event-Related Potentials During Sleep

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Little is known about olfactory responses during sleep. Aim of the study was to investigate whether cortically generated chemosensory event-related potentials (ERP) can be recorded during sleep. Chemosensory function during sleep was assessed in 15 healthy female volunteers. An overnight polysomnography was performed to assess nocturnal sleep and to classify sleep stages. Simultaneously, chemosensory ERP were recorded using air-dilution olfactometry. H_2S (4 ppm) was used for olfactory and CO_2 (40% v/v) for trigeminal stimulation. Chemosensory ERP could be recorded during sleep for both olfactory and trigeminal stimuli although ERP were not consistently present at all sleep stages. Compared to baseline, latencies of olfactory ERP were longer and amplitudes were larger during light sleep and slow wave sleep. For trigeminal stimulation N1 latencies were longest during REM sleep compared to baseline. In this study we were able to demonstrate that both trigeminal and olfactory ERP can be recorded during sleep. This suggests that chemosensory stimuli are processed on a cortical level during sleep.

120. Study of Olfactory Bulb Time Response by Using Multivariate Image Analysis Tools

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In the study of the functionality of the Olfactory Bulb (OB), the recording of images can be of utility to achieve better comprehension of its mechanisms. This process of image recording is not easy, and specific methodologies must be developed, both from the preparative aspects at a tissue level and from microscopy image recording. The information obtained in this way provides not only spatial information, concerning to the OB parts active during the sensing stimulus. Additionally, it can provide temporal information related with this one, if the data acquisition tool is fast enough. With the development of microscope spectrometers, the use of Data Processing methods commonly used in chemometrics such bilinear and three-way modelling have experimented an increase on its application onto this specific kind of data. The expansion in this field has been followed by the development of more generic image processing applications based on these methods, and their further application to chemical problems. In this work, it is presented the application of data processing methods such Multiway Principal Component Analysis (MPCA) and Multivariate Curve Resolution (MCR) to different series of images, recorded after the stimulation of the OB from frogs (*Rana temporaria*) stained using a voltage-sensitive dye with different odorants. The response of these stimulus was recorded using a fast CCD device attached to a epifluorescent microscope, to follow the response in real time. The results obtained present both techniques as a diagnostic tool to evaluate the temporal evolution of the signal after the

stimulus, to discriminate if there exist different sources of oscillation and if it is possible to relate these sources of oscillation with zones of the image corresponding to glomeruli.

121. Investigation of the "Sniffin' Sticks" Odor Identification Test in Japanese Subjects

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Clinical olfactometry is not standardized all over the world. Several different tests have been developed in different countries. The UPSIT is frequently used in the USA, "Sniffin' Sticks" are used in many European countries, and T&T olfactometry (T&T) is common in Japan. The aim of this study was to investigate the usefulness of the "Sniffin' Sticks" in Japanese subjects. T&T was also performed in parallel with the "Sniffin' Sticks" on the subjects who consulted with complaints of smell disorder. Normosmic Japanese subjects ($n = 200$) were studied using the original and a modified "Sniffin' Sticks" 12-item odor identification test (oriental type) with soy sauce odor replacing the odor of cloves which is rarely known in Japan. These tests (12 common ordants, multiple forced choice from four verbal items per test) were applied to a group of 200 normosmic volunteers (78 male,122 female, mean age 37.7 years, range 12–82 years). Average scores with the standard and modified "Sniffin' Sticks" versions were 9.38 ± 1.43 and 9.53 ± 1.43, respectively ($P < 0.01$). The 50th percentile of the distribution of scores of original tests was 9 and oriental type test was 10. The lowest identification scores for all odors were obtained for liquorice (37%), and leather (45%). Results from "T&T" correlated to results from the "Sniffin' Sticks" (Spearman test, $n = 66$, "T&T" detection threshold vs. "Sniffin' Sticks": rs = −0.51, $P < 0.001$; "T&T" recognition threshold vs. "Sniffin' Sticks": rs = −0.54, $P < 0.001$). It is concluded that the Japanese 50th percentile score was lower than that obtained in Germany. If two or three odors unfamiliar to Japanese people are replaced with more familiar ones, "Sniffin' Sticks" will also become a significant clinical assessment of olfactory performance in Japanese patients.

122. Influence of Learning Parameters on Acquisition of Odor Preferences in Humans

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Whereas some aspects of olfactory hedonism in humans are innate, others are formed during development. Although it is admitted that such hedonic representations emerged from associative learning during the human life, learning parameters that are prominent for such modulation are not yet known. The present study investigated the influence of three learning parameters (the number of trials during learning, learning duration and the route of stimulation during learning) on the acquisition of odor preferences in human adults. Sixty-four human subjects (20–30 years old) randomly assigned to four groups (defining four experiments) were tested. Odors were presented orthonasally in Exp1-2-3 and retronasally in Exp4. All experiments included three sessions. In a first session, participants were asked to rank 8 food odors from the most pleasant to the most unpleasant. In a second session, the most neutral odors (i.e. ranked 4 and 5) were randomly presented in an associative learning procedure with either water (CS−) or with a pleasant sweet solution (CS+). The third session was identical to the first one. In Exp1, CS+ was associated only once with the US. In Exp2 and Exp4, CS+ was associated three times with the US (taste). Results showed that CS+ was significantly more pleasant after learning in Exp2 ($P < 0.05$), but not in Exp1 and Exp4 ($P > 0.05$). In Exp3, CS+ was associated only once with the US but the total duration of learning was identical to that used in Exp2. Results showed no significant modulation of olfactory hedonism ($P > 0.05$). In sum, this study showed that a neutral smell can acquire positive emotional features after being paired with a pleasant taste. This modulation is dependent on the number of associations during learning and the route of stimulation.

123. Olfactory Detection Thresholds in Hunger and Satiety

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There are some published reports in the literature that olfactory performance is related to hunger and satiety. However, contradictory results make a clear statement on the influence of hunger on olfactory sensitivity difficult. The primary aim of this study was to investigate whether olfactory detection thresholds correlate with different states of satiety. Using the Sniffin' Sticks test battery sensitivity to a non-food odor (n-butanol) and a food-related odor (isoamyl acetate) were tested. Twenty-four female subjects with normal olfactory acuity performed the test when hungry (after an overnight fasting) and when satiated (after a breakfast with standardized food). No significant change in olfactory detection threshold for the non-food odor n-butanol ($T(23) = -0.21$, $P = 0.84$), but a significant change in olfactory detection threshold for the food-related odor isoamyl acetate was found ($T(23) = -2.37$, $P = 0.03$). The detection threshold for isoamyl acetate was lower in satiety compared to the hungry condition. The perceived pleasantness of isoamyl acetate was significantly lower in satiety ($T(23) = -2.58$, $P = 0.02$). Subjects' ratings regarding valence, arousal, and alertness as well as intensity ratings of the odors did not reveal any significant difference between the hungry and the satiated condition. In summary, the results indicate that the actual state of satiety has effects on olfactory detection thresholds of food-related odors, but not on olfactory detection thresholds of non-food odors. A higher sensitivity to food-related odors in satiety was detected.

124. On How the Brain Processes Crossmodal Integration of Intranasal Stimuli

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Most odorants, in addition to the olfactory system, also activate the intranasal trigeminal system. It is known that both senses interact, a fact supported by psychophysical and electrophysiological means. Recent studies have shown that pure trigeminal stimulation activate somatosensory circuits as well as brain regions traditionally thought of as primary olfactory areas. The aim of the present study was to compare fMRI activation following stimulation with (1) a relatively selective olfactory stimulant (phenyl ethyl alcohol [PEA]) and trigeminal stimulant (carbon dioxide [CO_2]) with little or no activation of the olfactory system, presented separately and (2) a mixture of both stimuli (CO_2/PEA) presented simultaneously. A total of 15 male subjects (31–59 years) participated in the study. Odors were presented monorhinally to the right nostril in a block design. The control contrast (CO_2/PEA − AIR) revealed areas implicated in the processing of both olfactory and trigeminal stimuli. When the mixture was contrasted with the sum of the single compounds [CO_2/PEA − (CO_2 + PEA)], activations in integration centers (e.g., right superior temporal sulcus) and in secondary olfactory areas (left medial and lateral orbitofrontal cortex) were detected. The opposite contrast [(CO_2 + PEA) − CO_2/PEA] did not reveal any significant activation. In conclusion, a mixed olfactory/trigeminal stimulus appears to lead to higher cortical activations than the sum of its parts. Further, the observed activation reflects an interaction between the two sensory systems.

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125. Developmentally Regulated Expression of OBP (Olfactory Binding Protein) Identified in the Aerial Olfactory System of *Xenopus laevis* and *Xenopus tropicalis*

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Olfactory Binding Proteins (OBP), commonly associated with aerial olfaction, are currently found in mammals olfactory mucus, but have never been identified in fish. It is not clear yet if the absence of OBP in fish olfactory system is due to phylogenetic differences, or due to functional differences linked to adaptation of the olfactory system to an aerial environment. Adult *Xenopus laevis* and *tropicalis* olfactory system are organised into two olfactory chambers, which are thought to be devoted respectively to aquatic and aerial olfaction. This specificity provides us with the opportunity to test this alternative hypothesis. We have identified for the first time an Olfactory Binding Protein in *X. laevis* and *tropicalis*. A reverse transcription and 3′ RACE strategy has been applied and yielded two products, which were cloned and sequenced. These cloned sequences were used to analyse the expression pattern of the gene in the olfactory system of two *Xenopus* species: *X. laevis* and *X. tropicalis*. We showed that in both *X. laevis* and *X. tropicalis*, XOBP transcripts are only present in the aerial chamber supporting the idea that OBPs are an adaptation to aerial olfaction. In this poster, owing to the production of specifics antibodies, we present preliminary results regarding the expression pattern of OBP proteins during development.

Reference: Julie Millery, Loïc Briand, Valérie Bézirard, Florence Blon, Claire Fenech, Laurent Richard-Parpaillon, Brigitte Quennedey, Jean-Claude Pernollet, Jean Gascuel. 2005. Eur J Neurosci 22:1389–1399.

126. Odor Imagery in Noses and Student Experts as Assessed by Event-Related fMRI

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Cerebral imaging studies have demonstrated that visual, auditory or motor mental imagery involves a subset of the regions activated during actual perception. In the present study, we sought to extend this finding to the sense of smell using event-related fMRI. Since the ability to imagine odors is not the privilege of everybody, we tested experts in perfumery. Twenty-five subjects including 11 students of the international school of perfumery (ISIPCA) and 14 Noses known for creating perfumes participated to the experiment. Four neuroimaging sessions (two for odor perception and two for imagery) were performed. Twenty pure odorants commonly used by experts were used and presented twice in each perception session while their names were presented twice in each imagery session. During perception sessions, odors were delivered to subjects from an olfactometer during 20 epochs of 12 s each alternated with 20 epochs of rest. During imagery sessions, the subjects had to imagine the odor whose names were presented and had to indicate at the end of each trial whether they could imagine it or not using a two-key press buttons. Individual statistical analyses were performed using the general linear model with regressors being the canonical hrf and its time derivative using SPM2 software. Individual results were entered in a random-effect analysis for group statistics. Overall, 92.9% of the odors were reported to be imagined. During perception, bilateral activation was found in PC in both groups. During imagery, preliminary results showed bilateral activation in PC in both groups, thus suggesting that neural substrates of odor imagery and perception are overlapping, and that as for Noses, student experts could be rapidly enough very gifted to imagine odors with training.

127. Ca2+-Activated Currents in Vomeronasal Sensory Neurons—A Potential Role in Primary Signal Transduction?

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The mammalian vomeronasal organ (VNO) plays an essential role in the detection of chemosensory cues that convey significant information about gender, status, and individuality. Recent findings provided first insight into the basic molecular mechanisms underlying vomeronasal signaling. Receptor activation in vomeronasal sensory neurons (VSNs) ultimately leads to generation of action potentials and an increase in intracellular Ca2+, presumably via G protein-mediated activation of phospholipase C and generation of the second messenger molecules IP3 and DAG. We recently showed that DAG, in turn, activates a nonselective cation conductance that is critical for membrane depolarization and significantly impaired in mice lacking the TRPC2 ion channel. However, potential roles

of IP3 and/or TRPC2-dependent Ca2+ influx in vomeronasal signaling remain unresolved. Using electrophysiological methods, we studied the occurrence of Ca2+-dependent currents in freshly dissociated mouse VSNs. Defined intracellular Ca2+ increases, established via whole-cell patch-clamp configuration, activated channels of distinct ion permeabilities. All cells tested showed Ca2+-dependent potassium channels. Pharmacological inhibition of these channels revealed cation conductances in about 50% of VSNs. In a small fraction of cells, ionic exchange experiments revealed an additional chloride conductance. To investigate a potential role of Ca2+-activated conductances in primary signal transduction, we applied defined Ca2+ concentrations to the cytosolic side of membrane patches excised from the dendritic endings of VSNs. In a population of these patches we isolated a cationic Ca2+-dependent current. On-going studies will characterize this conductance to reveal its molecular identity.

128. Differential Role of Olfactory Bulb (OB) Muscarinic Acetylcholine Receptor (MAChR) Subtypes in Conditioned Odor Aversion (COA) in the Rat

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Cholinergic innervation to the OB plays a critical role in olfactory learning. It has been shown that nonselective muscarinic receptors antagonists such as scopolamine (Scop) impairs acquisition of odor guided learning in several tasks in the mouse, rat and ewe. However, little is known about the specific participation of MRs subtypes in these processes. The aim of this study was to evaluate the contribution of M1 and M2 MAChR into the OB to COA learning. Male Wistar rats were bilaterally implanted with guide cannulae within the OB for ic injection. Independent groups were used for each manipulation. After 1-week recovery period, they were water deprived during 24 hrs, and then trained for COA as follows: The first day, rats were offered water for ten minutes. On day two and three, they were offered water in the presence of vanilla or almond scent, respectively. On day three, rats were bilaterally injected with Scop 0 (saline control group), 15, 30, 60 mg, pirenzepine (Pir) 0, 15, 30, 60 mg or Gallamine (Gal) 0, 15, 30 mg, 15 min before drinking. Five min after drinking they were ip injected with LiCl 0.15 Mol, 2% body weight. Two days later preference test was performed by offering water in presence of both the scents. Results show that 60 mg Scop and 30 mg Pir induced learning deficits ($P < 0.001$ and $P < 0.05$, respectively), and that the M2 antagonist Gal produced no effects. These data suggest that M1AChRs are critically involved in acquisition of COA.

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129. Detection of Potato Pathogens Using an SPME-Enose

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The risk that pathogens present in imported potatoes may infect indigenous crops has highlighted the need for instrumentation to detect the presence of unwanted pathogens before a shipment is unloaded. Brown rot and ring rot in potatoes are quarantine diseases and are notifiable in the EC. We have pioneered a new approach based on detecting volatile chemicals from infected potatoes. For both brown/ring rot infected tubers disease-specific volatile markers were tentatively identified, but these were of very low concentration and the relative intensities of compounds varied considerably. Due to the complexity of detecting unique markers in the field, a strategy of profiling the entire headspace emitted from potatoes was adopted. For field use a robust system of sampling and measurement was required. A robust method of preconcentrating the volatile chemicals using solid phase microextraction (SPME), as well as instrumentation based on an array of metal oxide semiconductors for detection and classification of infected tubers was developed. This system behaves like an electronic nose (Enose) and is capable of real-time sampling, measurement and recognition. The portable Enose incorporates a system for desorption of potato headspace volatiles from a SPME fibre directly onto the sensor array; together with electronics and software that includes a neural network for online recognition. Testing demonstrates that the system can discriminate each disease from the controls and is not cultivar dependent. The use of a custom-built headspace bar device and conducting polymer coated fibres to discriminate between infected and noninfected tubers is under investigation.

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130. Intraspecific Communication through Chemical Signals in Female Mice: Reinforcing Properties of Nonvolatile Male Sexual Pheromones

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In rodents, intersexual attraction seems mediated by the vomeronasal organ (VNO), which detects nonvolatile chemosignals. In this context, the aim of this work is to demonstrate that, in contrast to other chemosignals used for intraspecific communication, VNO-detected (nonvolatile) male sexual pheromones are rewarding to female mice. To do so, we first compared the dynamics of the chemoinvestigation displayed by female mice towards bedding soiled by other females (F), males (M) and castrated males (CM) and towards the synthetic odorant citralva. Females investigated citralva as much as clean bedding, whereas CM-, F- and M-soiled bedding where intensely explored with a different habituation profile, M-soiled bedding being explored throughout the experiment. Next, we explored the reinforcing properties of bedding soiled by different conspecifics, by checking their ability to induce instrumental place preference. Only bedding soiled by adult, intact males induced place preference. Finally, we analysed the chemoinvestigation of female mice in M-soiled versus CM-soiled bedding two-choice preference tests, as well as the acquisition of place preference after repeated tests of this kind, performed in two different experimental situations. One group of females had full access to the bedding, whereas in a second group access to the nonvolatiles was prevented. Female mice show preference for male-derived, testosterone-dependent, nonvolatile pheromones that induce preference for the location

of the cage where they are presented systematically. This constitutes the first demonstration of the reinforcing properties of nonvolatile (VNO-detected) sexual pheromones in mammals.

131. Expression Of OBP-1F in Rat Olfactory Mucosa Is Regulated by the Nutritional State

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In order to study the relationship between olfaction and nutrition in the rat olfactory system, we developed a mRNA differential display analysis of olfactory mucosa (OM) isolated either from normal fed (NF) or 48 h starved animals (FD) (pool of 4 rats). Almost one hundred potentially regulated molecules were isolated, profiled into functional categories and spotted on a nylon membrane using a ''Virtek'' robot. The resulting macroarray was also enriched in a subset of neurotransmitters. The expression level of these molecules was checked by hybridization with radioactive labelled complex targets of OM isolated either from NF rats (n = 4) or FD rats (n = 4). After a statistical interpretation carried out with ''R'', we highlighted 16 molecules differentially expressed in OM between the two studied nutritional states. One of them, OBP-1F (Odorant binding protein, family 1F), was selected for further characterisation, because of its greatest level of differential expression (more than fivefold decrease in food-deprived rats vs. normal food rats); moreover, this molecule displayed a great interest, because of its broad implication in the olfactory peri-receptor events. Q PCR analysis of OBP-1F expression into OM confirmed our transcriptional data based on macroarray. Finally, we propose that the down regulation of OBP-1F gene expression into starved rats might be correlated to a modulation of olfactory function.

132. Electrophysiological Identification of Mouse Taste Bud Cell Types

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Mammalian taste buds consist of four cell types, type I to IV cells. Type I cells are supportive. Type II cells are taste receptors expressing taste transduction machinery such as G-protein–coupled taste receptors. Only type III cells have chemical synapses with taste nerves within taste buds. Type IV cells are precursors of other cell types. The classification of these taste bud cells (TBCs) requires electron microscopy or the immunohistostaining with cell type–specific marker proteins. Therefore, it has been impossible to identify the cell type of given TBCs while they are electrophysiologically investigated. In the present study, we investigated the relations between the voltage-gated currents of TBCs and their cell types with peeled lingual epithelia of mice containing fungiform papillae. We labeled TBCs electrophysiologically investigated with biocytin, and identified cell types with antibodies against IP3R3 (a type II cell marker) and SNAP-25 (a type III cell marker). Nonimmunoreactive cells to these antibodies were taken as type I or IV cells (type I/IV cells). Among various voltage-gated currents, the magnitude of TEA-insensitive outward currents was significantly larger in type II cells than type I/IV and III cells, though there was no significant difference in the magnitude of total outward currents between type II and III cells. The magnitude of TTX-sensitive currents was significantly smaller in type I/IV cells than type II and III cells. These results enable to estimate the cell type of living TBCs, and to select physiology experiment protocols suitable to the cell type.

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133. Time-of-Day and Discrete Spatial Cues Changes Act as a Context in Taste Recognition Memory

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Previous results in our lab have demonstrated that the time of day may act in a similar way to an spatial context in taste recognition memory (Morón et al. 2002. Learn Mem 9:218–223). A change between preexposure and conditioning interferes with the retrieval of the safe taste memory, reducing the latent inhibition effect (Manrique et al. 2004. Neurobiol Learn Mem 82:77–80). The present experiment explored the ability of discrete spatial cues (burette position) to induce a similar effect. Four groups of Wistar rats were preexposed to a saline solution (0,9%) and subjected to later conditioning (LiCl 0.15 M; 2% b.w.). In two groups conditioning took place either at a different time of day or using a different burette's location inside the home cage than preexposure and testing. Simultaneous temporal and spatial cues changes and control for other features of the previous habituation procedure were added in additional groups. The results showed that those groups subjected to a singular change (time of day or burette location) exhibited similar saline aversions than those subjected to both changes simultaneously. It can be suggested that both the change of time of day and burette location act as a temporal and spatial context respectively using a taste aversion learning task.

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Session 3

Symposium 7: Neural Mechanisms of Taste and Smell

134. Molecular Insight into the Peripheral Events of Human Oral Sensations

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The recent identification of receptor proteins that transduce sensory information from the oral mucosa into gustatory or trigeminal

nerve activity now allows a detailed molecular analysis of their characteristics and provides new insights into several aspects of orosensory coding. We studied candidate human taste and trigeminal receptor proteins in recombinant expression systems and compared their functionality to data derived from isolated receptor cells, and to psychophysical data from human sensory evaluation. Results from an analysis of candidate umami receptors, and a comparison of the findings to studies performed with other gustatory receptors will be described. A comparison with data obtained for the trigeminal cold-sensitive receptor hTRPM8 reveals that for the latter we observed a 1:1 relationship of potency in vitro vs. in vivo for all identified agonists, whereas this might be more difficult to establish for candidate taste receptor ligands. Several factors appear to contribute to this observation and will be discussed.

135. Tracking the Neural Mechanisms of Somato-Sensory and Taste Interactions from Taste Buds to Cortex

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Using fMRI, Cerf et al. showed that taste and somato-sensory stimuli activated the same cortical areas in humans. Activation in Rolandic opercula did not covary with other activated areas in the case of oral somato-sensory information whereas the insular inferior areas did not covary with others in the case of taste: different information is encoded though most neurons integrate both senses. Boucher et al. showed a contribution of the dental trigeminal nerve to the taste signal in rat Nucleus Tractus Solitarius neurons. We show in humans that dental deafferentation (DD) contributes to an elevation of taste thresholds on the tongue in regions close to DD confirming the potentiating role of the trigeminal innervation on taste. By recording both the lingual and the chorda tympani whole nerves simultaneously in the hamster and using taste and somato-sensory stimuli, Lugaz suggested that responses recorded in the gustatory nerve might partly depend on the presence of a simultaneous signal in the lingual nerve and vice versa. Experimental results suggested that these interactions may occur at the peripheral level of 1st order neurons. A further study showed that using mixtures of tastants which, in the human, reduce the irritating effect of capsaicin and, in the rat, turns the avoidance to capsaicin into a preference for the mixture including capsaicin, considerably modifies the response of each nerve: neither the taste nerve nor the lingual nerve respond the same to capsaicin or to capsaicin within the mixture. Whether these modifications of taste and lingual nerve responses depend on neurophysiological interactions (a cross talk between nerve fibres), or from events at the receptor level is an open question.

136. Location and Properties of the Primary Gustatory Area in Humans

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MEG has fine temporal and spatial resolutions for cortical activation with short latency, effective in the localization of the primary sensory cortex. We measured gustatory-evoked magnetic fields and found activations at the transition area between the parietal operculum and insula (area G) and at the bottom of the central sulcus (CS) with the shortest latency (110 ms) for 1 M NaCl, and activation exclusively at area G with 250 ms for saccharin.

By means of fMRI, we measured rCBF changes caused by repeated short duration pulses of a 1 M NaCl solution. This form of stimulation has generally been used for the detection of activities in the primary sensory areas using fMRI or PET in visual, auditory and tactile sensations, although different from the one using long lasting stimuli in previous studies on gustation-induced rCBF changes. Individual analysis revealed activations at area G and frontal operculum in 8 of 11 participants, and CS in 3 in ($P < 0.05$ corrected). Group analysis also revealed significant activations at both area G and CS. The results have good consistency with the findings by MEG. Since it has been reported in rats that trigeminal nerve is activated by 0.4 M NaCl, the question still remains whether taste or trigeminal stimulation resulted in activation of CS in our MEG and fMRI experiments. To answer this question, we used participants with chorda tympani on both sides sectioned in the surgery of bilateral cholesteatoma and measured the magnetic fields evoked by 3 M NaCl presented to the tip of the tongue. We found, however, no remarkable response, which indicates that activation of CS reflected that of taste nerves but not trigeminal one. Thus, it is most likely that CS constitutes one of the primary gustatory cortices.

137. Neural Mechanisms of Taste, Smell, and Flavour

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The orbitofrontal cortex contains the secondary taste and olfactory cortices, in which the reward value of the taste, smell, sight and texture of food in the mouth is represented, as shown by neuronal recordings in macaques. The primary taste cortex combines taste, oral texture, and oral temperature inputs, but not olfactory or visual inputs. The visual and olfactory representations in the orbitofrontal cortex are built by visual to taste and olfactory to taste association learning. The ability of different neurons to respond to different combinations of these inputs underlies the basis of flavour. With human fMRI, it has been found that olfactory and taste inputs can combine nonlinearly in the orbitofrontal cortex and anterior cingulate cortex to produce a representation of umami flavour from glutamate taste and a savoury odour. This leads to the concept that umami can be thought of as a delicious flavour, made delicious by a combination of a glutamate taste and a corresponding savoury odour, which are brought together only in and after the secondary taste and olfactory cortices in the brain. The representation of flavour in the orbitofrontal cortex is hunger dependent. This neurophysiological and human functional neuroimaging evidence thus shows that the orbitofrontal cortex is involved in representing the reward value and subjective pleasantness of taste, odour, oral texture, and flavour. Moreover, unpleasant odours, tastes and flavours are represented in separate locations in the human orbitofrontal cortex that the corresponding pleasant representations, and cognitive inputs can reach down into these systems to modulate their responses to chemosensory stimuli.

Rolls, E. T. 2005. Taste, olfactory, and food texture processing in the brain, and the control of food intake. Physiol Behav 85:45–56. Some papers are available at www.cns.ox.ac.uk.

138. Different Cerebral Activation Produced by a Ligand of a Human V1R-Type Receptor and of Rose Odor

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Aim of this study was to compare activation of a ligand of a human V1R-type receptor and of phenyl ethyl alcohol (PEA; rose odor). Study 1 used event-related potentials (ERP), study 2 used functional MRI. In both a computer-controlled olfactometer was used (OM6b, Burghart Instruments, Germany); 23 healthy subjects (11f; mean age 25; 20–38 years) participated in study 1; 17 subjects (9f; 26; 23–29) participated in study 2. The two odors were rated as equally intense, and received similar hedonic ratings ($P > 0.11$). Subjects were right handed and normosmic and had no nasal pathology. Women were investigated on days 5–10 of the menstrual cycle. Study 1: ERP were recorded to a ligand of a human V1R-like receptor (LIG) or PEA. ERP were recorded in response to stimuli of 200 ms duration (interval 40 s; flow 8 l/min). Latencies of the ERP's late positivity were significantly shorter in response to LIG ($F(1,21) = 7.93$, $P = 0.01$) compared to PEA. The difference was more pronounced at frontal recording sites. This indicated that early processing of LIG and PEA differs although intensity and hedonic ratings were similar. Study 2: Subjects were examined in a 1.5 Tesla MRI scanner (Siemens, Germany). Stimuli were presented in blocks (24-s air, 24-s odorant; 1-s stimulus duration, 2-s ISI, randomized). Preliminary data showed differences in cortical representation, pointing to a higher involvement of limbic structures (cingulate cortex, amygdala) and orbitofrontal cortex for LIG. The data suggest that differences exist in activation patterns based on stimulation of a V1R-type receptor agonist and a PEA receptor both of which is expressed in the human olfactory epithelium.

139. Identifying Odorants in Mixtures: Synthetic Task Instructions Favour Performance Compared to Analytical Task Instructions

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In a large number of studies, humans have shown great difficulty identifying three or more odorants in odorous mixtures. Generally, these studies involved the analytical task of identifying odorants in mixtures of odorants. Recently, it was suggested that single odorant contributions cannot be retraced from the neural code the mixture produces, because this code already reflects holistic stimulus properties at precortical stages of processing. This suggests that analytical task instructions (the bottom-up task of identifying single components in a mixture) could lead to poorer identification performance than synthetic instructions (the top-down task of identifying aroma notes in a unitary aroma). An apple and an orange model aroma, each consisting of 5 odorants, and a set of 8 single odorants, 4 from each aroma model, were presented to volunteers in a classroom setting. Results showed better performance for the 57 subjects receiving the synthetic instruction than for the 45 subjects receiving the analytical instruction. Subjects in the synthetic condition produced more correct identifications for the constituents of the apple model and less false identifications for the components of both the apple and the orange model. These outcomes suggest that the cognitive representations of a complex mixture and its constituents have a substantial effect on the way in which a complex percept is analysed.

Symposium 8: From Reception to Perception: Mechanisms of Olfactory Coding

140. Odorant Specificities of Human Olfactory Receptors from Different Phylogenetic Classes

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Humans are able to detect and discriminate myriads of structurally diverse odorants owing to only several hundreds of olfactory receptors (ORs), using a combinatorial code in which, one OR recognizes multiple odorants and different odorants are recognized by different sets of ORs. In addition to their agonist role, odorants could also behave as antagonists for other ORs, thus complexifying the combinatorial coding. OR genes are classified in two major phylogenetic classes. Class I ORs, originally identified in fish, have been suggested to be evolutionary relics in humans, while class II ORs were subsequently found in vertebrate species. However the pseudogene fraction among the fish-like class I ORs (52%), considerably lower than that observed for terrestrial class II ORs (77%), strengthened the idea than human class I receptors could be functional. We deciphered the odorant repertoire of two human ORs belonging to class I and class II, respectively. For this purpose, we set up an HEK293 cell-based assay, in which 100 odorants were screened using air-phase odorant stimulation at physiological doses. We showed that the class I OR52D1 receptor 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. In contrast, we revealed that the class II OR1G1 receptor is broadly tuned towards odorants of 9–10 carbon chain length, with diverse functional groups. We also demonstrated the existence of antagonism between odorants at the level of OR. OR1G1 antagonists were observed to be OR specific and structurally related to its agonists, with shorter size.

141. Odorant Receptors: Determinants for Wiring?

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The axons of olfactory sensory neurons (OSNs) expressing the same odorant receptor (OR) typically converge on two glomeruli in the olfactory bulb (OB), one located in the medial, one in the lateral hemisphere. Gene targeting experiments indicated that altering the coding regions of OR genes influence the projection of the affected axon population. The notion that the OR is a critical determinant for axonal guidance implies that odorant receptor proteins which are primarily located in the cilia of OSNs for odorant recognition may also be present in the axons of OSNs. This view was in fact confirmed using receptor-specific antibodies which specifically stained receptor-specific glomeruli and their approaching axonal fibers. All OSNs expressing a given OR can be categorized into two subpopulations: one projecting to the medial, the other to the lateral glomerulus. Retrograde tracing experiments demonstrated that these two subpopulations are spatially segregated in the olfactory epithelium. Thus, in addition to the OR type expressed by an OSN, also its spatial position within the epithelium appears to determine axonal projection. To scrutinize this notion, the projection of OSNs from the septal organ (SO) was analyzed. The results indicated that all SO axons terminate in glomeruli exclusively positioned in the posterior, ventro-medial aspect of the bulb. Whereas small population enter glomeruli which are also innervated by ôsisterö axons from the olfactory epithelium, larger populations targeted in distinct SO-specific glomeruli supporting the view of an interplay between OR-type and the spatial position as determinant for axonal projection patterns.

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142. Response Profiles of Individual Olfactory Glomeruli in the Main Olfactory Bulb of *Xenopus laevis* Tadpoles

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A number of past studies have focused on olfactory responses of amino acids at the level of both the olfactory epithelium and the olfactory bulb (OB) of aquatic species. However, the recording and analysis of responses to a high number of odorants of many individual olfactory glomeruli is to date missing. Here we report on amino acid-evoked responses of individual glomeruli in the OB of *Xenopus laevis* tadpoles. We first show that only glomeruli in the lateral half of the OB respond to mucosal application of amino acids and that in cases where an individual glomerulus responds to more than one amino acid, the intraglomerular appearance of the [Ca2+]i responses of different amino acids is virtually identical. We further recorded the response profile to 15 amino acids of a high number of individual glomeruli, show that there is a strong tendency of glomeruli to respond to few rather than to many amino acids and that the response frequency of basic and aromatic amino acids is explicitly higher than that for the other two subgroups of amino acids. Surprisingly, the glomerular response profiles were partly contradictory to the response profiles of olfactory receptor neurons of the same species obtained in a previous study. As a last point we thoroughly compare the two datasets and give some possible explanations of these partly inconsistent results.

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143. Processing of Odor-Evoked Activity Patterns in the Olfactory Bulb

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Neuronal circuits in the olfactory bulb transform distributed, yet chemotopically organized, patterns of glomerular activation into time-varying patterns of output activity across mitral cells. Associated with this transformation are multiple computations that have been examined previously in our laboratory in the intact olfactory bulb of zebrafish. To analyze the network activity underlying these computations, we measured odor responses across large numbers of neurons by 2-photon imaging after bolus loading of a calcium indicator. Mitral cells were distinguished from local interneurons by a transgenic fluorescent marker. Firing rate changes were reconstructed from calcium signals by temporal deconvolution, which dramatically increased the temporal resolution of activity measurements. The results revealed that response properties of interneurons differ substantially from those of mitral cells. Moreover, the topological relationship between glomerular input patterns, mitral cell activity patterns, and interneuron activity patterns changes as the olfactory bulb output is reorganized so that the chemotopic organization of glomerular activity patterns is maintained only partially in the mitral cell activity patterns. The basic properties of the measured activity patterns were reproduced in a simple model of the olfactory bulb circuitry. Together, experimental data and modeling results provide new insights into the mechanisms by which neuronal circuits in the olfactory bulb process odor-evoked activity patterns.

144. Accurately Predicting Olfactory Perception from Odorant Structure

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Although it is agreed that physico-chemical features of molecules determine their perceived odor, the rules governing this relationship remain unknown. A significant obstacle to such understanding is the high dimensionality of features describing both percepts and molecules. We applied the statistical method of principal component analysis (PCA) to reduce dimensionality in both odor percepts and physico-chemical descriptors for a large set of molecules. This allowed us to create a perceptual space for odorants, and a corresponding physico-chemical space for their molecular structure. The primary dimension of perceptual space was odorant pleasantness, and the primary dimension of physico-chemical space was molecular compactness. Strikingly, the primary axis of physico-chemical properties predicted the primary axis of olfactory perception. This allowed us, in a series of experiments using 100 subjects, to accurately predict the pleasantness of novel molecules by their physico-chemical properties alone. Pleasantness is traditionally thought to reflect an internal state, with no obviously linked parameter in nature. Our findings, in contrast, suggest that pleasantness is an

olfactory metric, that like colour and pitch, is an internal representation that is lawfully and rationally linked to nature, corresponding to an axis of maximal discriminability amongst biologically relevant molecules.

Symposium 9: Behavioral Reactions to Attractive and Aversive Chemostimulants in Man and Animals

145. Does Flavor Perception Reflect Nutrient Value?

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Chemical sensory systems (taste, smell, chemical irritation) evolved in part to insure the consumption of foods and beverages that are beneficial and to prevent ingestion of harmful substances. For example, for taste, it is widely believed that sweet substances are innately preferred because they signal the presence of calories whereas bitter substances are innately avoided since they may also be poison. Consistent with the belief that the hedonic sensory properties of a stimulus signal its nutritional benefits, over 50 years ago Fisher suggested that the oral cavity might act as a pharmacological preparation in situ. That is, chemical senses sensitivity (e.g. the threshold for a bitter compound) might reflect the pharmacological activity of a compound (e.g. the more bitter the compound the more potent it is pharmacologically). We have recently extended this idea to investigate the potential value of an unusual property of certain extra virgin olive oils: they contain a compound, oleocanthal, which irritates the throat but has little oral irritation. This pattern of irritation resembles that of the anti-inflammatory compound ibuprofen leading to the hypothesis, confirmed using in vitro assays, that oleocanthal also has anti-inflammatory activity. Long-term consumption of olive oils with oleocanthal may provide health benefits and this unusual irritation, regarded as a positive sensory attribute in olive oil, signals the presence of this benefit. An unanswered question is how an apparently negative sensory property (throat irritation) has come to be perceived as attractive.

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146. Taste- and Odor-Induced Facial-Expressions in Man and Animals

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Sensory processes result in feelings and sensations that cannot be shared or measured. Information on quality and intensity of the perceived sensation is required in order to have reliable indicators of perceived sensations for clinical and research purposes. Cognitive reports describing and rating sensations are restricted to conscious, cooperative human examinees. Among stimulus-dependent motor or secretory manifestations, which can serve as indicators, we have found that stimulus-dependent facial expressions are highly indicative of both quality and intensity of taste and odor sensations. These distinct, differential, fixed features can be elicited already in the perinatal human infant, prior to any food-intake experience and they do not recede with age. Since these facial expressions, indicating ôindifferenceö, ôacceptanceö and ôaversionö can be elicited even in neonates born with severe CNS developmental malformations, it was concluded, that the reflex is controlled at subcortical brain-levels and was named gusto-facial-, naso-facial-reflex, respectively. For adult human examinees (n = 160) the cognitive hedonic- self-estimates of the perceived taste and odor sensations correlate with hedonic estimates of their video-recorded facial displays, assessed by nonbiased evaluators. Among the normal and diseased subpopulations tested, the results obtained on autistic children are of special interest. Experiments on different animal species revealed, that chemo-stimulus–dependent oral and facial displays are not restricted to man, but many animal species display motion features, indicative of ôacceptanceö and ôaversionö. Our studies show that taste-induced facial displays of monkeys and apes are very similar, almost identical, to those of neonate human infants.

147. Behavioral Responses of Bacteria to Chemostimuli

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Bacterial populations may encounter a large spectrum of environments during their life cycles. Due to their small size and relative simplicity, their ability to adjust the environment to their needs is very limited. Instead, they apparently adopted a strategy of moving from our environment to another. Chemotaxis as well as other types of taxis (e.g. thermotaxis and phototaxis) enable bacteria to approach (and remain in) beneficial environments and escape from hostile ones. Bacteria such as Escherichia coli do it by modulating their two main swimming patterns; smooth swimming in a rather straight line (a run) and a brief but abrupt turning motion (a tumble). In the absence of stimuli (an attractant or a repellent), the bacterial cells execute a random walk, composed of runs and tumbles with essentially no net vectorial movement. When in a stimulus gradient, the random walk is biased towards the attractant or away from the repellent due to the modulation of the direction of flagellar rotation. The behavioral and molecular mechanisms by which this is achieved will be reviewed.

148. Contextual Learning about Odors and Flavors during Childhood

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Odors and flavors can acquire specific meaning early in life that can persist throughout the lifetime. In this talk, I will present research findings that suggest that the infants' preferences for retronasally-presented odors are mediated by experience. These early experiences may also result in the generation of olfactory memories through associative learning processes. Age-appropriate tasks were used to assess older-aged childrenÆs liking, identification and preference for a variety of odors ranging in hedonic valence and familiarity. These studies revealed that the hedonic responses to the odors of alcohol and tobacco were related to the drinking and smoking habits, respectively of their parent. Such findings a suggest that early learning about alcohol and tobacco is based on sensory experiences at home and anchors it to the emotional context in which their mothers drink and smoke.

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149. Chemical Stimuli-Response Coupling in Neonates: Experience-Dependent or Independent?

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Human and nonhuman infants display nonrandom facial–oral responses to a variety of odorous/sapid stimuli. These responses take the form of approach/withdrawal (or appetence/aversion) actions, and are interpreted as overt signs of underlying positive/negative affect. As they can be elicited in newborns before explicit postnatal reinforcement, such dichotomous responses were first considered to rely on automatic, genetically programmed, learning-independent processes. Although such unconditional responses are certainly effective, especially with intense stimuli, there is evidence that odorants that are not unpleasant to adults (e.g. vanilla, milk) can trigger disgust-like facial displays in newborns, depending on the prior experience they had with them and on their metabolic state. The conditional character of neonatal coupling between stimuli and affective responses is further substantiated by the effect of fetal chemosensory experience on hedonic responses in newborns: aversive odorants or tastants can be rendered less aversive, and neutral stimuli can gain clear attractiveness, through fetal exposure. The case of sweet taste will be examined in the light of the above results. Although it is reputed to elicit innately positive responses in term or preterm newborns, it will be examined as a possible outcome of prenatal exposure effects. Some psychobiological processes underlying this prenatal shaping of the brain's ability to extract the affective impact of chemostimuli will be discussed.

150. Seasonal Variations in Sensitivity to Androstenone

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Throughout the animal kingdom, many species alter their perception of chemical signals in relation to mating in synchrony with seasonal changes. However, in humans few chemosensory-related seasonal fluctuations have been identified. One example where fluctuations have been observed is in individuals with seasonal affective disorder, who display seasonal fluctuations in olfactory thresholds. The aim of the present study was to determine whether young healthy adults show a seasonal fluctuation in sensitivity to the endogenous steroid androstenone (AND). Sensitivity to the odor of phenyl ethyl alcohol (PEA) was also tested for comparison. Over a 1-year period, 240 subjects were tested, 20 (10 women) each month. Subjects were university students with no known history of smell disorders or allergies, and all had identifiable threshold for both compounds. Detection thresholds were tested for one substance or the other in counterbalanced order, followed by a 20-min questionnaire on seasonal behaviour, and then threshold testing for the remaining odor. The results indicate that subjects tested in March, April and May were significantly more sensitive to AND than subjects tested in other months, whereas no differences were observed in sensitivity to PEA. In addition, when subjects were grouped by gender, these findings were significant only for women. The results suggest that humans are susceptible to seasonal variations in sensitivity to an endogenous odor, similar to variations observed in other animals.

Oral Session 3: Vomeronasal Organ and Pheromones

151. Modifications in Exploratory Behavior And LH Release in Female Mice Induced by Exposure to Male MUP

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In rodents, the release of urine in the environment allows the emission of substances that can alter the behavior and hormonal status of conspecifics. In rats (1), we demonstrated that male urine induce modifications in the neurotransmitter release in the amygdala nuclei that receive inputs from the accessory olfactory system, followed by a LH surge in the plasma. This suggests an amygdala-mediated link between chemosensory stimulation and hormonal modulation, induced by male rat urine or by the rat urinary protein, alpha-2u. In the first experiment, we explored if a similar effect was present in mice, by exposing female mice to the Major Urinary Proteins (MUP) isolated from adult male urine. Adult estrous females spent a greater time in the proximity of the MUP-containing vial compared to control, an effect most probably due to some of the volatiles bound by MUP. In the second experiment, we investigated the effect of MUP presence on the behavior on a larger timeframe, in addition plasma LH was determined at the end of the experiment. When MUP was introduced in the cage of females, estrous females displayed a behavioural pattern similar to that of estrous rats, characterised by a reduction in sleeping time. Also the total investigation time was higher for the MUP-anointed stimulus, compared to control, and effect most evident in diestrus females. Three hours after MUP introduction in the cage, plasma LH was determined via EIA: an increase in LH was detected in MUP-exposed females, most evident in diestrus females. These data show that also in mice, the exposure to MUP, purified from adult male urine, can attract females and induce a LH surge.

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152. Smell Plays a Role in Detecting Oestrous Females in the Tammar Wallaby (*Macropus eugenii*)

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Odours are important to synchronize behaviour and hormones of the potential mating partners in many mammals. So far these effects have been shown only in one marsupial, *Monodelphis domestica*. We have investigated the role of smell in reproductive synchronization in another marsupial, the tammar wallaby (*Macropus eugenii*). The

tammar is a small macropodid marsupial that inhabits 6 offshore islands in South Australia and Western Australia. During the breeding season, males establish a rank order that determines mating order. Individual males associate with females throughout the period of gestation. Females come into oestrus about 1 hour after birth, and are receptive for up to 8 hours. During this time males spend considerable time sniffing the urogenital opening (UGO) and the pouch of females. This study investigates whether odours or secretion from the pouch or UGO are attractive to males when a female is in oestrus. Tammars have a well developed olfactory epithelium and vomeronasal organ, connected to the nose and mouth via the nasopalatine ducts. Male tammars were tested for their behavioural preference to sniff swabs taken from the UGO or pouch of oestrous females compared to swabs taken from nonoestrous females. The mean time spent per investigation of samples from oestrous and nonoestrous females were compared. Males ($n = 8$; $P = 0.488$) did not show preference for either oestrous or nonoestrous samples taken from the pouch, whereas males appeared more interested in UGO samples from oestrous females than those from samples of nonoestrous females ($n = 8$; $P = 0.059$). This study suggests that odours from the UGO may be important for the sexual checking behaviour of male tammars.

153. Role of the Main Olfactory Epithelium in Mediating the Effects of a Putative Pheromone in Humans

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Mammalian pheromones influence behavior and/or hormonal state in conspecifics, often through the vomeronasal organ (VNO). Human putative pheromones have been identified, but whether their effects are mediated through the main olfactory epithelium (OE) or the vestigial VNO remains controversial. Here we test whether the OE mediates the effects of smelling the putative male pheromone 4,16-androstadien-3-one (AND). Smelling AND influences autonomic nervous system activity and mood in women. We set out to quantify these effects, and then ask whether we could negate them by physically and chemically blocking the putative VNO (VNOblock) but not the main OE. In a double-blind study on 27 women scheduled 28 days apart, VNOblock was applied selectively before smelling AND or CONTROL. Session order was counter balanced, and AND/CONTROL were matched for intensity/pleasantness. This design allowed us to assess the differential impact of VNOblock on the physiological, endocrine and psychological response to AND and CONTROL. To date our analysis on these 27 subjects shows that: 1) AND induced specific effects on physiology (temperature, skin conductance and motion) and mood (positive mood and sexual arousal) along with previous reports; 2) VNOblock had minor effects on the main OE as shown by intensity ratings and UPSIT scores performed with and without the VNOblock; 3) the effects of AND on physiology and mood were either greatly reduced or suppressed by VNOblock. Our results points against a role of the human main OE in processing the physiological and psychological effects of AND in humans. However more experiments assaying the impact of VNOblock on the sniff volumes will be performed by ECRO meeting to insure that the main OE was equally exposed to AND.

154. Expression of Epithelial Membrane Transporters in the Developing Mouse Vomeronasal Organ

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In order to clarify the chemical maturation of neural structures of the mouse vomeronasal organ (VNO) during development, in the last years we investigated the expression of several molecules therein, with special reference to neuropeptides, neurofilament proteins, and nitric oxide synthase. To further characterize the developmental patterns of the VNO, we investigated the expression of two important epithelial membrane transporters namely, the cAMP-activated chloride channel (CFTR) and the water channel protein aquaporin 4 (AQP4), in embryos and postnatal mice. Embryos were collected at embryonal (E) days 13–19; pups were sacrificed at postnatal (P) days 8, 15, 21, and at 2 months of age. After paraformaldehyde fixation, decalcified specimens were embedded in paraffin, sectioned, and immunostained by the ABC method. Results showed that CFTR is first expressed at the apical border of the vomeronasal sensory epithelium at E19 with a maximum at P8–P15 and a clear reduction by 2 months of age; CFTR is also expressed in blood vessels and in cells of the vomeronasal glands as well as in cells of the nasal respiratory epithelium. AQP4 was first found in cell clusters at E19 and, with increased intensity, at P8 to 2 months of age in cells throughout the vomeronasal sensory epithelium. AQP4-positive cells were also found with the same timing in cells of the nonsensory vomeronasal epithelium as well as in the basolateral membrane of cells in the nasal respiratory epithelium. The preliminary results presented here show for the first time that key membrane transporters are differentially expressed in the vomeronasal sensory epithelium during development. CFTR seems to subserve a hitherto unrecognized function in the developing sensory epithelium.

155. The Role of Vomeronasal Organ in Reception of Predator Scents

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Chemosensory detection may be an important aspect of predator avoidance strategy for many mammals. In our earlier studies we examined the influence of predator chemical cues derived from feral cat urine on reproductive output of rodents: rats, mice and voles. Animals responded to predator chemical cues with reduced litter size and skewed sex ratio. The reduction in litter size in rodents exposed to predator urine was attributable to suppressed progesterone levels affecting the implantation of embryos. In more recent study we analyzed patterns of c-fos activation in receptor tissue of vomeronasal organ in mice in response to stimulation with different predator excretes: urine, faeces, saliva. Also we used as stimuli single compounds isolated from predator excretes. We compared

patterns of activation in vomeronasal receptor tissue depending on time course exposure and hormonal status of the signal recipient.

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156. Functional Neuronal Processing of Olfactory Kin Recognition

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Olfactory-based kin recognition in humans has previously been demonstrated, but the cortical structures involved are still unknown. By means of positron emission tomography (PET), we sought to elucidate the neuronal substrate behind olfactory-based kin recognition. Twelve women participants were scanned while smelling either body odors from their biological sister, a close friend, strangers, a mixture of perceptually similar common odors, or clean air. A screening session revealed that participants were well able to identifying the individual body odors. All odorous stimuli were perceived to be matched in both intensity and pleasantness inside the scanner. Initial analyses of the imaging data indicate that kin recognition (sister vs. friend) is primarily mediated by cortical regions commonly associated with social recognition such as the medial frontal cortex. In addition, known body odors (sister + friend) seem to be processed differently from unknown body odors (strangers). Body odors from known individuals activate occipital regions associated with body perception. Taken together, these preliminary results suggest that olfactory kin recognition is mediated by cortical regions associated with social cognitive processing.

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Poster Session 4

157. The Effect of Osmotic Pressure on Salt Taste Responses in the Bullfrog Glossopharyngeal Nerve

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Taste stimulations change chemical compositions and osmotic pressure on the taste epithelium. We investigated the effect of osmotic pressure on bullfrog taste nerve responses to inorganic salts where the osmotic pressure was increased by the addition of either urea or sucrose to stimulating salt solutions. Both urea and sucrose alone elicited negligible neural responses up to 1 M. The magnitude of tonic-response to 0.5 M NaCl was increased by a factor of 2.7 ($n = 9$) with 1 M urea. The addition of 1 M urea to the concentration series of NaCl increased the magnitude of tonic-response without changing its threshold concentration. In contrast, the magnitudes of tonic-responses to 1 mM CaCl2 were suppressed by urea at concentrations higher than 0.6 M. The control concentration–response relation for CaCl2 peaked at 1 mM. The addition of 1 M urea suppressed the peak magnitude of the response to CaCl2 at concentrations lower than 30 mM and increased the response magnitude with increasing CaCl2 concentrations. Sucrose similarly modified the response magnitude to these salts as urea did. The addition of 1 M urea to various salts of 0.5 M differently increased the response magnitude of the salts. The extent of increase depended on the differences between the mobility of each cation and anion forming the tested salt. These results suggest that the hypertonic solution increases the conductance of tight junctions in the taste epithelium, and enhances currents generated by the diffusion potential across the taste epithelium, which depolarize or hyperpolarize the basolateral membrane of taste receptor cells and modify the neural responses.

158. Role of Phosphatidylcholine Molecular Species in School Recognition in the Catfish *Plotosus lineatus*

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Group is formed by members who communicate via a distinctive signal, so called social signal. Chemical signal play an important role in the recognition of familiar individuals, although their nature is poorly characterized. The catfish *Plotosus lineatus* forms a dense school immediately after hatching and their school recognition is under the control of chemical signal emitted by school members. However, the chemical signal governing this recognition (school recognition substance, SRS) has long been unknown. Here we show that the school recognition of *P. lineatus* is governed by school-specific phosphatidylcholine (PC) profile. First, we found that *P. lineatus* responded only to PC originated from familiar school, but not to those from unfamiliar schools. Quantitative high performance liquid chromatography analysis of PC molecular species revealed not only a complex mixture of PC molecular species but also the school-specific PC profiles; further, principal component analysis of the quantified peaks revealed the presence of a wide variety of PC profiles. Finally, we showed that modified PC profiles by adding synthetic PC molecular species no longer elicited school recognition activity in *P. lineatus*, concluding that *P. lineatus* recognizes familiar school via school-specific PC profile. We report the first identification of a chemical signal underlying group recognition in vertebrates, which may provide insight to research the species-specific signal perception, cue-triggered behavior expression, and decision-making processes in animal communication.

159. Electron Microscopic Analysis of the Effect of Synapse Formation on Accessory Olfactory Bulb Neurons by Coculture with Vomeronasal Neurons

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A coculture system of accessory olfactory bulb (AOB) neurons and vomeronasal (VN) neurons was established for studying the functional roles of AOB neurons in pheromonal signal processing. The effect of VN neurons on the development of AOB neurons was examined in a coculture system. The densities of dendritic spines were

lower in the coculture than in single culture. The ratio of the density of synaptophysin-immunopositive spine/total spine density was larger in the coculture than in the single culture. The volume of spine head was larger in the coculture than in single culture. In the present study, we examined the effect of VN neurons on the development of AOB neurons using electron microscope. The synapses on dendritic shafts were decreased and the spine synapses on dendrites were increased in the coculture. The ratio of symmetrical synapses in total synapses was larger in the coculture than in single culture. The synapses between AOB neurons and VN neurons were recognized in the coculture. These observations suggest that synapse formation of AOB neurons is regulated by synaptic contact with VN neurons.

160. Modulation of V2R Receptor Expression in Cultured Vomeronasal Neurons by the Interaction with Cocultured Accessory Olfactory Bulb Neurons

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Pheromones are detected by unique receptors in the vomeronasal organ (VNO), and then affect endocrinal status and behavior in various kinds of animals. Although it is well known that the VNO mediates sexually and developmentally different behavioral and neuroendocrine responses, the cellular mechanisms regulating these functions are not much understood. The differences in pheromonal effects might be in part due to differential expression of pheromone receptors. Previously, we reported that vomeronasal neurons (VRN) in culture form a spherical aggregate with a central cavity, referred to as a vomeronasal pocket (VNP). We also reported the maturation of each VRN in the VNP was induced by coculture with dissociated accessory olfactory bulb (AOB) neurons. To characterize the receptor expression, we examined two representatives (VR1 and VR4) from different subfamilies of the V2R family of pheromone receptors using cultured VRNs. A western blotting analysis showed the expression of VR1 and VR4 in the VNP was induced and increased with days in coculture, an effect which was not observed in the VNP-alone culture. Moreover, we applied charged compounds in mouse urine iontophoretically into the cavity of VNP using a microelectrode and analyzed VNP response by a Ca2+ imaging method with or without cultured AOB cells. When urine compounds were ejected into the VNP cocultured with AOB cells with a current of -2 μA, subpopulation of VRNs clearly showed long-lasting Ca2+ increases. Injections of a current below -5 μA alone had no effect to the VNP. Such Ca2+ increases were not observed without AOB cells. These results indicate that VRNs result in expressing pheromone receptors by interacting with AOB neurons in coculture, and then acquiring responsiveness to compounds in urine.

161. Experience with Na-Cyclamate, but not Acesulfame-K, Induces Increased Taste Sensitivity for Glucose

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Experience with fructose induces increased discrimination ability for glucose. We studied the effects of experience with Na-cyclamate or acesulfame-K on glucose discrimination. Subjects were randomly assigned to one of three treatment groups—Na-cyclamate (40 mM), acesulfame-K (0.4 mM), or distilled water. They briefly treated their tongues with one of the blue-colored isosweet sweeteners or blue water each day for 10 days. On day 11 or 12, they tasted paired samples of green glucose (17.5, 27, 43, 65, 100 mM) and water and indicated the ''sweetener'' in each pair. There was a significant difference among the groups ($P < 0.004$). Subjects treated with Na-cyclamate discriminated glucose at lower concentrations than those treated with water ($P < 0.013$) or acesulfame-K ($P < 0.002$). There was no difference between water and acesulfame-K treated subjects ($P > 0.5$)(Wilcoxon Kruskal–Wallis). Of Na-cyclamate treated subjects, 79% discriminated glucose 17.5 mM and all higher concentrations, while only 28% of water treated, and 19% of acesulfame-K treated subjects were able to do so. Most discriminated 42 or 65 mM and higher concentrations. The results suggest that Na-cyclamate and glucose share a common mechanism, while the differential effects suggest an induction locus more peripheral than the shared mechanism. Viewed in light of in vitro responses of human sweet receptor subunits to fructose, glucose, acesulfame-K and cyclamate, it would appear that experience via hT1R2/hT1R3 can but does not necessarily lead to, while experience via hT1R3 alone may be sufficient for, the induction. Further work with various sweeteners could clarify the induction locus.

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162. Ligand Specificity of Odorant Receptors

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Odorant receptors belong to class A of G protein–coupled receptors (GPCRs) and detect a large number of structurally diverse odorant molecules. A recent bioinformatic analysis of class A GPCRs suggests that structural features are conserved across them, in spite of their low sequence identity. First site-directed mutagenesis experiments performed on one of ORs (MOR174-9) provide information, which helped to identify nine positions involved in ligand binding. Based on these results, we have aligned the sequences of 29 ORs for which data on ligand binding are available. Our modelling provides a rationale for these positions in most of the considered odorant receptors and helps to identify other amino acids, which could be important for odorant binding. Our findings are consistent with experimental results and suggest that all ORs may share similar 3D structure and differences in their recognition profiles are due to the substitution of side-chains at several key positions. Finally, our results allow making predictions for site-directed mutagenesis experiments, which could also validate our model.

163. Computational Neuroanatomy of Insect Antennal Lobes: Tools and Applications

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The insect antennal lobes (ALs) are cerebral structures made of several individually identifiable spherical neuropil, called glomeruli. The glomeruli house all synaptic connections between olfactory receptor neurons and central neurons. Much remains to be learnt on their role in the coding of odours. In the past two decades, technical advances enabled to simultaneously record functional and anatomical information. Meanwhile, image analysis and computational neuroanatomy have also been progressing. We benefited of those advances to develop a software tool adapted to the visualization and analysis of ALs scanned as stacks of confocal images. Our tool, developed with the Matlab language, includes four components. Component 1 aims at manually segmenting the glomeruli on the confocal images. Component 2 provides realistic 3D reconstructions of the AL. Using quantitative criteria, component 3 standardizes the measurements by correcting for orientation and size. These corrected data result in a schematic representation of ALs, which is convenient for reliably identifying a given glomerulus in different specimens. Component 4 allows a quantitative analysis of the variability in size and position and an automated identification of glomeruli. The processing chain is now being used for creating a 3D map of the AL in the male moth *Spodoptera littoralis*. We intend to use this map in neurophysiological experiments to identify the glomeruli innervated by individual AL neurons. In many species, our specialized software can facilitate the creation and analysis of AL maps, ease the interpretation of the anatomical correlates of molecular and physiological data and thus contribute to a better understanding of the structure–function relationships in AL glomeruli.

164. Impact of Hedonic Appreciation on Memory for Custard Dessert

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Memory plays a fundamental role in food expectation and perception. In a study performed by Sulmont-Rossé et al. consisting in the recognition of a previously learned target among distractors varying in sweetness or sourness, participants showed a better memory for unpleasant samples (i.e. less sweet and more sour) than for pleasant samples (i.e. sweeter and less sour). Nevertheless, this study was not specifically designed to study the impact of liking on food memory. To answer this question, 2 pleasant and 2 unpleasant custards were produced. Their hedonic values were checked by several groups of young adults during preexperiments. Then, at the occasion of 2 snacks offered on 2 consecutive days, 44 different young adults received 2 of these 4 custards, that is, 1 pleasant and 1 unpleasant, under the cover story of an hunger-feeling study that ensured an incidental learning. One week later, participants were confronted with the 4 custards, namely the 2 custards previously eaten and the 2 custards not previously presented. Participants were asked to decide whether or not they ate these custards during the snacks (memory task) and then to rate their liking of these custards (hedonic rating task). The results of the hedonic rating task confirmed the expected custards classification (i.e. pleasant vs. unpleasant). Concerning the memory results, in the line of previous studies, the positive d' obtained, showing a good memory capacity of participants, depended mostly on the distractors rejection than on the targets recognition. Indeed participants were unable to recognise the targets (hits rate = 0.50), and this whatever their hedonic value. But they correctly declared the distractors as different from the targets, particularly in the case of unpleasant distractors.

165. Mutual Expression of Family-C Vomeronasal Receptors, V2Rs

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Pheromone receptors are expressed in the sensory epithelium of the mouse vomeronasal organ. They are assigned to two superfamilies of G-protein–coupled receptors, namely V1Rs and V2Rs. V1Rs colocalize with the Gai2-subunit in the apical vomeronasal neurons, whereas V2Rs with the Gao-subunit in the basal ones. According to their sequence homology, V2Rs can be classified in three families, namely A, B and C of respectively 52, 6 and 4 functional members. Our previous studies reported that receptors of family-C, the most divergent among the three families, coexpress with receptors of family-A and -B in the basal vomeronasal neurons. This observation seems to partially contrast with the ''one receptor-one neuron'' hypothesis that is a typical feature of the modality of expression of olfactory and V1R receptors, in which an individual neuron only expresses one receptor type. Indeed, although colocalized with family-C receptors, family-A and -B receptors are mutually expressed in nonoverlapping populations of vomeronasal neurons. The puzzling problem is whether basal neurons observe an analogous modality for the expression of family-C receptors ('olfactory receptors' modality) or, differently, if they allow multiple genes of this family being expressed in an individual neuron ('bitter taste receptors' modality). Aiming at solving this problem, we have characterized the expression of functional members of the family-C and report that these receptors are expressed in different and nonoverlapping populations of vomeronasal neurons. These results suggest that, in the basal neurons of the vomeronasal organ, family-C V2Rs are expressed by the same modality as family-A and -B receptors, thus suggesting a common but parallel transcriptional programming of V2R genes.

166. Role of the Chorda Tympani and Glossopharyngeal Nerves in Oral Linoleic Acid Detection in Mice

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Behavioural studies with rats and mice have shown that linoleic acid is a preferred stimulus in a two-bottle preference test, however it is still debated whether this stimulus is somatosensory or mediated by gustatory nerves. In order to address this question, we designed an experiment in which mice underwent bilateral transections of the glossopharyngeal (IX) and/or the chorda tympani (VII) nerve before being tested for linoleic acid preference and digestive secretions. Three groups of mice: sham-operated (SO), glossopharyngeal denervated (GL-X) and chorda tympani-glossopharyngeal double denervated (CT/GL-X) mice were subjected, 10 days after surgery, to a two-bottle preference test with a choice between 2% linoleic acid (77μM) in xanthan gum or xanthan gum in water. Controls including an evaluation of the number of remaining taste buds were

performed on the denervated animals to assess the surgery. Our results show that as opposed to the GL-X group, the CT/GL-X denervated group had diminished preference ratio for linoleic acid after 30 minutes, while both groups showed greatly reduced preference for linoleic acid after 48 hours, possibly highlighting postingestion effects. Moreover, we report that the rapid and sustained rise in the flux of pancreato-biliary secretions, normally triggered by an oral linoleic acid load in sham-operated mice with a ligatured oesophagus, was diminished in GL-X and CT/GL-X mice. Taken together, these results point to a role for the VII and IX nerves in mediating linoleic acid preference as well as the pancreato-biliary response following oral stimulation by linoleic acid.

167. Do Just Noticeable Differences of Components Concentrations Modify Qualitative Perception of an Odor Blending Mixture?

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In a recent publication, Zou and Buck demonstrated that binary odorant mixtures can stimulate cortical neurons that are not stimulated by their individual component odorants. They suggested that this observation could explain the perception of novel odor in a mixture containing several odorants. At a perceptual level, Barkat suggested that an optimum ratio of concentrations of two odorants (ethyl caproate and furaneol) can lead to the emergence of a Pineapple note in the mixture. In a recent study (data unpublished yet) we observed that a ternary mixture of definite proportions of ethyl isobutyrate, ethyl maltol and allyl ionone was judged as more typical of a Pineapple note than the individual component odorants. It was concluded that this mixture (further called target) elicits a novel odor through an odor blending process. In the present study, we address the question of the necessity to mix definite proportions of odorants to elicit a novel odor in a mixture. Starting from the composition of our target mixture, four concentration levels of each component were chosen to elicit just noticeable differences. Each combination of these concentration levels were used to design sample mixtures. Following a repeated paired-comparison method, a panel of 15 subjects evaluated intensity, Pineapple typicality and pleasantness of each sample mixtures, delivered with an olfactometer, as compared to the target mixture. Results show the impact of just noticeable differences of components concentrations on the qualitative perception of a novel odor (Pineapple) in a ternary odor mixture.

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168. Increase in Anhedonia Level in Menopausal Women Is Accompanied by a Shift of Olfactory Function

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Menopausal women between 55–65 years of age exhibit psychological and hormonal changes that are reflected in modification in experiencing sensory pleasure. In the present study we set out to characterize whether that modulation of anhedonia level was accompanied by a modification of olfactory function during that period of life. Thirty-two menopausal women subjects (55–65 years of age) without neurological diseases and not under menopausal substitutive treatment were tested. Anhedonia level of each subject was assessed with the Physical Anhedonia Scale, a 61-item true/false inventory that attempts to access a wide variety of positive physical experiences. Subjects' olfactory performances were estimated using the European Test of Olfactory Capabilities (ETOC). After completing the localisation and identification tasks included in the ETOC, subjects sniffed each odorized vial that they properly localized and rated compound intensity and pleasantness on a 1–9-point scale (from "1: not at all intense or pleasant", to "9: extremely intense or pleasant"). Results showed that anhedonia level was negatively correlated with olfactory function ($F(1,31) = 5.982$, $P = 0.0205$) and nearly negatively correlated with the percentage of odors perceived as pleasant ($F(1,31) = 4.023$, $P = 0.0540$). Control analyses revealed no significant relationships between physiological age ($F(1,31) = 0.322$, NS) or menopausal age ($F(1,31) = 0.371$, NS) and olfactory function. This result suggests therefore that affective perturbations occurring in menopausal women are accompanied by both a decreased olfactory function and a shift in olfactory hedonism affecting mainly its pleasant side.

169. The Neural Representation of Odor Is Modulated after Trigeminal-Olfactory Associative Learning in Humans

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Odor perception is characterized by a high degree of plasticity in humans: odor hedonics and odor intensity are modulated by various factors such as stimulus concentration and contextual information. In the present study we set out to examine whether the presence of a pure trigeminal stimulus during odor encoding may modulate odor intensity and hedonics on both behavioral and cortical levels. Fourteen human subjects (10f, 19–35 years of age) were tested in a within-subjects design including two sessions. First, an associative learning was used whereby (1) a pure odorant (CS+) was presented with a 200 ms pulse of CO_2 (US), and (2) a pure odorant (CS−) was presented alone. Odorants and CO_2 were presented using a computer-controlled air-dilution olfactometer. Second, the pure odors were presented alone. During that session, subjects were asked to rate odor intensity and hedonics after each trial. Olfactory event-related potentials were simultaneously recorded at different electrode sites including Cz and Pz (sites known to have maximum amplitudes for trigeminal [Cz] and olfactory stimuli [Pz], respectively). Results showed that CS+ intensity ratings increased in 8 subjects and decreased in 6 subjects. On the cortical level, a group effect was observed for P2 amplitude reflecting an increase in P2 amplitude for the " increase in CS+ intensity " group vs. the " decrease in CS+ intensity group " in Cz ($t(12) = 2.12$, $P = 0.03$) but not in Pz ($t(12) = 1.35$, $P = 0.90$). This result suggests therefore that the presence of a pure trigeminal stimulus (CO2) during odor encoding alters the neural representation of a pure odor. Thus, the neural representation of odors combines the odor itself but also contextual information (trigeminal in the present case) presented during encoding.

170. Is Life and Death of Olfactory Neurons Controlled by a Knob and Ciliae Specific Pool of RNA?

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Olfactory sensory neurons (OSN) are highly polarized cells located in the nasal cavity. Ciliae emerging from the dendrite knobs lie in the olfactory mucus and are directly exposed to external environment. As for other neurons, the dendrite extremities are very distant from the nucleus, and local synthesis of proteins from specific pools of dendrite RNA may provide an efficient and fast mechanism to deliver proteins where and when they are needed. Local translation might also exist in the dendrite knobs of OSN. We isolated rat knobs and ciliae compartment from total epithelium, and purified enough RNA to be able to build a compartment-specific cDNA library composed of a limited number of cDNA. Specific enrichment in dendrites relative to whole OSN was assessed for several genes by differential real-time quantitative PCR methods. Out of more than 250 different RNA species present at high level in the OSN dendrite compartment, one third is encoding proteins that may protect neurons against environmental aggressions. We found several neuron maturation, apoptosis or survival genes, some of them known to be involved in neuro-degenerative diseases. Interestingly, an important fraction of the cDNA library encodes ribosomal and translation factors. This may indicate the existence of local translation regulatory loops. We found RNAs for nucleo-cytoplasmic transport and transcription factors in dendrites, suggesting that translation in the external compartment of OSN might also influence nuclear transcription of a number of genes in response to environment. Environmental factors as well as the physiological or pathological states may thus regulate olfactory neurons turnover, survival and death through an activity-dependent located translation of dendrite RNA pool.

171. The Spanish Society of Sensory Sciences and the Percepnet Symposia, Two Strong Sensory Initiatives

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Sensory sciences have gained their place inside the general scientific landscape. An increasing number of meetings are being held, but due to the great variety and complexity of interests (scientific, technological and economical), most achievements remain in a great deal inside the scopes of the disciplines that generate them, and many opportunities for knowledge sharing are missed. This is the rationale behind the creation of the Spanish Society of Sensory Sciences (SECS), gathering scientists and professionals that join efforts to contribute to the advancement of sensory sciences and their applications. SECS intends to promote research and teaching in the field of sensory sciences, contribute to fruitful discussion, collect and divulgate information, promote the curricula of academic and professional collectives and keep contact with other national and international societies and institutions with similar purposes. This initiative was created and promoted by the participants of the Percepnet Symposia (PS), whose third edition (PS2006) will be held in Barcelona on July 2006. The PS meetings focus on sensory sciences with a transdisciplinary insight, defining their needs, limits and possibilities. The PS2006 theme will be ''Perceiving the future: science and industry facing the sensory challenge''. What are the possibilities offered by the knowledge of human perception? Which are the cognitive aspects associated with it? By which means can we intervene in our environment? Those are quite recent questions that will be raised in PS2006 through its four strengths: debates, colloquia, sensory workshops and sensory experiences. All they represent a unique opportunity to share experiences and reflect about senses.

172. A Coated Quartz Crystal Microbalance (QCM) Odor Static Sensing System Construction for Olive Oil Flavors

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A coated QCM static sensing system construction has been developed to analysis volatile organic compounds frequently found in some positive and negative organoleptic olive oil attributes. The film-coated is composed by an adsorbent material onto a substrate which improves adherence and homogeneous distribution of adsorbent layer. Polyvinyl chloride (PVC) as substrate film and tetrahydrofuran (THF) as solvent have been used, analysing twelve PVC/THF relations (0.25–50 mg/ml range) and seven dropped volumes (0.5–10 ml range). Sensor responses are measured as the stabilised oscillation frequency shift of the coated QCM sensor. PVC/THF = 1 relation and 10 ml dropped volume have been selected as QCM substrate film because they give the firmest sensor responses and constitute the greatest PVC film area. Operating parameters, such as measurement chamber temperature and sensor stabilisation time, have been optimised to improve the selectivity of the coated QCM sensor. After 15 minutes of sensor stabilisation time, volatile organic compounds, such as acetic acid, 1-octen-3-ol, 2-butanone, (*Z*)-3-hexenyl acetate, hexanal and undecane, are injected in a closed thermostat chamber, where coated QCM sensor is placed inside. OV–275 (1 mg) is used as adsorbent material. Changes in the oscillation frequency is registered each 5 seconds during 25 minutes. Measurement chamber temperature at 30 °C. For acetic acid and (*Z*)-3-hexenyl acetate the highest frequency shifts are found at 30 °C. 2-butanone, hexanal and 1-octen-3-ol exhibit great frequency shifts at 35 °C, but there are low differences with oscillation frequency shifts at 30 °C. The adsorption of undecane shows double frequency shift at 35 °C than 30 °C, but this nonpolar volatile does not interact with polar OV-275 polymer.

173. Working Memory for Odors

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Little is known about working memory for odors. Dade et al. compared working memory for odors and faces in a two-back same-different task and found similar levels of performance. Because the

odorants in their study were highly identifiable odorants, verbal coding is likely to have supported the memory performance. In our study, using a similar two-back task, we compared odors that could be verbally described to a variable degree. Twenty-two paid participants (16 women), with a mean age of 28.09 (SD = 9.17) judged a series of 36 odors with regards as to whether the odor on the current trial (trial n) was the same the as the one presented two trials previously (trial $n - 2$). The probability of an odor on trial n to be same as the one on trial $n - 2$ was 0.33. In a second round of presentations of the same odors, complementary judgments of the odorants' perceived intensity, familiarity and verbal category were also required. Results indicate that memory performance varied with level of familiarity and, in particular, the level to which the odor could be verbalized, with higher levels yielding higher memory performance. Odors that were easier to verbalize yielded an $A' = 0.91$ whereas the odors that where poorly verbalized yielded an $A' = 0.79$ in the two-back task. Memory performance was impaired if the odor on trial $n - 1$ could be verbalized compared to when it could not. Altogether the results suggest that working memory for odors can be observed. Moreover, the results indicate that the verbalization of the percept is advantageous but not necessary to observe performance levels above chance in a two-back task.

174. Effect of Odor Experience on Rat Piriform Cortex Plasticity

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Olfactory system encoding of odors has been hypothesized to be similar to visual face or object encoding. Perceptual odor objects are hypothesized to be synthesized from olfactory bulb-encoded features by cortical circuits through experience. Here, we tested whether experience with a triple odor mixture would modify cortical coding of the mixture and its components. Animals were trained with odor discrimination task where the S+ was a mixture of acetic acid, limonene and eugenol. Component ratios in the mixture were derived from Laing and Francis for a mixture that humans have difficulty identifying the individual components. S-odorants were the individual components or clean air. After reaching behavioral performance criterion, single-unit recordings made from the anterior piriform cortex (aPCX) and the posterior piriform cortex (pPCX) in urethane-anesthetized rats. Odor naïve rats served as controls. Responses to the mixture and components, as well as the novel odor isoamyl acetate were analyzed. The odor response magnitude, breadth of tuning, and correlation analyses of population odor responses were determined. After odor familiarization, aPCX neurons were more narrowly tuned to odorants whereas pPCX neurons were more broadly tuned, population responses of aPCX neurons to the mixture became more distinct (decorrelated) from responses to the mixture components whereas pPCX population responses to the mixture became more similar. The results suggest aPCX and pPCX would play different roles in the processing of familiar odors and are consistent with an experience-dependent encoding of synthetic odorant identity in aPCX and an experience-dependent encoding of odor quality in pPCX.

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175. New Insights into Odor Processing Mechanisms Using Realistic Cell Properties and Connectivity

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Most studies of cortical networks use simplified cells and connectivity to avoid long simulation times. Recently we have shown with a realistic model of the olfactory mitral cell that this approach cannot be applied to the olfactory bulb, because the long primary dendrite is essential for separating, by some 400–600 μm, the input processing that occurs in the glomerular dendritic tuft from the output processing that occurs in the soma and secondary dendrites. Those simulations provided evidence for the role of gap junctions in synchronizing activity in mitral cell glomerular tufts to form a glomerular processing unit. Building on this study, we are currently analysing the contributions of inhibitory periglomerular and granule cell interneurons, with realistic physiological properties and synaptic connectivity, to shaping the mitral cell odor responses. The results show that both types of interneuron can mediate odor refinement through lateral inhibition, but with interesting differences. PGCs give stronger Mach band-like edge enhancement. GCs provide for selective inhibitory control of activity in individual mitral cell lateral dendrites with minimal effect on soma and other lateral dendrites. Some properties can be shown to depend on the dendritic architecture and cannot be obtained with reduced or artificial networks. An important realistic temporal constraint is that these contributions take place during a single respiratory cycle, which has been shown behaviorally to be sufficient for odor discrimination The results give new insights into the specific computational roles of PGCs and GCs in processing the odor maps in the glomerular layer for output to the cortex.

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176. Physiological Characterization of G Protein Coupled to Umami Receptors in Mouse Taste Receptor Cells

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Taste receptor cells (TRCs) accept taste stimuli. Recently, candidate umami receptors are identified molecular biologically in TRCs, and it is believed that their receptors are G protein–coupled receptor (GPCR) and coupled to taste tissue-specific G protein subunit α-gustducin (Gαgust). However, it is no physiologically evidence in TRCs that the GPCR and Gαgust participate to umami receptive. In order to clarify the signal components that participate in umami transduction, we recorded activation of TRCs by umami stimuli using whole cell patch clamp and Ca2+ imaging in mouse TRCs. When TRCs were treated with G protein inhibitor, GDP-β-S, TRCs lost umami-induced inward currents. And, many TRCs did not increase intracellular Ca2+ level to umami stimuli with Gαi inhibitor, *Pertussis* toxin treatment. Moreover, when TRCs that responded to umami stimuli were stained using immunohistochemistry, a few TRCs expressed Gαgust. In mouse TRCs, we demonstrated that umami stimuli might be accepted by GPCRs and the GPCRs might bind to mainly members of Gαi protein family. Furthermore, we showed that a few umami responses might be transduced by Gαgust in Gαi family.

177. Concurrent TAL: Aversive Processing via Capsaicin-Sensitive Vagal Afferent Fibres

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Taste aversion learning (TAL) is a type of learning characterized by rejection of a gustatory stimulus as a consequence of its pairing with visceral discomfort and malaise. TAL can be established in the laboratory following a concurrent procedure in which two flavoured stimuli are offered at the same time in each session for several minutes; the ingestion of one flavour is associated with the simultaneous intragastric administration of an aversive substance, whereas ingestion of the other is associated with an innocuous product. Previous studies with vagotomized or medullary afferent vagal axotomized animals demonstrated the involvement of the vagus nerve in concurrent TAL. The aim of this study was to examine the role of capsaicin-sensitive vagal afferent fibres in this modality of TAL in rats. Capsaicin is a neurotoxin that selectively destroys weakly myelinated A-delta or unmyelinated C afferent fibres, and it has been shown that around 99% vagus abdominal afferents are unmyelinated C-fiber. In this experiment, the neurotoxin was applied perineurally on the oesophagus in the subdiaphragmatic region. After 1 week of recovery, the animals (control and capsaicinized) were given a choice of two flavours (strawberry and coconut) for 7 minutes. The ingestion of one flavour was paired with simultaneous intragastric administration of hypertonic sodium chloride (NaCl) and the other flavour with physiological saline. This experimental procedure was prolonged over three trials. In agreement with previous studies in vagotomized or afferent axotomized animals, the results showed that perivagal administration of capsaicin blocked acquisition of concurrent TAL. In contrast, control animals effectively learned to avoid the flavour associated with hypertonic NaCl.

178. The Role of Vomeronasal Organ in Reception of Androstenone in Inbred Strains of Mice

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Sex boar pheromone androstenone was used as model odorant in our studies. Specific anosmia to androstenone (inability to smell the odorant) affects about 50% of adult humans. An animal model for this phenomenon has been developed using inbred strains of mice CBA/J (CBA) and NZB/B1NJ (NZB). Using Y-maze paradigm we estimated sensitivity of NZB and CBA mice to androstenone. NZB mice could detect 0.1% androstenone in mineral oil, but not 0.05% androstenone (a powerful odorant for people who smell androstenone). CBA mice could detect androstenone at a concentration 2000-fold more diluted. In more recent study we investigated possible involvement of vomeronasal organ in reception of androstenone. Patterns of c-fos expression were recorded in response to androstenone stimulation (0.1% w/v) in receptor tissue of highly sensitive to the compound CBA mice and in almost insensitive to the compound NZB mice. We observed activated cells in basal and apical zone of CBA mice. In NZB mice pattern of activation was observed only in apical zone. The data obtained indicate the involvement of vomeronasal organ in reception of androstenone in both strains of mice. Different distribution of activated receptor cell in CBA and NZB mice explains in part differences in sensitivity to the odorant.

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179. c-Fos Expression Is Induced by a Familiar Nonfood Related Odour in the Rat Olfactory Bulb and Is Increased by Fasting: Possible Implication of Orexins?

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Most animals depend on olfaction for food search and choice, and olfactory performances depend on nutritional status. For example, in rat olfactory bulb (OB), relations between nutrition and olfaction were suggested from results of electrophysiological studies: in anaesthetized animal, fasting induced an increase of mitral cell single-unit responses to both food and nonfood odour. We have chosen to quantify c-Fos expression as a marker of cellular activity in OB of fed or fasted rats submitted to different odours. After exposure to food odour, c-Fos expression significantly increased in OB of rats fasted for 48 h as compared to rats fasted for 4 hours; in contrast, the responses to a nonfamiliar odour (isoamyl acetate IA) were similar in the 2 groups of rats. As familiarization with odour altered c-fos expression in rat OB, we compared c-Fos expression in OB of rats submitted to IA either as a non familiar odour or as a familiar odour and fasted for 4 hours and for 48 hours. Whereas the rat nutritional status did not modified the OB responses to ''non familiar'' IA, after familiarization, the responses were significantly increased by food restriction. As orexins (OX) fibres and their receptors were present in rat OB and as OX level increased in OB after 48 h of food restriction, we hypothesised that the effect of nutritional status on OB responses could be due partly to these peptides. By immunohistochemistry, we determined if neurons expressing c-Fos also expressed OX receptors. We used quantitative PCR to estimate food restriction effect on gene expression of OX and their receptors. The implication in the increased responses to familiar odours of orexin1 receptors are tested by treating fasted rats with Ox1R antagonist SB334867.

180. Crossmodal Associations and Interactions between Olfaction and Vision

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We studied crossmodal links between olfactory and visual information processing. In Experiment 1, we investigated the existence and stability of olfactory–visual associations indirectly using a variation of the Implicit Association Test (IAT). Participants made speeded discrimination responses to a series of unimodal targets (strawberry or spearmint odour, a pink or a turquoise colour patch) by pressing one of two response keys. The assignment of stimuli onto the two response keys was varied in order to generate both compatible (e.g., strawberry and pink) and incompatible (e.g., spearmint and pink) response mappings. Odour–colour pairings sharing a stronger

crossmodal association resulted in better performance than those sharing a weaker association. In Experiment 2, we investigated the extent to which vision modulates olfactory information processing. Participants made speeded odour discrimination responses (strawberry vs. lemon) while viewing irrelevant visual information (a black line drawing of a square, a lemon, or a strawberry coloured either white, yellow, or red). The accuracy of participants' odour discrimination responses was significantly influenced by odour–colour compatibility: In particular, responses to the lemon odour were significantly less accurate when a red picture was presented than when a yellow picture was presented. By contrast, the congruency of the images (square, lemon, or strawberry) had absolutely no effect upon performance. Taken together, these results demonstrate both that odour–colour associations are stable enough to be highlighted indirectly and also that colour cues appear to exert a much stronger compatibility effect on olfactory discrimination performance than do pictorial cues.

181. Involvement of Dopamine in the Aversive Processing Induced by Intracranial Electrical Stimulation

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The general involvement of dopamine in brain reward as well as brain aversive processing is well established. Previous work in our lab has shown that lesions of the external lateral parabrachial subnucleus block concurrent taste aversion learning. In this study we demonstrate that electrical stimulation of this pontine subnucleus induces concurrent conditioned place aversion in a rectangular maze subdivided into three different compartments. Tiapride, a dopaminergic D2/D3 antagonist, impaired the aversive behaviour induced by electrical stimulation of the external lateral parabrachial subnucleus. A follow-up experiment showed that the effect cannot be explained in terms of a motor disruption induced by tiapride, since the number of crossings and rearings before the administration of the dopaminergic antagonist were similar to the number observed under its effect.

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182. GnRH-Immunoreactive Innervation of the Olfactory Bulbs in Female Mice during the Estrous Cycle

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In male hamsters, intracerebroventricular injections of GnRH partially restore the deficits in mating caused by vomeronasal (VN) lesions. This suggests that the expression of sexual behaviour is controlled by sexual steroids but also modulated by GnRH through VN- or olfactory-dependent mechanisms. We explore this possibility by analysing the distribution of GnRH-immunoreactive (GnRH-ir) fibres in olfactory and VN centres of ovariectomized female mice treated with vehicle (oil), estradiol, progesterone and estradiol + progesterone. Three hours after the last injection, animals were perfused with fixative and their brains were processed for GnRH immunohistochemistry. GnRH-ir cell bodies were found in the medial septum-diagonal band, medial preoptic area (around the vascular organ of the lamina terminalis), parts of the bed nucleus of the stria terminalis and anterior lateral hypothalamus. A few isolated GnRH-ir cell bodies were seen in the accessory olfactory bulb and in the taenia tecta. In the olfactory and VN systems only the olfactory bulbs showed substantial innervation, mainly found in the glomerular and outer plexiform layers of the accessory olfactory bulb (AOB, especially in its anterior part) and in specific locations of the main olfactory bulb (MOB). Acetyl cholinesterase histochemistry was performed in parallel sections of the bulb to explore the possible association of GnRH fibres with the atypical sexually dimorphic glomeruli of the MOB. Quantification of GnRH-ir innervation in the olfactory bulbs using computer-assisted image analysis is used to explore its putative modulation by sexual steroids.

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Session 4

Symposium 10: Revisiting the Functional Dichotomy in the Mammalian Olfactory System

183. Synergism, Potentiation And Inhibition Among Odorants—Examples from Olfactory and Human VN1-Type Receptors

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The human nose is exposed to odours that are shaped in many cases rather by mixtures of odorants than by a single odorant. For example, the key aroma compounds from food or human skin emanations are quite diverse, but also include homologous series of aliphatic odorants, that vary over size and functional groups. When tested with individually applied odorants, olfactory receptors (ORs) display specific odorant recognition profiles. At the receptor level, related odorants can antagonize each otherÆs effect. The complex actions of odorant mixtures on OR, however, are poorly understood. In 96-well Ca2+ imaging FLIPR experiments, we identified specific C7-C11 aliphatics as best agonists for all five human VN1-type receptors (VN1Rs), when expressed in HeLa/Olf cells. In binary mixtures with agonists, related odorants with the same functional group, but with a carbon chain length <C7, or >C11 acted as antagonists. Related odorants with a different functional group but similar length had no effect by themselves, but potentiated the effect of the agonist. For example, VN1R1 responded specifically to aldehydes with decanal as best agonist. Hexanal and undecanal concentration-dependently inhibited the decanal responses, while decanol had no effect by itself, but potentiated the decanal response about 2-fold. Similar effects were observed with the other four human VN1Rs. Aliphatic aldehydes and alcohols, both agonists on a receptor from the main olfactory epithelium, showed synergistic action in binary mixtures. Synergism, potentiation, and antagonism may

thus account for the dominating or masking of odorants in complex mixtures. Our observations with binary mixtures anticipate an even higher level of complexity of odorant coding at the level of ORs in the nose.

184. Expression of *Xenopus* Vomeronasal Receptor Genes (V1R) in Main Olfactory System

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In rodent, 100–200 V1Rs are specifically expressed in the vomeronasal organ (VNO) and are generally considered to be responsible for pheromone reception. However, a single V1R gene was expressed in olfactory epithelium (OE) of fishes, which lack VNO structure. Phylogenetically, VNOs first appeared in amphibians. To examine when the vertebrate V1R repertoire expands and functions as pheromone receptors in VNO during the course of evolution, we analyzed the genomic database of amphibian *Xenopus tropicalis* and identified 21 intact V1R sequences. We cloned all of these sequences from the genome and found that the expression of these genes was not detected in the VNO, but most of them were expressed in the OE of the middle cavity (MC), which is supposed to be specialized for detection of water-soluble odors. These results strongly suggest that V1R repertoire expanded after fish-amphibian species divergence by gene multiplication and sequence diversification, and raise the possibility that V1Rs function as receptors for water-soluble pheromones in the MC. Since the axons of the olfactory neurons in the MC project to the main olfactory bulb, it is possible that pheromonal information detected in the MC is transmitted and processed via the main olfactory system.

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185. The Ventrolateral/Ventromedial Region of the Olfactory Bulb: An Olfactory Fovea?

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In earlier work, we showed that glomeruli in the ventromedial and ventrolateral olfactory bulb (OB) differentially responded to odortypes (urine from mice differing at the major histocompatibility complex [MHC]). More recently, we found that CNGA2−/− mice respond to putative pheromones, implying the presence of cAMP-independent transduction pathways. We studied the responsiveness of individual glomeruli in CNGA2−/− mice by detecting odor-induced Fos expression in periglomerular cells (odor maps). While a subset of glomeruli activated by putative pheromones were necklace glomeruli (in which the second messenger is cGMP), the majority of active glomeruli in CNGA2−/− mice were not. Furthermore, we found that the TRPM5 channel, a member of the PLC pathway in taste cells, is coexpressed with CNGA2 in a subset of OSNs projecting to ventromedial/ventrolateral glomeruli that respond to putative pheromones and urine. While the olfactory deficits in TRPM5−/− mice are relatively mild, we find that mice defective for both TRPM5 and CNGA2 have a dramatic phenotype including severely diminished OBs and missing glomeruli in discrete areas. These data imply that the PLC/TRPM5 and cAMP pathways are coexpressed in a subset of urine-responsive OSNs. Interestingly, these OSNs are targeting their axons to the same ventromedial/ventrolateral OB areas we previously implicated in odortype discrimination. In a related vein of research, we have discovered that mice deficient for MHC receptors display altered targeting of OSN axons to glomeruli in the ventral OB. This suggests that the MHC plays a role in establishing the odor maps in the glomerular layer of the OB.

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186. Parallel Processing of Social Cues by the Main and Accessory Olfactory System

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The traditional concept that common odors are detected by the main olfactory system whereas the vomeronasal system acts as a specialized pheromone sensor has recently been challenged by various groups. Comparing the functional properties of both systems by a combination of molecular, electrophysiological, imaging, and behavioral approaches, we report here that the mouse main and accessory olfactory systems detect, in part, overlapping sets of molecular cues that regulate social behaviors (e.g. urinary pheromones, MHC peptide ligands). Both systems can process social chemosignals of volatile as well as nonvolatile nature, showing remarkable sensitivity with detection thresholds in the subnanomolar range. However, coding strategies and tuning properties of individual sensory neurons in MOE and VNO differ significantly and system-specific signal transduction pathways are used for the detection of these social cues. Moreover, apical and basal vomeronasal neurons apparently employ layer-specific transduction mechanisms. In behavioral tests using mice with both genetic and surgical lesions, selective stimulation of each system by the same cues triggers distinct sexual and social behaviors. In summary, our findings give rise to a model that involves parallel processing of the same molecular cues in both systems. Differential activation of each system might therefore lead to distinct behavioral outcomes.

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187. Cells in the Vomeronasal Organ Express Odorant Receptors but Project to the Accessory Olfactory Bulb

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In addition to the main olfactory system, most mammals possess a well-developed vomeronasal system that is considered to be

specialized for detecting pheromones. Within the vomeronasal organ (VNO), two populations of sensory neurons are distinguished; cells in the apical layer expressing V1R-receptors and cells in the basal layer expressing V2R receptors. Recent evidence indicates that the VNO of mice not only responds to pheromonal compounds but also to general odorants. RT-PCR and in situ hybridization studies indicated that certain members of the OR gene repertoire are expressed in the VNO. These genes expressed in the VNO were found to be scattered over several genomic clusters and were concomitantly expressed in cells of the MOE. Gene-targeted mice which coexpress histological markers with the receptor mOR18-2 allowed to unravel characteristic morphological differences between OR-expressing cells in the VNO compared to the MOE. Experiments towards a molecular phenotyping of these cells revealed that the OR-cells in the VNO did not share elements of the signal transduction machinery typical for MOE neurons, such as ACIII or Golf, but rather expressed TRPC2 and Gi. Visualizing the axonal processes of VNO cells expressing distinct ORs revealed that they projected to the accessory olfactory bulb (AOB). Axon fibers were visible in the anterior AOB where they converged into glomerular-like structures. The finding that distinct OR-types are expressed in cells which are located in the apical layer of the VNO and have the typical features of VNO sensory neurons including the projection to the AOB suggest that this population of sensory cells may be a novel facet in the complexity of the chemosensory system.

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188. Feedback Loops Link Odor and Pheromone Signaling with Reproduction

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Pheromones can have profound effects on reproductive physiology and behavior in mammals. To investigate the neural circuits underlying these effects, we used a genetic transneuronal tracer to identify neurons that synapse with GnRH neurons, the key regulators of reproduction. We then asked whether the connected neurons are presynaptic or postsynaptic to GnRH neurons, and analyzed their responses to chemosensory cues. Surprisingly, these experiments indicate that GnRH neurons receive pheromone signals from both odor and pheromone relays in the brain, and may also receive common odor signals. Moreover, feedback loops are evident whereby GnRH neurons could influence both odor and pheromone processing. Remarkably, ~800 GnRH neurons communicate with ~50,000 neurons in 53 functionally diverse brain areas, with some connections exhibiting sexual dimorphism. These studies reveal a complex interplay between reproduction and other functions in which GnRH neurons appear to integrate information from multiple sources and modulate a variety of brain functions.

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Symposium 11: The Olfactory System in Teleosts. From Sensory Neurones to Behaviour

189. Optical Imaging and Molecular Characterisation of Receptor Gene Repertoires in the Zebrafish, *Danio rerio*

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To understand the perception of odor quality, it is essential to establish the ligand spectra of individual olfactory receptors, as well as the nature of the receptor repertoires that are activated by particular olfactory stimuli. We employ the model system zebrafish to study these problems using molecular biological and physiological methods. The response properties of many olfactory receptors can be visualized simultaneously by optical imaging of neuronal activity in the olfactory bulb, since olfactory receptor neurons expressing the same olfactory receptor converge onto common glomeruli. Odor responses can be measured selectively in the presynaptic compartment of glomeruli by introducing a calcium-sensitive dye exclusively into olfactory receptor neurons, via axonal transport from the nose. We have used this technique previously to demonstrate chemotopy and combinatorial representation of odorants. Furthermore we derived estimates about the structural requirements and minimal number of different zebrafish olfactory receptors that respond to amino acids, a major component of feeding stimuli in fish. We now have characterized the zebrafish olfactory receptor gene repertoire (class C), which appears to represent the best candidate for the family of amino acid receptors. We report that about fifty olfactory class C genes segregate into two opponent groups with respect to evolutionary constraints, phylogenetic position, and expression pattern. We also characterized a novel olfactory receptor gene family which may constitute pheromone receptors.

190. A Novel Olfactory Receptor Gene Family in Teleost Fish

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Pheromones mediate sexual and social communication between individuals. Reproduction in fish is governed by a handful of identified pheromones, which actually are sexual hormones that double as ligands for as yet unidentified olfactory receptors. In mammals two large families of up to 200 vomeronasal receptor genes (V1Rs and V2Rs) may serve as pheromone receptors. V2R receptors have been recognized as an evolutionary old family—with about 50 members present already in several fish species, but an extensive search of the fish genome so far has unearthed only a single V1R-like receptor gene. Thus the V1R family appeared to be evolutionary rather recent, originating as a family with tetrapods. We now identified five ancestral V1R-related genes, with orthologues detected for each gene in every teleost species analysed (zebrafish, two pufferfish, medaka). These genes form a coherent family in the phylogenetic analysis, containing in a single branch the whole mammalian V1R superfamily. Expression pattern, phylogenetic position and family size support a role as olfactory pheromone receptors.

191. Parallel Pathways in the Fish Olfactory System; Morphology, Physiology and Behaviour

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The olfactory organ of teleosts consists of three morphological types of olfactory receptor neurones ORN; namely ciliated and microvillous sensory neurones and crypt cells. 1) Axons from the ciliated ORN project to relay neurones in the medial part of the olfactory bulb; and axons of these neurones form the medial part of the medial olfactory tract, mMOT. The mMOT mediates the alarm reaction in crucian carp. 2) Axons of the microvillous ORN project to relay neurones in the lateral part of the olfactory bulb; and axons of these neurones form the lateral olfactory tract, LOT. The LOT mediates feeding behaviour. 3) The axons of the crypt cells project to relay neurones in the ventral part of the olfactory bulb; and axons of these neurones form the lateral part of the medial olfactory tract, lMOT. The lMOT mediates behaviours related to reproduction. A gender distinction is evident from physiological recordings of the spike activity of the relay neurones in the ventral part of the bulb responding to sex pheromones. In crucian carp the male bulbar neurones respond specifically to each of the four sex substances, while the neurones in the female olfactory bulb do not. These studies on the crucian carp show that the teleosts olfactory system is composed of distinct elements constituting three parallel pathways from the epithelium to the telencephalon.

For review see Hamdani, D Ving. 2005. Functional organization of the olfactory system in fish. In: Ladich et al., editors. Fish communication. p 223–257. Supported by the Norwegian Research Council.

192. Antalarmin Inhibits Alarm Reaction in Crucian Carp (*Carassius carassius*)

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Many fish species perform alarm reactions when exposed to olfactory cues from injured conspecifics. In the crucian carp this response includes characteristic components of avoidance behaviour such as escape and freezing. The peripheral parts of the olfactory system implicated in the alarm reaction have been described, but the central mechanisms involved are largely unknown. Corticotrophin-releasing factor (CRF) is central in the mediation of behavioural and physiological reactions to fear and stress, but its role in nonmammalian vertebrates needs further investigation. The involvement of CRF in the crucian carp alarm reaction was studied by intraperitoneal injections of the nonpeptidergic CRF1 antagonist antalarmin. One hour after injection, the fish were exposed to skin extract from conspecifics. The treatment resulted in an inhibition of both escape and freezing behaviour, while vehicle treated individuals displayed fully expressed alarm reactions. These observations suggest that the CRF1 receptor is involved in the crucian carp alarm reaction, and give support to the idea that CRF might be a component in fear induced behaviour among vertebrates.

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193. Seasonal Variations in the Appearance of Crypt Cells in the Olfactory Epithelium of the Crucian Carp

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The crypt cells in the olfactory epithelium are sensory neurones implied in responses to sex pheromones in crucian carp. Some inconsistencies in appearance of these cells were suspected to be related to the reproductive cycle. Therefore, the seasonal variations were studied in a group of 100 crucian carps caged in an outdoor pond exposed to natural conditions. Fish were sampled every month; sex, gonadal stage, age and length were determined, and aliquots taken of blood and bile. The fish were perfused with paraformaldehyde, and crystals of a neural tracer, the fluorescent carbocyanine dye (DiI) were applied to the olfactory bulbs. Inspection of the olfactory epithelium in microscope slides revealed that the number of crypt cells in the olfactory epithelium was drastically reduced during the period from August to December from several hundred to less than 10 per olfactory rosette. In the same period the crypt cells were found to migrate from their usual appearance at the surface, towards the basal lamina of the sensory epithelium, indicating that they were not exposed to the environment. These findings demonstrate an unpredicted variation in appearance of the olfactory sensory neurones in vertebrates that has not been observed before. The findings have implications for our understanding of the function of the fish olfactory system and fish ecology.

194. Olfactory Discrimination, Chemotopy and Perception in Fishes

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Differential glomerular activity patterns in the zebrafish olfactory bulb enable olfactory discrimination of amino acids, whereas highly similar patterns do not enable the discrimination. As indicated in the glomerular activity patterns, zebrafish discriminate nearly every conditioned amino acid from every other amino acid. Two exceptions were predicted from a recent electrophysiological study and confirmed in behavioral studies; that is, zebrafish cannot discriminate L-Phe from L-Tyr or L-Ile from L-Val. Catfish also discriminate most amino acids and their mixtures, but they cannot discriminate L-Ile from L-Val, which indicates similar olfactory systems in unrelated fishes. Since receptor potentials of spontaneously inactive olfactory receptor neurons (ORNs) add up into EOG amplitude, equal EOG amplitudes indicate equal effectiveness level of different olfactory stimuli. Binary mixtures prepared from unequally stimulatory amino acids are initially detected as the more stimulatory component of the mixture. Catfish can detect modifications of the binary mixture percepts caused by the minor component only after additional experience with the mixture. In conclusion, any single amino acid, their binary and complex mixtures are always detected as singular percepts that are refined with experience. Components of mixtures, except for the temporary perception of the more stimulatory components of binary and ternary mixtures, are not detected. Using smell alone neither single amino acids

nor their mixtures can be classified as either pure odorants or mixtures. Percepts of olfactory stimuli that exist in inner world of the brain do not contain odorant complexity information.

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Symposium 12: Appetitive and Aversive Aspects of Taste Sensation

195. Inflammation Regulates the Interferon Signaling Pathways in Taste Buds

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Taste disorders, including hypogeusia, ageusia and dysgeusia, contribute to malnutrition, cachexia, depression and a compromised quality of life. The common causes of taste disorders include infection, chronic inflammation, medication, radiation and aging. To investigate possible mechanisms involved in the development of taste disorders, we studied the expression of inflammatory pathways in mouse taste tissues. Interferons are multifunctional cytokines produced during viral and bacterial infection. By regulating the expression of numerous genes, interferons affect cell proliferation, differentiation and apoptosis. Using RT-PCR analysis we showed that genes involved in both type I and type II interferon signaling are expressed in mouse taste tissues. In situ hybridization and immuno-fluorescence studies showed that the interferon-γ receptor IFNGR1 is preferentially expressed in the taste buds of γ interferon-fungiform, foliate, and circumvallate papillae. Within taste buds, IFNGR1 is expressed predominantly in a subset of taste cells that also express NCAM, whereas, the expression of IFNGR1 and α-gustducin is largely non-overlapping, suggesting that IFNGR1 is expressed mostly in the type III taste receptor cells. Furthermore, inflammation induced by intraperitoneal injection of double-stranded RNA (poly[rI:rC]) or bacterial endotoxin lipopolysaccharide (LPS) upregulates the expression of interferon-inducible genes in taste tissues. The expression of Mx1 and IRF1 is elevated to a higher level in the taste epithelium than in the surrounding nongustatory lingual epithelium. Together, these data suggest that inflammation can activate the interferon signaling pathways and alter gene expression in mouse taste tissues.

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196. Identification of Agonist Interaction Sites in the Human Bitter Taste Receptor hTAS2R46

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It is commonly assumed that the bitter taste receptors of the hTAS2R gene family enable the recognition of bitter compounds to caution us against the ingestion of poisonous food. However, it is not clear how humans can perceive thousands of structurally diverse bitter substances with only 25 bitter taste receptors. As little is known about the structure–function relationship of hTAS2Rs and their agonists, we want to elucidate the structural basis for the bitter taste receptor–agonist interactions. The receptor hTAS2R46 is activated by many bitter substances present in plants used for the production of food and alcoholic drinks. The receptor responds to a number of sesquiterpene lactones and diterpenoids. Furthermore, hTAS2R46 is a sensitive bitter taste receptor responsive to strychnine. For the characterization of the hTAS2R46 agonist binding domain, we utilize closely related bitter receptors, for example, hTAS2R43 and -R44. We hypothesize that sequence differences within variable regions of extracellular loops (ec) and transmembrane domains (TM) in highly homologous receptors relate to different agonist profiles. Calcium imaging of heterologously expressed hTAS2R44/46 chimeras revealed that ec and TM contribute to specific interactions with agonists. In these receptor parts we subsequently mutated several residues from the hTAS2R46 aa sequence to the corresponding hTAS2R44 aa sequence and vice versa. Using this strategy we identified residues that determine the activation of hTAS2R46 by its agonists absinthin, strychnine and denatonium, as well as residues for the response of hTAS2R44 on aristolochic acid. To identify additional residues that influence receptor–agonist interaction we will introduce these positions into less related bitter receptors, for example, hTAS2R47.

197. Differential Involvement of Insular Cortex and Basolateral Amygdala in Aversive and Appetitive Taste Memory Formation

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During feeding, the subject learns to associate the sensory characteristics of food, particularly the taste, with the postingestive consequences. When ingestion of a novel taste solution is followed by a visceral malaise, the subject will avoid further consumption of this taste solution. Conversely, if the novel taste consumption leads to an energy intake or even an absence of negative consequences, the intake of that taste solution will subsequently be enhanced. We will focus on recent investigations with one-trial aversive (conditioned taste aversion) and appetitive learning paradigms in rats using the sweet taste saccharin 0.1%. The role of insular cortex and basolateral amygdala in gustatory and visceral information processing will be addressed. Using cellular imaging, microdialysis and pharmacological approaches, I will present recent evidence suggesting that some neurochemical (cholinergic, glutamatergic and b-adrenergic receptor activation) and cellular (immediate early gene c-fos and protein synthesis) mechanisms in these forebrain areas are common and others different in aversive and appetitive taste memory formation.

198. The GABAergic System in the Ventral Pallidum Is Involved in the Retrieval of Conditioned Taste Aversion in Rats

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When rats drink a taste solution and are followed by malaise, the rats acquire aversion to the taste solution (conditioned taste aversion, CTA). Hedonics of taste solution shifts from ingestive to aversive as a function of CTA. Recently, we found that the ventral pallidum (VP), a part of the reward system, plays a role in hedonics of taste. Therefore, to elucidate the role of VP on CTA, we examined the effects of microinjections of a GABAA receptor antagonist, bicuculline, into the VP at the retrieval of CTA. In Experiment 1, rats received a pairing of saccharin or QHCl (conditioned stimulus, CS) with an i.p. injection of 0.15 M lithium chloride (unconditioned stimulus, US) using a single-bottle method. After this conditioning, vehicle or bicuculline was bilaterally infused into the VP before the reexposure to the CS on the test day. The bicuculline-injections significantly increased the saccharin intake, but not QHCl. In Experiment 2, we tested the effects of bicuculline-injections on the behavioral responses to saccharin CS using an intraoral infusion test. The bicuculline-injections decreased the aversive responses and increased ingestive responses to saccharin CS on the test day. Therefore, the administration of bicuculline into the VP might shift the hedonics of saccharin CS from aversive to ingestive. In Experiment 3, we investigated the release of GABA in the VP during the retrieval of CTA using the microdialysis technique. It was found that the presentation of saccharin CS increased the GABA release in the VP. These results suggest that the activation of GABAergic neurons in the VP may be involved in the reduction of intake with aversive responses to reexposure to saccharin CS after CTA.

199. Fat Flavor Preferences in CD36, Trpm5 and Gustducin Knockout Mice

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The orosensory appeal of high-fat foods has been attributed to their texture and odor, but several findings suggest a ôfattyö taste may also exist. The CD36 fatty acid transporter is a candidate fat taste receptor due to its affinity for long-chain fatty acids, its presence in taste cells and the lack of linoleic acid preference in CD36 knockout mice. The present study measured preferences for nutritive (soybean) and nonnutritive (olestra) oils in CD36 mice and C57BL/6J (B6) controls. Trpm5 and gustducin (Gus) knockout mice were also studied. Gustducin and Trpm5 are part of the signaling pathway involved in sweet, umami and bitter taste transduction and may also contribute to fat taste signaling. In 48-h 2-bottle tests with dilute soybean oil emulsions (0.313–1.25%), CD36 and Trpm5 mice showed weak or no oil preference whereas B6 and Gus mice strongly preferred oil to emulsifier vehicle. At higher concentrations (2.5–20%) CD36 and Trpm5 mice developed significant soybean oil preferences which may be mediated in part by the known postoral reinforcing effect of fat. Two-bottle tests with olestra (0.313–2.5%) revealed significant preferences for the nonnutritive oil in B6 and Gus mice but no preference in CD36 and Trpm5 mice. The lack of preference for dilute soybean oil emulsions in CD36 and Trpm5 mice supports a gustatory contribution to fat orosensation. However, the finding that these mice also failed to prefer olestra oil, which is assumed to provide texture rather than taste cues, requires explanation. The preferences observed in Gus mice indicate that gustducin, despite its colocalization with CD36 in taste cells, is not critical to the orosensation of nutritive and nonnutritive oils.

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200. Integration of Orosensory and Postingestive Stimuli for the Rewarding Effects of Dietary Oil

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Mice showed a strong preference for dietary oil and long chain fatty acids in voluntary intake of oil in rewarded (reinforcement) conditioning of CPP test, which has been used to evaluate the reward effects of addictive drugs. Artificially synthesized oil (fatty acids sorbitol ester: ASO), low in calories because it is not digestible by lipase, had no reward effect, even though mice still preferred the indigestible fat over the control in a two-bottle choice test. Palatability of the ASO was similar to corn oil over 30 min in the short-term two-bottle choice test in mice. However, mice did not continue to eat the ASO in the long-term two-bottle choice test, which included postprandial feedback effects. Moreover, the ASO did not act as a reinforcer in the CPP test. Mice with 0.1 ml of corn oil placed into their stomachs just before conditioning showed reinforcing effects on taking the ASO in the CPP test. These results suggests that the postingestible effects of corn oil are involved in the long-term preference and reinforcing effects. On the other hand, intragastric administration of medium-chain fatty acid triglyceride (MCT) instead of corn oil with voluntary intake of the ASO did not show any such reinforcing effects against the ASO. Administration of intravenous fat-transfusion just before conditioning did not showed reinforcing effects on taking the ASO in the CPP test. These data suggest the chemical recognition of the long-chain fatty acid in the gastrointestinal tract may be connected in the postingestive effects in the CPP test.

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Oral Session 4: Sweetness Research

201. Homology Models of the Sweet Taste Receptor: A Study of their Interactions with Different Classes of Sweet Compounds

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Significant progress has been made in the last few years in our understanding of the mechanism of sweet taste chemoreception. Functional dimeric receptors for coexpression of T1R2/T1R3, activated by a variety of sweet compounds (carbohydrates, amino acids, peptides, proteins, synthetic sweeteners) have been obtained, supporting the idea that there is a single receptor for sweet taste. The 3-D structure of such receptor is still unknown. All possible dimers formed by combinations of the human T1R2 and T1R3 subunits, modelled on the A (closed) or B (open) chains of the extracellular domain of the metabotropic glutamate receptor were derived by homology modelling, their ligand binding sites identified, probed by docking a large set of sweeteners representative of all classes of sweet compounds and calculating the free energy of ligand binding consistent with their relative sweetness. Our results showed that the closed form can host a smaller number of compounds than the open one. At least 3 of the 4 compounds that can bind to active sites of closed protomers show synergy with other sweet compounds, suggesting that, although the binding in a single subunit is sufficient for receptor activation, the binding of a ligand in the second subunit increases the response. We demonstrated that sweet proteins can bind to a secondary site. A confirmation of the ligand binding sites identified and of the predictive power of the model came from the good prediction of the sweetness of some sweet compounds recently reported in the literature. Our models are consistent with experimental observation on sweeteners' taste, including synergy and can help to design new sweeteners.

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202. Modeling and Interpretation of Gustatory Response Curves by a Statistical Physics Treatment for Sweet Molecules

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The purpose of this study is to give an analytic expression of the gerbil gustatory response relative to some sweet molecules using a statistical physics treatment by the grand canonical ensemble. This method allowed firstly to study the stimulus-receptor site interaction, secondly to better understand the taste mechanism and to interpret this phenomenon at the microscopic level. This work took the electrophysiological responses curves as a support which are taken from the literature. The activity of the whole gustatory nerve (Chorda Tympani) of the gerbil is considered. We have chosen four curves giving the responses according to the concentrations of stimuli molecules (sucrose, tetracholorogalactosucrose, maltose and maltitol). Some simplifying approaches are considered to drive the theoretical model of the concentration–response curve. The physicochemical parameters involved in the analytical expression have then a physical significance. We attempt to interpret the behavior of these physicochemical parameters for the four sweet molecules deduced by numerical simulation.

203. More than Sweeteners: Temporal Perception of Natural and Synthetic Sweeteners

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The great interest of research on chemoreception and chemesynthesis is explained by the fact that food and pharmaceutical industries are always searching for novel compounds to be used as additives or substitutes of molecules available on the market. In a previous study it was evidenced that the compound (±)-2-(3-hydroxy-4′-methoxyphenyl)-3,1-benzoxathiane (HMB), that is, a synthetic Phyllodulcin derivate, is characterised, besides its sweet taste, by a Liquorice-like flavour and a Cooling sensation that persist over time. The aim of the present study was to evaluate the temporal perception of the natural compound Phyllodulcin and its synthetic derivate HMB for the sensory characteristics mentioned above by using the time-intensity methodology. For Sweetness and Cooling sensation compounds available on the market, i. e. sucrose for Sweetness and WS3 for Cooling sensation, were used as references. A trained panel of 14 subjects, 6 females and 8 males (mean age: 27.1 ± 3.2 years), rated the temporal perception of the sensory attributes by using an unstructured vertical line scale anchored at the extremes with "weak" (corresponding to 0) and "strong" (corresponding to 10). Panel reliability was estimated by means of noncentred PCA. Results evidenced that, Phyllodulcin and HMB were defined by similar curves and the two sweeteners showed shapes similar to reference compounds for all the attributes evaluated, except for Sweetness. Phyllodulcin and HMB were characterised by a shorter persistence and a shorter time to extinction with respect to sucrose. Results observed suggest that Phyllodulcin and HMB could be considered as interesting additives to be used as sucrose substitutes and as possible cooling enhancers.

204. Genetics of Sweet Taste in Carnivores

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Sweet taste is transduced primarily by one receptor, a dimer of two proteins, T1R2 and T1R3 (genes symbols Tas1r2 and Tas1r3). Mutations in either protein could have a spectrum of consequences, from little or no alterations, to complete loss of activity of the receptor. A particularly striking example of the effect of mutations on the sweet receptor has been found in various species of carnivores. These responses to sweet stimuli range from apparent indifference as in the cats, to profound avidity, such as in the dog. Our findings suggest that cats cannot taste sweet due to pseudogenization of Tas1r2. This indifference to sweets is in marked contrast with that of dog, possessing a functional T1R2. It is very likely that a mutation of Tas1r2 in Felidae occurred after the divergence of Feliformia and Caniformia. We hypothesized that this mutation is not uniform across all species of Feliformia, with the result that some species retain all or part of the ability to taste sweet while others are sweet taste blind. Our basic procedures are to perform behavioral studies in selected species of Feliformia to evaluate their ability to taste sweet stimuli and to examine the molecular basis for sweet perception in carnivores by characterizing sweet receptor genes in these same species. Three species within Feliformia, each with distinct food choices, have been selected: genet, hyena and mongoose. Several sugars and artificial sweeteners have been chosen and two-choice tests have begun. DNA sequences of sweet receptor genes are being obtained by PCR. Differences in taste behavior will be

correlated with differences in gene sequence and structure to determine critical regions for sweet taste avidity and thereby add to our understanding of the evolution of sweet taste.

205. Sweetness Enhancement by Ethyl Butyrate at Subthreshold Concentrations

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Central sensory integration at a subthreshold level was investigated by studying sweetness modulation by retronasal olfaction using odorants at a subthreshold level. In a first experiment, 9-naïve subjects evaluated the impact of 6 odorants at a suprathreshold concentration on sweetness of a sucrose solution. The odorant having the highest (ethyl butyrate) and the lowest (maltol) boosting impact on sweetness enhancement were selected. Another panel of 12 naïve subjects performed a second experiment with the two selected odorants at subthreshold concentrations. The detection threshold of the subjects for these two odorants was determined. The impact of odorant at subthreshold level on sweetness was investigated using a liquid delivery system based on a 4-channel HPLC pump. The apparatus allowed to deliver continuously a 25 ml/min flow sucrose solution during 180 s with five different odorant concentrations while keeping constant the sucrose concentration. At a subthreshold level, only ethyl butyrate significantly enhanced the sweetness of the sucrose solution. This study highlights that retronasal olfactory perception induced by odorants at subthreshold levels can enhance significantly sweetness. If validated in real drink or food, this finding may be key in the current food industry context of sucrose content reduction.

206. Aroma Network Presentation

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A thematic and dinamized network of technology transfer has been constituted about aroma and fragrances (2003XT 00041). The network was financed by the Agència de Gestió d'Ajudes Universitàries i d'Investigació (AGAUR) of the Generalitat de Catalunya. Nine investigation groups distributed in different national (UB, UPC Terrassa, URV, UZ), international (University of Udine) universities, investigation centers (CSIC of Barcelona) and research and development units in some companies (Dallant, S.A. and Freixenet S.A.) belong to the network. This network gathers diversity of researchers dedicated to the study of the aromas from different points of view and different objectives. For this reason, the network offers experience and complete information in different topics as isolation, identification, quantification, formulation, and perception of the aroma. The cross-disciplinary character, infrastructure and human potential allow approaching ambitious projects that gather, economic and investigation interest, in the same platform. Network objectives embrace recognition and increasing interest in organizing the investigation around the researcher association who belongs to different competent scientific areas from the same scientific field as aromas and fragrances. Specifically, the purpose of the network is coordinate and join the efforts of the different investigation groups which works in different aspects of aroma and essential oils in order to dinamize the investigation and development in that field. Optimize the use of the existing resources in the different groups, specially in relation to infrastructures, in order to offer service to the companies of aromas and/or food, pharmaceutical and cosmetic as to those dedicated to the development of sensors. Present the possibilities of collaboration and promotion of technology to the companies of the sector in order to establish contracts of investigation, technical advising or formation.

Panel Discussion 5: Virgin Olive Oil and Sensory Analysis

207. Virgin Olive Oil and Health

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Because high plasma cholesterol levels are a risk factor for coronary diseases, there is widespread support for a dietary approach that prevents this type of disease by reducing plasma cholesterol. Approximately half of the Spanish population currently presents with blood cholesterol values above 200 mg/dl and cardiovascular disease (CVD) remains the prime cause of death in our country. A healthy and balanced diet can impact on cardiovascular risk factors, reducing body mass index and blood pressure, improving the lipid profile, controlling glycemia and reducing thrombosis risk. Epidemiological studies have shown that dietary factors are responsible for the large differences in cardiovascular mortality among countries. The habitual presence of virgin olive oil in the diet is highly advantageous to health because of its oleic acid and antioxidant content. Lesser components of virgin olive oil also have beneficial effects against CVD. Oral or parenteral administration of vegetable sterols and stanols reduces levels of total cholesterol and cholesterol associated with low density lipoproteins, raises levels of cholesterol associated with high density lipoproteins and also has an antioxidant effect The reduction is probably largely due to inhibition of intestinal cholesterol. Numerous epidemiological studies have shown the protective effect of virgin olive oil in the diet against certain types of cancer. One of the greatest risks for the formation of malignant tumours is cellular oxidative status. Thus, the greater the susceptibility of the cell to oxidation, the higher is the cancer risk. Colon-rectal, prostate and breast cancers show the closest relationship with diet. A study of Spanish women who consume a large amount of virgin olive oil showed that they had a low risk of developing breast cancer. In Italy, a similar study related the lower breast cancer risk to a higher intake of monosaturated fatty acids, since 80% of the oil consumed in the country is virgin olive oil. It can be concluded that the health advantages of the habitual consumption of virgin olive oil derive from its composition, both its lipid profile and its lesser components, and especially from the synergic effect among these components, endorsing virgin olive oil as the main source of lipids in our diet.

208. Nutritional Properties of Virgin Olive Oil and Sensory Analysis

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The acidic profile of the extra virgin olive oil and the presence of secondary components guarantee olive oil as the first source of lipids in our diet. The saponifiable fraction, greater than 95%, is formed principally by triglycerides and six of the main fatty acids, oleic, stearic, palmitic, palmitoleic, linoleic and linolenic with enough amounts for covering the human body needs. The secondary components, reaching 1.5%, are not less important. Some of them are beneficial for the human health, others help to oil preservation and others are responsible of extra virgin olive oil aroma and palatability. Previous research and epidemiologic reports since the eighties have shown that including extra virgin olive oil in the diet protects against the oxidative damage and several diseases, such as cardiac ischemia and cancer. Squalene, sterols, tocoferols, phenolic components and mainly the synergic effects among the individual components protect against low-density lipoproteins oxidation and free radicals-induced damage. The organoleptic assessment of the virgin olive oil applied only to the classification of virgin olive oil according to the appearance of the fruit attribute and the intensity of faults, assessed by a selected and well trained tasters panel according to CE N° 796/2002 (6 May 2002). Assessed by this method, extra virgin olive oil is recommended to be included in the diet because the preservation of its properties and health benefits. Nevertheless, an additional method with a different rating sheet is used for sensory analysis, which allows us distinguishing among different extra virgin olive oils. The proposed speakers will cover these aspects.

209. Biosensor Measurements for the Assessment of Bitterness and Pungency of Virgin Olive Oil

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Bitterness and pungency, sensory quality attributes of virgin olive oil, are related to the presence of phenolic compounds. Fast and reliable alternatives for the evaluation of sensory attributes and phenolic content are desirable, as sensory and traditional analytical methods are time consuming and expensive. In this study, two amperometric enzyme-based biosensors (employing tyrosinase or peroxidase) for rapid measurement of polar phenolics of olive oil were tested. The biosensor was constructed using disposable screen-printed carbon electrodes with the enzyme as biorecognition element. The sensor was coupled with a simple extraction procedure and optimised for use in flow injection analysis. The performance of the biosensor was assessed by measuring a set of virgin olive oils and comparing the results with data obtained by the reference HPLC method and sensory scores. The correlations between the tyrosinase- and peroxidase-based biosensors and phenolic content in the samples were high (r = 0.82 and r = 0.87, respectively), which, together with a good repeatability (rsd 6%), suggests that these biosensors may represent a promising tool in the analysis of the total content of phenolics in virgin olive oils. The correlation with sensory quality attributes of virgin olive oil was lower, which illustrates the complexity of sensory perception. The two biosensors possessed different specificity towards different groups of phenolics, affecting bitterness and pungency prediction. The peroxidase-based biosensor showed a significant correlation (r = 0.66) with pungency.

Poster Session 5

210. Olfactory Neurons as Landmine Detector

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Today more than 80 countries are affected to some degree by antipersonnel landmines. It is estimated that their presence cause more than 40 causalities each day. They are indiscriminating and inhuman. The global landmine problem needs to be tackled from many different angles. Action is needed to remove mines from the ground. In this regard, animals such as dogs, little pigs and giant rats, which exhibit a characteristically sensitive olfactory system, have been of crucial help for their detection in the field. However the use of live animals for mine detection imposes an ethical problem and the search for other sensitive, nonanimal, devices is desirable. A critical step in the design of such devices is the understating of how the chemicals present in mines interact with the olfactory system and how the olfactory system processes this information. Using electro-olfactogram (EOG) we recorded the response of mouse olfactory epithelium to explosive like cyclotrimethylenetrinitramine (RDX) and 2,4,6-trinitrotoluene (TNT) and also to some volatile compound of land-mine caps. Further, to elaborate on the response of olfactory neurons to explosives (TNT and RDX), we used Calcium Imaging on dissociated neurons to monitor the increase in the level of intracellular calcium. These preliminary results show that volatile compounds released by landmines cap and explosives can elicit a response in a population of isolated OSN. In conclusion this could be a first step in identification of a set of OR that could be used to create a biosensor useful in a fast and safe recognition of landmines.

211. Scaffolding Proteins as Possible Organizers of the Constituents of Rat Olfactory Transduction

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Odorants induce excitatory or inhibitory responses, as increases or decreases in firing rate, respectively. Both response types can be generated by a single ORN and are thought to involve G-protein–coupled odor receptors that trigger a cAMP cascade, activating cyclic nucleotide gated channels. A Ca2+ influx through such channels activates depolarizing Ca2+-dependent Cl– channels, in the first case, and hyperpolarizing Ca2+-dependent K+ channels, in the second situation. Since cAMP and Ca2+ increases are confined to the cilia, it seems likely that the chemotransduction cascade constituents are organized into complexes, as in other transduction

systems. Such organization might be attained by scaffolding proteins. We investigated the presence of scaffolding proteins in olfactory cilia. By Western blots and immunohistochemistry we found ProSAP1 and 2, of the ProSAP family, CASK, SAP97 and SAP102, from MAGuK family, and mLIN-7. Immunoprecipitation assays suggested an interaction between ProSAP2 and CaMKIIα, an enzyme that regulates the transduction cascade, contributing to the response termination. The epithelial SAP102 migrated at an apparent lower molecular weight (MW) in Western blots than expected ($\sim$80 vs. 100 kDa). By RT-PCR we detected three new splicing variants of SAP102, which had not been previously described. Translation analysis showed that one of them might correspond to lower MW band in the Western blots analysis. We conclude that specific scaffolding proteins are present in olfactory epithelium. All but SAP102 are enriched in cilia, where they may have a key role in chemotransduction.

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212. Identification of Inhibitors of the Bitter Aftertaste of Artificial Sweeteners

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Artificial sweeteners assist in weight management, prevention of dental caries, and control of blood glucose for diabetics. But the two widely used sulfonamide sweeteners, saccharin and acesulfame K, possess an intrinsic lingering bitter aftertaste, which increases at higher concentrations and limits their use. Understanding the aftertaste mechanism allows for the development of novel flavorants that may be used to improve consumer acceptance for this family of artificial sweeteners. By functional expression experiments in human embryonic kidney cells it has been shown that the sweeteners specifically activate two members of the family of human bitter taste receptors, hTAS2R43 and hTAS2R44, at concentrations known to stimulate bitter taste. Based on these findings, we generated an assay suitable for high throughput screening of substances capable of modifying the bitter aftertaste of the sweeteners. We used HEK-293T cells stably expressing the chimeric G-protein subunit, G16gust44, to couple activation of the stably expressed TAS2R receptors to the release of Ca2+ from intracellular stores, which can be measured using a fluorescent imaging plate reader (FLIPR). A diverse chemical library of 12,000 compounds has been screened for their ability to inhibit the response of the two bitter taste receptors to saccharin or acesulfame K. Concentration–response curves have been performed to determine the specificity and the potency of the initially identified hits. We will present our findings on biochemical and sensorial analysis of these candidate bitter masking molecules, which are targeted for reducing the bitter aftertaste of sulfonamide sweeteners.

213. Neural Representation of Olfactory Mixtures in the Honeybee Antennal Lobe

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Natural olfactory stimuli occur as mixtures of many single odorants. We studied whether the representation of a mixture in the brain retains single-odor information (elemental rule) and how much mixture-specific information it includes (configural rule). To understand mixture representation in the honeybee brain, we used in vivo calcium imaging at the level of the antennal lobe, and systematically measured odor-evoked activity in 24 identified glomeruli in response to four single odorants and all their possible binary, ternary and quaternary mixtures. Qualitatively, mixture-induced activity patterns always contained glomeruli belonging to the pattern of at least one of its components, suggesting a high conservation of component information in olfactory mixtures. Quantitatively, glomerular activity saturated quickly and increasing the number of components resulted in an increase of cases in which the response of a glomerulus to the mixture was lower than that to the strongest component (''suppression''). This shows global inhibition in the antennal lobe, most likely acting as overall gain control. Single components were not equally salient (in terms of number of active glomeruli), and mixture activity patterns were always more similar to the more salient components, in a way that could be predicted linearly. Thus, although a gain control system in the honeybee antennal lobe prevents saturation of the olfactory system, mixture representation follows essentially elemental rules.

214. Behavioral and Neurophysiological Studies of the Conditioned Food Aversion Elicited by the Physical Properties of Food as CS

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It is known that taste can act as a conditioned stimulus (CS) for conditioned food aversion. In this study, in order to examine whether or not the temperature and hardness of food can be a CS, we conducted behavioral and neurophysiological experiments in Wistar rats. The following results were as follows: (1) The rats subjected to aversive conditioning to 5 °C or 40 °C distilled water could learn to avoid these CSs, but they did not avoid any taste stimuli. (2) The rats subjected to aversive conditioning to 5 °C or 40 °C 0.1 M sucrose developed a generalized avoidance to sucrose at any temperature. (3) When rats familiarized to 25 °C 5 mM saccharine-Na (Sacc) were subjected to aversive conditioning to 5 °C or 40 °C Sacc, they avoided the respective CS, but they did not generalize it to any other stimuli even if having the same temperature as the CS. (4) The rats which had undergone transection of the taste nerves (chorda tympani and glossopharyngeal nerves) could acquire the conditioned response to the temperature of the CS. (5) The rats with bilateral lesions of amygdala could not acquire the conditioning. (6) The rats subjected to aversive conditioning to hard or soft pellets without taste, which had same ingredients, did not avoid same hardness of food with 0.1% Sacc. (7) The rats subjected to aversive conditioning to hard or soft pellets with Sacc avoided any pellets with Sacc, even if their hardness were different from CS. These results suggest that rats can be conditioned to

temperature and hardness aversion, that rats give priority chemical stimuli over physical stimuli to acquire the conditioned food aversion, and that amygdala plays an important role to acquire the conditioned food aversion elicited by the physical stimuli as well as taste stimuli.

215. Preference for Astringent Foods: Link with Oral Parameters

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Polyphenols are nutritionally desirable however they often present undesirable sensory properties such as bitterness and astringency. A better understanding of the physiological basis of their perception might help to promote their consumption. We studied perception and liking for a pear juice naturally rich in polyphenol (procyanidol content 1.52 $g\,l^{-1}$) and therefore clearly astringent The objectives of this study were twofold. First, liking of 10- to 11-year-old children for the astringent juice were studied in relation to their oral physiology and to their sensitivity to astringency. Second, the evolutions of liking and of oral parameters were studied following a period of $\sim$30 days of exposure to the astringent juice. To explain individual variations in liking for the astringent juice, different parameters were examined: papillae number and perceived astringency; as well as variations in saliva flow, pH and composition (e.g. proline-rich proteins content) before and after consumption of the astringent juice. To examine the evolution of liking following consumption, children were provided with bottles of the astringent juice they were requested to drink at home, five times per week during 6 weeks. During this period, children came to the lab every other week to score their liking for the astringent juice. Results showed that initial liking for the astringent juice are positively associated to perceived astringency but not to oral parameters (saliva flow and pH). Saliva flow and pH following stimulation by the astringent juice appear remarkably stable throughout the study. Repeated exposures to the astringent juice were not associated with an increase in liking.

216. Everyday Human Olfaction: An Ethnographic Approach

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''Cognitive and sensory anthropology'' is an emergent subfield of social sciences that aims to provide an ecological look at the human sensory knowledge. Broadly ignored in social sciences, smell is only discussed as a hidden and unused sense, at least in the occidental area. Our ethnographic research aims to show how this hypothesis is largely missproductive, in revealing the complexity of olfactory experience in the course of mundane life. A subsequent question is how smell perception is simultaneously culturally and naturally constraint? In this presentation, we will propose two linked lines of investigation based on data provided by our current doctoral study among French population (open interview focused on the perception and production of odorous events at home, during culinary and hygienic tasks and corporeal smell detection). First, we will describe how smells are conceptualized in the case of pragmatics activities. Many studies reveal the paucity of Indo-European vocabulary. However, the contextual nature of human smell memorization should be reevaluated and invite us to pay more attention to the referential nature of the olfactory verbal evocation. Second, we will discus the nature of human olfactory niches, built in the course of everyday domestic tasks. Constant control of smell environment is a central assignment, described in terms of homeostatic safeguarding. This reveals the importance of the contextual congruence between particular range of events, emotional arousal and perceptual cues as an evaluation tool. Finally, we advocate for the hypothesis of the prototypical perception of smells shaped within the activity of a categorical-like evaluation of the perceptual context, where culturally shared expectation seem to play a determinants role.

217. Ecto-ATPase in Surface of Vertebrates Olfactory Epithelium: Properties and Possible Functions

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The ecto-ATPase activity was determinated on nondestructive olfactory epithelium surface of different vertebrate animals with using biochemical and electron cytochemical methods. The properties of this enzyme were different from ATPases, which take part in energy transduction and ions transport processes. It was defined, that this ATPase had a wide substrate specificity and its activity depended on Mg2+ and Ca2+ ions. The EDTA and EGTA made a strong inhibitor effect and high concentration of ATP too. The enzyme activity was insensitive to action of mitochondrial inhibitor – oligomycin and Na,K-ATPase inhibitor – ouabain, but its activity depended on some SH-reagents, NaF and La3+ ions. The effect of calcium and magnesium concentrations on frog olfactory epithelium ecto-ATPase activity was studied. It was shown that ecto-ATPase was more sensitive to Ca2+ concentration than Mg2+. This results are discussed in relation to possible involvement of this ecto-ATPase in Ca2+ exchange by excitation mechanism of the olfactory cell. The molecular forms of ecto-ATPase from some vertebrate animals olfactory epithelium surface structures (cow, mouse, frog) were studied by electrophoresis method in gradient PAAG. The enzyme activity was observed in two molecular forms, which had a glycoprotein subunits. Among surface phosphohydrolases in cow and mouse olfactory epithelium were found isoforms of alkaline phosphatases, but in frog olfactory epithelium this activity was related only with ecto-ATPase. The research of the ultrastructural localization in the frogs olfactory epithelium demonstrated, that the ecto-ATPase activity was in the external part of the olfactory cilia membranes and in olfactory mucus near the olfactory knobs.

218. Nasal Pathways into CNS for Different Transmitters

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In our earlier investigations the ability of different compounds such as protein, neurotransmitter, neuropeptide to penetrate into CNS by intranasal delivery was demonstrated. The penetration

is realized through the system of axoplasmic transport. For example, the intranasal administration of GABA, a compound, which does not easily cross the blood–brain barrier, inhibited intermale mice aggression. Very low doses (0.5 mg/kg) during 15 minutes reduced intermale aggression and this effect was long lasting: aggression was suppressed for 3 days after 0.5 mg/kg intranasal dose, and for 10 days after 10 mg/kg intranasal dose. The intraperitoneal injection had only a mild effect in reducing intermale aggression when given at a high dose of 100 mg/kg. Similar experiment on this behavioral model were carried out using another inhibition transmitter – glycine. Its effect was weaker GABA one. Intranasal delivery of glycine in dose 5 mg/kg had suppressed male aggression only by 40% as compared with control experiments. The intraperitoneal injection of larger increased dose (50 mg/kg) had no effect on intermale aggression, because of glycine not being a compound, which easily cross the blood–brain barrier. Such a light weak effect of glycine on males behavior observed by intranasal administration might be related with lower concentration of its synapses in olfactory bulbs, and so that smaller participation in aggressive reaction. In summary, the nasal cavities and olfactory bulbs appeared to be in important link, not only in term of olfactory transduction but also via direct intranasal delivery for use to deliver compounds.

219. Odor Attitudes Uncovered Implicitly

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Beliefs and attitudes about odors and exposure effects can influence odor perception in an implicit manner. So far, researchers have tried to map these odor attitudes by using self-report measurements, and thus by asking people to think explicitly about their attitudes. Objective: To measure odor attitudes in an implicit way, we developed an odor version of the Implicit Association Test (IAT) Validation of this odor-IAT will be discussed. Methods: Three experiments were conducted to test the odor-IAT, which is a computerized reaction time task, during which participants have to associate words from the concept ''odor'' with positive and negative words, by pressing the corresponding keys on a computer keyboard. Participants not selected on specific odor attitudes (Experiment 1 and Replication Experiment 2) and participants who frequently use aromatherapy products (Experiment 3) completed the test. Implicit odor attitudes were inferred from examining response latencies and error rates. Results: In general, lower response latencies and error rates were observed during phases of the test where ''odor'' had to be associated with positive words, reflecting an overall positive attitude toward odors in Experiment 1 and 2. This odor attitude was even more positive within an aromatherapy group in Experiment 3 compared to participants who reported to never use such products. In conclusion, the odor-IAT was capable of uncovering odor attitudes in an implicit manner.

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220. Neurogenesis of Dopaminergic Neurons in the Adult Mammalian Olfactory Bulb: A Possible Source of Cells for Neural Repair Strategies

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Contrary to previously held beliefs, the adult brain is in fact capable of generating new neurons that can integrate into its complex circuitry. Recent researches have demonstrated that neurogenesis constitutively occurs in two specific regions of the adult mammalian brain, olfactory bulb (OB) and hippocampus. In the OB there is a significant number of dopaminergic (DA) precursors, originated from the subventricular zone and migrated following the rostral migratory stream. The properties of these cells has been studied with the patch-clamp technique in a transgenic animal model expressing GFP under the tyrosine hydroxylase (TH) promoter. Using BrdU we have first demonstrated that, in regions not normally occupied by DA neurones (mitral and external plexiform layers, ML and EPL) there are cells in which the transcription of the TH gene occurs in the absence of significant translational activity. We have studied the functional properties of these cells, showing that they seem to reflect different degree of maturation towards the DA phenotype as they become progressively closer to their final destination, the glomerular layer. In fact, cells in the EPL are autorhythmic, as are mature DA neurons in the glomerular layer, whereas TH-GFP cells in the ML are not. Furthermore, the cells in the EPL are synaptically connected to the olfactory nerve, whereas those in the ML are not. A new technique, based on dielectrophoresis, is being developed to sort immature DA neurones. It is hoped that these cells, present in a region easily accessible with surgical techniques, expanded in vitro and induced to differentiate towards the DA phenotype, could be a convenient source of neurons for cellular replacement strategies to treat neurodegenerative diseases affecting DA systems.

221. Four Interactive Tools Applied to Olfactory Studies, Available on the Net

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Among various classical tools for handling experimental data in our chemosensory studies, we have developed four original programs which are now free available in self service on the following web site: http://paul.laffort.free.fr/. 1. 3D olfactory space. This tool is also available, in a slightly different presentation, on the site: http://www.u-bourgogne.fr/ENSBANA/d.valentin/. It displays a 3D representation of mutual olfactory similarities of 141 odorants as evaluated by human subjects (about 10 000 similarities), with 88% of variance. Each odorant is identified by its name, its highest semantic descriptor out of 146 and out of 16. The later identification is underlined using 16 different colors. 2. MIG (Mixtures Intensities Generation). This algorithm allows the drawing of *iso*-effect curves, named isobolograms, using the Gamma' model of synergy and inhibition used in olfaction and taste. It could also be fruitfull in pharmacology. 3. MMA (Multiplicative Matrix Analysis). This statistical tool allows testing theories each time a matrix of experimental data for a given phenomenon can be expressed as a product of two theoretical matrices. This algorithm has recently be applied to improve the definition of solvation parameters of solutes, which could be also involved in the recognition of the odorants by the olfactory receptors. 4. SMT (Simplified Molecular Topology). The solvation parameters, in their recent updated definition, can be experimentally established via gas–liquid chromatography. They can also be evaluated from the molecular structure, which is the purpose of the SMT algorithm.

222. Dynamic Changes Associated with Odor Stimulation in the Rat Olfactory Bulb Revealed by Bold fMRI

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Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) has recently been applied to the rat olfactory system. In the olfactory bulb (OB), reproducible and relatively specific odor-induced spatial activity patterns have been demonstrated. These results support previous imaging studies showing that odors elicit activity within glomerular layer domains. Recently, number of studies also pointed out the temporal aspects of these glomerular odor representations. To take advantage of BOLD fMRI to address this question we used short (5 s) odor stimulations combined with high time resolution sequences. BOLD responses to amyl acetate were acquired in the OB of anesthetized rats using a 7 T spectrometer. Odor stimulation elicited reproducible patterns in the glomerular layer with the medial and lateral regions containing the most intense signals. Interestingly, regions of interest analysis revealed a temporal organization in BOLD response within the pattern. fMRI protocols required repetitions of odor expositions and averaging of corresponding signals. Such a paradigm might lead to an habituation of olfactory neurons response. In order to evaluate the way it could affect the BOLD response, we compared variations across repetitions of BOLD and electrophysiological signals recorded on different groups of rats, in the same experimental conditions. Neither BOLD fMRI nor electrophysiological responses exhibited habituation processes. These results confirmed that BOLD fMRI is suitable for studying dynamic changes associated with odor responses. They also pointed out the OB as a good model to study the relationship between measured fMRI signals and electrophysiological activity of the underlying neuronal elements.

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223. Different Options for the Study of the Vomeronasal System in Mammals: The Sheep as an Interesting Model

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It has long been suggested that some very well-known effects on reproduction (Lee-Boot, Whitten, Bruce and Vandenbergh) are mediated by the vomeronasal system (VNS). More recently, pheromones-VNS specificity has been accepted, with some nuances. So far, most studies published in relation to this subject have been carried out using mice, a species in which the three structures of the system are perfectly developed. However, there is huge morphological diversity in the olfactory systems (main and accessory) of the Mammalian Class. Therefore, it seems reasonable to consider the likelihood that mammals without a VNS – or with a poorly developed one – are also be able to detect pheromones. It would be interesting to differentiate a true VNS from other ''pheromonal-receptors'', that is to say areas with capacity to identify pheromones. The three different parts of the VNS in sheep, a macrosomatic mammal, show some singularities worth bearing in mind, especially those related to the accessory olfactory bulb. This bulb displays prominent vomeronasal nerve and glomerular layers in sheep at all ages, including before birth, while other strata are less evident. The present contribution reports our preliminary results on morphological peculiarities of the VNS in sheep, as compared with the pattern in mice, and the corresponding functional implications are discussed. Irrespective of other considerations, following the argumentation of diversity, it is not surprising that the above-mentioned pheromones-VNS specificity has been questioned in recent publications which demonstrate the implication of the main olfactory system in the coding and processing of vomeronasal information.

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224. Volatile Organic Compounds of Thai Aroma Rice

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Aroma rice, for example, Basmati and Jasmine rice, is very popular among rice consumers in South and South-East Asia and is becoming famous in USA and Europe. Thailand is one of the major rice exporters; rice produced from Thailand possesses peculiar properties as aroma, appearance, and texture. One outstanding property of Thai rice, known as ''Jasmine rice'', is its fragrance. The characteristic flavour of this rice is principally contributed by 2-acetyl-1-pyrroline. There are many varieties of aroma rice produce in Thailand, among which Khao Dawk Mali 105 (KDLM105) variety is the most famous one. In this experiment, we investigated volatile organic compounds (VOC) of Thai rice (Oryza sativa) by using five varieties: KDLM105, RD15, PTN1, HSP and CNT1. All of them were planted in the experimental field in 2005. The first four varieties are aroma rice, the last one is a rice variety of high yield and good grain. The Jasmine rice bought from the retail store was also used. VOC released to the head space from dry stored and moistured-heated rice were sampled using solid phase microextraction (SPME). In order to optimise the device suitability, three different fibers, Carboxen/PDMS, Carbowax/DVB and PDMS/DVB, were chosen. The resulting compounds were analysed using GC–MS. In parallel, sensory evaluation of rice samples was carried out focussing on odour perception of the panel considering the parameters ''variety'' and ''ageing''. The majority of VOC identified as highly abundant were aldehydes. Hexanal, considered as an off odour, was detected even in recently harvested rice.

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225. Genomic and Behavioural Studies of Olfaction in *Tribolium castaneum* Larvae

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The beetle *Tribolium castaneum* is a model organism for understanding insect olfaction in a representative of the most numerous insect order. We have identified 25 *Tribolium* olfactory receptor (OR) genes from the genomic database, including the ubiquitous insect OR coreceptor (Or83b in *Drosophila*). Using bioinformatics and molecular genetic approaches, we have looked at the expression patterns in adults and larvae and found genes that are specifically expressed at each stage. We looked for similarities between these stage-specific expression patterns and those seen in *Drosophila* are examined, in order to test the hypothesis that there is a relatively conserved set of larval-specific genes in insects. We have also studied the olfactory responses of beetle larvae using simple behavioural tests, in order to identify ligand-specific responses we can subsequently disrupt using RNAi.

226. Monorhinal Detection Threshold as a Function of Nasal Airflow and Odorant Mucosal Retention Times

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Evidence from frog electrophysiology and human psychophysics suggest that both the mucosal retention time of an odorant, and the velocity with which it passes over the olfactory epithelium affect the magnitude of the olfactory response. Based on these results and the assumption that the threshold concentration required for detection is directly related to the magnitude of the olfactory response, we constructed a simple model of detection threshold as a function of airflow rate (FR) and sorption rate (SR): DT = 100([odorant]/[odorant]maxcov) − k(FR − SR)^2 + error + aSR + bSR^2 (a and b are scale factors). This model predicts that the high flow nostril is ''tuned'' to high SR odorants, having relatively lower detection threshold to these odorants than the low flow nostril, and vice versa. Previous work in our lab has shown that the high flow nostril indeed has a detection accuracy advantage for propionic acid (high SR) when the duration of the high and low FR sniffs are matched. In the absence of restriction, subjects were shown to increase their low FR sniff duration, which had the effect of equalizing the detection thresholds across nostrils. Here we aim to test this simple model by measuring monorhinal detection thresholds (using a maximum likelihood adaptive staircase). To date we have collected monorhinal detection thresholds to octane (low SR) from 32 naïve human subjects. We found no significant difference between the mean detection thresholds for each nostril, when sniff duration was equal. We found a trend (P = 0.0648) towards a low FR nostril detection threshold advantage among five subjects who sniffed longer with their low FR nostril. We plan to repeat this experiment for odorants of differing sorption rates in order to further test the validity of this model.

227. Promotor Motifs of Olfactory Receptor Genes Expressed in Distinct Topographic Patterns

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Olfactory sensory neurons are likely to express only a single receptor type from the large OR gene superfamily and depending on their spatial position, either in one of several broad areas or clustered within a small central region only distinct receptor types are expressed. These observations suggest a strict regulation of gene choice and transcription control. However, very little is known about the molecular parameters underlying these processes. Towards an understanding of the relevant mechanisms, attempts have been made to identify putative regulatory motifs within the promotor region of spatially expressed OR genes. Therefore, the genomic sequences upstream from the coding regions of mOR262 genes, which are expressed exclusively in clustered cells, were studied. It was found that all mOR262 genes share a conserved stretch of DNA of approximately 150 bp located immediately upstream of the transcription start site. A few OR genes of other families which are also expressed in clustered olfactory sensory neurons, were found to comprise upstream motifs which are highly related to those characteristic for mOR262 genes. These data suggest that the identified elements may be involved in governing the unique topographic expression pattern. Genes encoding class-I receptors are expressed in the dorsal region of the epithelium and promotor studies revealed that they share multiple O/E-like binding sites as a prominent feature. Yeast one-hybrid experiments led to the identification of O/E-type transcription factors which bind to the putative promotor region of class-I genes and are expressed in the olfactory epithelium. The findings support the concept that distinct elements in the promotor region of OR genes may determine their congeneric expression pattern in the epithelium.

228. Gut Hormones in Chemosensory Systems—A Chemosensory System in the Gut?

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Peptidergic hormones secreted by cells of the gut convey information about the nutritional state of the body to the brain thus governing feeding behaviour. Appetite and satiety are strongly affected through the action of the gustatory and olfactory system; in addition palatability and the smell of food vary with the metabolic situation. It has been suggested that the 'appetite hormones' may also affect the chemosensory systems. We have performed RT-PCR studies indicating that receptors for these hormones appear to be expressed in both the olfactory epithelium as well as taste buds. This was confirmed using immunohistochemical techniques for the leptin receptor Ob-R and the obestatin receptor GPR39. In the gut, a variety of intestinal processes, such as secretion, resorption and motility, are affected by the chemical composition of the gut contents. Thus, precise chemosensory monitoring of the luminal contents is of great importance, however, the relevant cell types and the molecular mechanisms are still elusive. The so-called ''brush cells'' in the intestinal mucosa are considered as candidate chemosensory cells due to their morphological similarity to taste receptor cells of the tongue. We have confirmed that some of these cells express the G-protein alpha-gustducin which is known to be involved in sweet and bitter taste transduction. Alpha-gustducin expressing cells were found to be scattered throughout the intestines; however, they are especially abundant in the cardia of the stomach where they are arranged in large cell clusters. In RT-PCR approaches, we have found that several members of taste receptor families are expressed in the intestine, which might act as chemosensors not only in the tongue, but also in the intestinal system.

229. Influence of Lipid Matrix on the Flavour Perception of Extra Virgin Olive Oil

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Flavour is a characteristic attribute sensorial of extra virgin olive oils. Usually, fruity, green leaf/grass and bitterness are more pronounced in some olive varieties and in oils obtained from nonripe fruits. However, during the ripening process the flavour of extra virgin olive oil decrease. So far, bitter taste has been associated to the presence of some natural phenolic compounds present in olive oils and absents in other ones, such as seed oils. Panel test is usually used to evaluate the intensity of these attributes, although several instrumental methods have been proposed to overcome the difficulties of methods based on a sensorial panel of tasters. In the present work, the effect of other factors in the sensorial perception of extra virgin olive oil flavour was studied. In special, the influence of lipid matrix unsaturation was evaluated. Lipid matrices obtained form both high and low unsaturated/saturated ratio were spiked with the same bitter extract, and tested in the panel test. Different spiking levels were evaluated, and significant differences were found at low and medium spiking levels. These results showed that the lipid matrix unsaturation degree determinate the sensorial perception of positive flavour in extra virgin olive oil.

230. Evolution of The "OR37" Subfamily of Olfactory Receptors: A Cross-Species Comparison

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Olfactory receptors (OR) of the OR37 subfamily are characterized by special features, 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 receptors in these species. Uniquely among OR subfamilies, genes encoding OR37 receptors are organized in 2 clusters. Cluster-I contains 5 genes sharing a high level of sequence homology (~90%). In line with the tendency of pseudogenization for human OR genes, the human cluster-I contains 4 pseudogenes; the mouse cluster-I only 1. Cluster-II comprises 3 genes in mouse and surprisingly 7 in human,. The sequence similarity of cluster-II genes is only ~60%. To gain 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, a member of insectivores, we have identified 21 OR37 genes which are related to cluster-I and cluster-II genes, respectively; indicating that the two cluster arrangement 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. All efforts to clone cluster-I–like genes from opossum failed; thus, cluster-II appears to be the original cluster and cluster-I evolved somewhere between 130 and 173 million years ago. Analyzing the egg-laying monotreme, Platypus, we were unable to identify any OR37 genes, however, a receptor was identified, which is a member of the OR-family 262; interestingly, all OR37-receptors are members of the 262 family.

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231. The Relative Contributions of Oral and CNS Processing to Food Texture Sensations

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Reaction times to detect and rate the intensity of various texture sensations were measured for custards and yogurts in a series of studies. The sensations reflected properties related to the surface (e.g., roughness, fattiness) or to the bulk of the oral food bolus (e.g., thickness, heterogeneity), as was established earlier. Facial and tongue muscle activity was measured electrophysiologically to quantify the amount of oral processing, whereas turbidity of rinse water was measured to quantify the degree of food breakdown as a result of the oral processing. Combined, the results from the various studies indicate that texture sensations receive qualitative input from oral processing and quantitative (intensity) input from CNS processing. 1. Oral processes; the duration, ranging between 0.5 sec (thickness) and 3 sec (melting), and exact nature of oral movements vary with texture sensations. Bulk sensations require shorter processing times and less complex oral movements resulting in less broken-down foods, than surface sensations. 2. CNS processes; the duration of CNS processing, ranging between 1.5 and 2.5 s is inversely related to the intensity of the sensation suggesting that information related to specific sensations is lost during central processing. The present results support our previous psychophysical and ultrasound findings that specific oral movements maximize specific texture sensations. Future research will focus on the specifics of oral movements and CNS responses in relation to sensations using behavioral and imaging techniques (e.g., ultrasound, articulography, EEG and fMRI).

232. Odor Representation Is Modified after Pairing a Neutral Odor with an Unpleasant Taste in Humans

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Olfactory hedonic representations are not solely innate, but can be acquired during development in humans. Sensory stimuli such as pleasant and unpleasant tastes paired with neutral odors during implicit learning may color affectively odor representation. In the present study, we focused on the effect of an unpleasant taste (quinine) on olfactory hedonic representations, and set out to characterize whether such hedonic modulation is dependent on the number of odor-taste trials encoded during learning. Thirty-two human subjects (22.2 ± 1.9 in average) randomly assigned to two groups (A and B) were tested in a within-subjects design including three sessions. In a first session, participants were asked to rank 8 food odors (garlic, coffee, chocolate, anise, pineapple, lemon, melon and kola) from the most pleasant to the most unpleasant. Once the ranking was done, they were asked to estimate odor intensity, familiarity and edibility using a scale from 1

(not at all) to 9 (very). In a second session, the most neutral odors (i.e. ranked 4 and 5) were randomly presented in an associative learning procedure with either water (CS−, control condition) or with a bitter solution (CS+, test condition). The third session was identical to the first one. In group A and group B, CS+ was respectively associated one time or three times with the US. Results showed that CS+ odor intensity (F (1,30) = 6.704, P < 0.015) and CS+ odor familiarity (F (1,30) = 5.194, P < 0.03) increased after learning in both groups. No significant differences were observed for the CS−. Thus, this experiment suggests that a neutral odor becomes more intense and more familiar after being paired with an unpleasant taste. Only one odor-taste trial is sufficient to elicit such modulation of odor representation.

233. Reaching Odor Blending in Binary Mixtures: A Matter of Typicality

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In previous studies, Barkat explored the field of the perceived quality in binary odor mixtures. We elaborated an experimental design so as to characterize odor harmony in a binary mixture designated as ''Pineapple blending'' (P-Blend). In this preliminary study, a mixture of Furaneol (fudge) and ethyl caproate (fruity) in precise proportions and specific concentrations was perceived as the best ''Pineapple odor'' by 100% of the subjects. In a second experiment, the validity of ''P-Blend'' was tested. For that purpose, we confronted P-Blend with a set of odorants eliciting pineapple odors or entering in pineapple odor formulations (i.e., 7 single compounds 1 binary mixture and 1 ternary mixture). Twenty subjects (23 ± 3 years old) participated in the experiment. They were asked to sniff all odorants in order to perform a qualitative ranking from what they consider to be the most prototypical pineapple odor (rank 1) to the worst (rank 10). Thereafter, subjects had to rate typicality and edibility for each odor on 9-point scales (bordered from not typical/edible at all to extremely typical/edible). Results showed that a single odorant evokes pineapple the best (i.e. allyl caproate). This compound is perceived as being the most typical and edible. The most remarkable result lays on the fact that single components such as ethyl isobutyrate and/or ethyl maltol are not regarded as good representative for pineapple odor when sniffed separately, but obtain a better rank when they are mixed together in a specific ratio. Our result underlines that, as recently suggested by Zhou and Buck; two single compounds that not evoke a specific target odor can elicit the perception of a novel odor, significantly much more typical of the target odor, when they are mixed together.

234. Expression of Olfactory Receptor Genes in a Heterologous Cell Line and Rat Olfactory Sensory Neurons

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In the past, ligand activation of mammalian olfactory receptors (ORs) has been studied in a variety of host cells. In heterologous cell lines the transport of the recombinant OR proteins to the cell surface is insufficient. Consequently, the percentage of functional ORs that are accessible to the odorants is low. To date, no uniform heterologous expression system has been identified that works for the majority of ORs. In the present study, human OR17-40 was stably expressed in HEK293 cells resulting in a robust in vitro expression of the receptor. Ligand activation of the receptor was studied by monitoring fluxes of the internal calcium concentration in a microtiter plate format. Ligand specificity of OR17-40 was consistent with data in the literature and receptor activation by agonists occurred in a dose-dependent manner. A series of structurally related molecules was tested for its ability to activate the OR and their EC50 values determined. Activation profiles of endogenous ORs can be studied by exposing primary olfactory sensory neurons (OSNs) to a specific family of odorants. This approach is possible because each OSN expresses only one or a small number of ORs. In the present study, sandalwood molecules, a family of perfume compounds with a relatively rigid chemical backbone, were used to profile endogenous rat ORs. The identified neurons expressed ORs that can discriminate between sandalwood odorants with slight differences in their molecular structures. One putative sandalwood receptor was identified using single-cell RT-PCR. The sandalwood OR was reintroduced into rat OSN using the Semliki Forest virus system and the activation profile of the recombinant OR in infected neurons confirmed.

235. An Open Source Software for Studying Dynamical Processes of Neuronal Activity

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Study of neuronal activity dynamic processes has received an increasing interest in the last decade. The role of oscillatory activities and cellular synchronizations in information processing has been largely described in several neuronal systems. Moreover, in the mammal olfactory system, these different levels of information processing are modulated by respiration. An important challenge in data processing is to correlate these different time scale–based signals (respiration, oscillating local field potentials, spiking activity). We propose an open source software, based on python, scipy (scientific module for python) and SQL database, able to perform a set of standard processings and analysis on electrophysiological signals such as filtering, time frequency analysis, spike detection, simple spike sorting (manual or automatic), ISI distribution, mean spike rate with sliding windows, spike coincidence analysis· · · Furthermore, the software offers two new analysis tools (using a system of plugins): a wavelet-based method for local phase extraction from a multifrequency oscillatory signal allowing to correlate spike activities and LFP oscillations (phase locking). The conversion of the respiratory cycle time representation into a phase representation of its different periods (inspiration–expiration). This phase representation can be used as a new ''time'' basis allowing to average olfactory neural events The use of SQL database for data storage permits a reliable data mining, and provides a powerful, and easy to use, tool for browsing and manipulating large amounts of data.

Sessión 5

Symposium 13: Chemical Mediation of Plant–Insect Interactions

236. Plant Diversity and Insect Antifeedants

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Plants are known to contain a diverse range of metabolites and some of these can modulate the feeding behaviour of insects and are involved in host-selection behaviour. However, despite the fact that scientists have been interested in the activity of compounds that influence host-selection behaviour there are more studies on the role of volatile compounds than nonvolatile compounds encountered by an insect when it bites into a leaf or root. This talk will provide an overview of the diversity of compounds, such as flavonoids, terpenoids, alkaloids and nonprotein amino acids, that have been studied, the methods used and the problems encountered. In some cases activity-based fractionation of extracts from plants has resulted in the identification of antifeedant compounds, whereas in other cases the active compounds have been isolated from extracts that where themselves not active. For example, extracts from plants containing coumarins are often very active and this activity increases during the isolation of the coumarins. In contrast, extracts from plants containing some of the different classes of diterpenoids such as the labdanes and neo-clerodanes do not always show potent antifeedant activity, despite the fact that the compounds when isolated from these extracts have antifeedant properties. Thus do these diterpenoids play a role in insect–host selection and do they have a different mode of action from the coumarins?

237. Metabolomics: Novel Tool for Studying Complex Biological Systems

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Functional genomics aims at understanding the role of all genes in the genomes of organisms that have been sequenced by:

- Reductionist approaches.
- Holistic approaches.

In systems biology instead of planning an experiment to proof a certain hypothesis, a large number of different parameters under different experimental conditions is measured, followed by an analysis that correlates changes with the external conditions. In this case the same methods as described for functional genomics can be used, as well as many other physical or morphological data. Based on possible correlations using, for example multivariate analyses, novel hypotheses might be postulated. It is thus also a holistic approach. For both fields a major technology required is the chemical characterization of the phenotype of an organism. In case of plants that means phytochemical analysis to measure the metabolome (the total of all metabolites in an organism). Chromatographic (LC(–MS), GC(–MS) or TLC) and physical (MS and NMR) methods are the tools. The combination of these methods seems the most appropriate, with NMR for a macroscopic view, followed by a targeted approach for a certain group of compounds by means of the other mentioned methods. All such methods require suitable data handling, data storage and statistical tools for dealing with huge data sets. In functional genomics and systems biology (e.g. plant resistance against insects) metabolomics is now a key technology.

238. Model Antifeedants Suggesting a Multifaced Neuroreceptor-Mediated Taste Regulation in Insects

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The silphinenes are unusual tricyclic sesquiterpenes derived from *Senecio palmensis*, a plant endemic to the Canary Islands. Previous studies demonstrated that these compounds have antifeedant and toxic effects in exposed insects, and are especially effective against Coleopteran pests, such as the Southern corn rootworm and the Colorado potato beetle (CPB). Molecular modelling studies suggested a structural similarity between the silphinenes and the known GABA antagonist, picrotoxinin. Ryanodol and related ryanodane diterpenes are rare compounds derived from the Canarian paleoendemism *Persea indica*. These compounds have strong antifeedant and toxic effects in insects and are specially active against the lepidopteran *Spodoptera littoralis*. The related compound ryanodine acts on CNS ryanodine insect receptors. *Delphinium*, *Aconitum* and *Consolida* spp. derived C19 diterpenoid alkaloids have strong insect antifeedant effects with molecular and species selectivity, specially towards CPB. Some of these compounds are known mammalian and insect nACh receptor agonists and antagonists. All these results indicate that multiple neuroreceptors are implicated in insect taste regulation, and that different insect species share common regulation mechanisms with variations according to their feeding adaptations.

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239. Assessment of Fungivorous Insect Antennae as Biosensors for Detecting Wood Rotting Fungi

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Trametes versicolor, *Gloeophyllum trabeum* and *Poria placenta* are known as wood destructive fungi, causing high economic losses in construction wood and wood composite products. To evaluate the quality of wood as raw-material for the production of high engineering property products, a reliable and sensitive detection of wood rotting fungi is needed. A rapid and nondestructive method for the evaluation of fungal infestation can focus on volatile organic compounds (VOC) released by fungi. Beech wood (*Fagus sylvatica*) was inoculated either with *G. trabeum*, *P. placenta* or *T. versicolor* and incubated at 22 °C for 1 week prior to sampling of VOC. Volatiles released to the head space were collected using solid phase microextraction (SPME), Carboxen/PDMS, and were analysed by GC–MS. Each fungus growing on beech released a specific pattern of VOC. The detected VOC can be categorised into two broad groups, aliphatic C5–C8 compounds and isoprenoids. Typical fungal odour compounds like 1-octen-3-ol and 3-octanone were produced by all three fungi. Two monoterpenes, alpha-pinene and

3-carene were found. However, dominant components with respect to abundance were sesquiterpenes. Sesquiterpene patterns were characteristic for each species. Moreover, 6-protoilludene was specifically produced by *G. trabeum* in high amounts. alpha- and beta-barbatene were typical and dominant VOC of *T. versicolor. Cis boleti*, an insect typically feeding on fruiting bodies of wood rotting fungi like *T. versicolor* was used for electroantennographic recordings of fungal volatiles. On the basis of these measurements the feasibility of a biosensor for the detection of wood rotting fungi was assessed.

P. Thakeow holds PhD fellowship by the Thai government and Chiang Mai University, Thailand.

240. Antennal Sensitivity to Odorants in Three Pierid Butterfly Species Reflects Behavioural Significance

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Intraspecific communication in *Pieris* includes volatile signals. Five male produced volatile compounds have been identified by a combination of chemical analysis (SPME and GC–MS) and behavioural studies; three emitted by the females and two by the males. Methyl salicylate (*P. napi* and *P. rapae*), indole (*P. rapae*) and benzyl cyanide (*P. brassicae*) originating from amino acids in the host-plants, produced by the male and transferred to the female during mating. They are used by the female as anti-aphrodisiacs, that is, mediating the mated female's unreceptiveness to males. The two geometrical isomers geranial and neral (citral) emitted from the wings of *P. napi* males are used as an aphrodisiac enhancing the female's willingness to mate (Andersson et al. 2006). The biological relevance of a volatile compound to the insect could be indicated by the peripheral sensitivity. The antennal sensitivity to the five signals was recorded by electroantennograms in both sexes of the three *Pieris* species. The sensitivity of the antennal responses to the odorants was in accordance with the described behavioural importance of the odorants as signals between sexes. The female antenna of *P. napi* was more sensitive to citral, both when compared to the conspecific male antennal responses and to both sexes of *P. rapae* and *P. brassicae*. The conspecific antiaphrodisiac odorant elicited stronger responses in the antennae of each of the three male butterflies compared to their conspecific females and both sexes of the two other species. This might indicate an evolutionary adaptation of peripheral sensitivity to biological important odorants.

Symposium 14: Olfaction and Food Intake

241. Olfaction and Food Intake: Electrophysiological and Neuroimaging Data in Human and Primate

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A bittersweet search for bitter: sweet interactions: cell to cell communication in the taste bud. The orbitofrontal cortex (OFC) contains the secondary taste and olfactory cortices, in which the reward value of the taste, smell, sight and texture of food in the mouth is represented, as shown by neuronal recordings in macaques. The activity of many olfactory neurons in OFC reflects the taste association of the odour, as shown during olfactory to taste association learning and reversal. The neurons represent the reward value of odour, for their responses decrease to zero when the macaque is fed satiety, and this implements olfactory sensory-specific satiety, which can be produced in part by smelling of the odour without ingestion for the normal period of a meal. In human functional neuroimaging fMRI studies, it has been shown that activation of OFC and adjoining anterior cingulate cortex by odours and by liquid food is hunger dependent, and indeed the pleasantness of the food is correlated with the degree of activation found. In both studies, it was shown that the modulation is sensory-specific, so that sensory-specific satiety is implemented in OFC. The pleasantness of odours is represented in human OFC, and flavor representations are formed by combining taste and olfactory inputs in OFC. Cognitive influences, such as a word label, can influence activations produced by olfactory stimuli in OFC. This neurophysiological and human fMRI evidence shows that OFC is involved in representing the reward value and subjective pleasantness of odours in a hunger including sensory-specific satiety way, and that OFC is thus involved in the powerful effects that odours have on the pleasantness of food and food intake.

242. Olfactory Inputs and Seasonal Changes in Energy Balance in a Primate

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Numerous studies have stressed the role of olfaction on regulatory mechanisms of food intake. To investigate the effects of olfactory cues or olfactory bulbs removal on energy balance, experiments were done in a nocturnal primate (*Microcebus murinus*). When exposed to short photoperiod (SP), this primate demonstrates rapid changes in energy balance as adaptive anticipatory response for winter survival. Mouse lemurs mainly use olfactory cues in social relationships and possess olfactory structures that are selectively activated by urine from conspecifics. Six intact males (controls), 8 bulbectomized males (BOX) and 8 males exposed to male urinary cues (U-exposed) were transferred to SP. Body mass, food intake and resting metabolic rate (RMR) were measured prior and after the photoperiod change. In controls, SP leads to rapid gain in body mass and increase in food intake, followed by a decrease in RMR and food intake 1 month after. BOX males constantly demonstrated a hyperphagic pattern. Even if SP exposure induced a body mass increase, RMR and food intake remained high and body mass-dependent. In U-exposed males, body mass gain was significantly reduced while RMR and food intake remained increased. In both BOX and U-exposed animals, the SP-mediated changes in energy balance were delayed. Other disturbances in seasonal/daily rhythmicity (behaviors, hormones) were previously exemplified in BOX or U-exposed mouse lemurs. Modification of responses to photoperiodic entrainment by changes in olfactory inputs strongly suggests that olfactory bulbs interact directly on brain structures controlling biological rhythms. This opens new perspective to understand the relationship between pathological

or age-related olfactory deficits and energy balance mechanisms in humans.

243. Visual Images of Fruits Affect Perception of Juices

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We humans use vision as a primary source of information about external world. We know that we also use vision in eating behavior: When we select and evaluate foods, we fully use a vision. There are many anecdotes about that, however, not so many academic studies studying about that. In this study, participants were required to drink four kinds of juices (i.e. apple, peach, orange and grape juices) under three conditions. Participants were asked to put an apparatus-like mask, which blindfolded participants and gave them a computer screen. In no image conditions, they drank a cup of juice without any visual images of fruits. In appropriate conditions, they drank a cup of juices with appropriate pictures of the juices. In inappropriate conditions, they drank with inappropriate pictures, for example, participants drank apple juice with a picture of orange. As a result, it was found that these visual stimuli enhanced flavor intensities and preferences when they were appropriate to the flavor. These results are compatible with those of preceding studies, which investigated the interactions of olfaction with gustation and/or vision and suggested that these interactions occur in cognitive level.

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244. Evidence for an Insulin/Insulin-Receptor System in the Rat Olfactory Mucosa

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Insulin is one of the major hormones controlling energy balance. Insulin receptors are found widely distributed in the central nervous system, with particularly high density in choroid plexus, hypothalamus and olfactory bulb. Insulin in the brain induces short and long term effects on food intake; furthermore, insulin could be a potential neuromodulator involved in cognitive process related to feeding. Olfaction is the most important chemical sense for prey detection and food intake regulation in vertebrates. It is influenced by physiological status and olfactory dysfunction is often associated with metabolic disorders. The first step of the modulation of odorant molecules detection and discrimination could take place at the olfactory mucosa level. We therefore investigate the expression of insulin and its receptor in the rat olfactory mucosa using RT-PCR and immunocyto and histochemical analysis. We showed that both preproinsulin (I and II) mRNAs and the two isoforms of the insulin receptor mRNAs (full length and exon 11 deleted) are expressed in the olfactory mucosa. Insulin receptors were located in all cell types of olfactory epithelium except in the immature neurones. Influence of metabolic status (starved vs. fed rats) on levels of insulins and insulin receptors mRNA expression in the mucosa was analysed by real time quantitative RT-PCR. Altogether, our data strongly suggest that insulin may be considered as a potential modulator of olfactory mucosa functions.

245. Breastfeeding and Experience with a Variety of Vegetables Increases Acceptance of New Flavours by Infants at Weaning

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In this study we compared the effects of dietary monotony vs. variety when vegetables were first introduced at weaning. Previous research demonstrated that experience with a variety of flavours can facilitate the infantÆs acceptance of a new food. Our aim was to look at effects of different temporal patterns of experience with variety and to compare the responses of breastfed and bottle-fed infants, in two nearby European regions (in Eastern France and Southern Germany). At weaning, infants were exposed to three different food variety regimens: (a) no variety (carrot purÚe for 9 days), (b) variety with daily change (artichoke, green beans, pumpkin for 9 days), and (c) variety with change every 3 days (artichoke 3 days, green beans 3 days, pumpkin 3 days). All purÚes were isocaloric. Before and after the 9-day home-exposure period we evaluated, in the laboratory, intake and acceptance of carrot (days 1 and 11) and of a new vegetable (courgette-tomato, on day 12). Moreover, we examined if the effect of variety was evident 11 days later, when another new vegetable was introduced (peas at day 23), and 1–2 months later when meat and fish were first introduced. Infants were videotaped during all feeding sessions. The infantsÆ reactions to new foods were measured by quantities eaten and by liking ratings (by mother and by an independent observer). Our results showed that breast-feeding facilitated the acceptance of new vegetables in both regions and for all new foods in Germany. Furthermore, exposure to flavour variety at weaning increased acceptance of new foods and the frequency of flavour change was more important than the number of vegetables.

246. Olfactory Processing and Food Status

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The relationships between olfaction and food intake has been known for several decades, yet the mechanisms underlying the modifications of the olfactory activity induced by the nutritional state remain unknown. Several food intake regulatory peptides are contained in the olfactory bulb. These peptides originate either in the blood circulation or in the hypothalamic feeding centres. The question of the functional links between olfaction and food intake was addressed through a multidisciplinary set of approaches which provides original data enlightening cross-talks between the hypothalamic feeding centers, olfactory pathways and metabolic peripheral events. The set includes electrophysiological, behavioural and immunocytochemical (IC) approaches. These studies aimed at both deciphering the action of food-intake regulatory peptides on the bulbar network and at objectifying how the nutritional states

influence the olfactory abilities of the animals. The electrophysiological data show that orexin and insulin acts on the olfactory bulb network, in vivo and in vitro results being in close agreement, while the IC studies precise the localization of the receptors of these molecules. The behavioural studies objectify that the nutritional status directly modulates the olfactory detection power of the animals: the hungry rats smell better. The icv injections of orexin and leptin are shown as mimicking the hungry and satiated states respectively. Further investigations are in process to approach the physiopathological aspects of food intake regulations by studying two other rat strains with opposite energy balance: genetically obese Zucker rats and lean Lou/C rats (low body weight).

Symposium 15: Olfaction and Taste: E.N.T. Aspects

247. Taste Function Measured by Electrogustometry (EGM) in Controls and Patients after Middle Ear Surgery

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Electrogustometric thresholds were recorded with a constant current generator delivering currents between 0.1 to 200 ÁA through a 5 mm diameter stainless steel spherical electrode on 13 patients (11 women and 2 men, 45.5 ± 15 y.o.) undergoing stapedectomy (middle ear surgery for otosclerosis implying chorda tympany injury). EGM thresholds were measured at 11 locations on the tongue as in Boucher et al. (in press). Subjects were measured prior to surgery and 3 times after surgery (8 days, 30 days and up to 6 months). Presurgery measurement served as a control for post surgery measurements for freshly operated side. As otosclerosis is most often a bilateral disease, patients need surgery on both sides. Thus, the unoperated side, prior to the second surgery, was used as a control for the operated side. A group of normal subjects also served as control. Results showed a massive impairment of sensitivity (thresholds increased by up to 100 ÁA, $P < 0.001$; df = 12) at all loci of the operated side, after surgery, which recovered progressively though not totally within the window of observation. The side of the tongue contralateral to surgery was also significantly but slightly affected. Palate loci recovered the best in all cases. There is some evidence that the previously operated patients had not fully recovered on the side they had been operated on, at the time of the surgery on contralateral ear (months or years later). After the second operation, patientsÆ sensitivity decreased on the freshly operated side, but there was no evidence of further loss on the already impaired previously operated side.

248. Olfaction in Patients with Nasal Polyposis: Effects of Systemic Steroids and Radical Ethmoidectomy with Middle Turbinate Resection (Nasalisation)

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Many patients with nasal polyps complain about severe hyposmia or anosmia. Our aim was to examine the role of steroid therapy and surgery in the treatment of hypo-anosmia in patients with nasal polyposis (NPS). The recovery of olfaction was measured (olfactory score: OS) using a visual analog scale. In the first study, 24 anosmic NPS patients (initial OS: 0.6 ± 0.5) received systemic oral steroids. After 1 week, OS significantly increased to 6.4 ± 1.5 and decreased after 2 months to 2.2 ± 1.5. All patients were operated bilaterally and received one depot steroid intramuscular injection of 80 mg triamcinolone the day after surgery and received nasal steroids during the whole study. One month after surgery, OS increased to 7.1 ± 1.6 and remained stable at 6, 9, and 12 months. In the second study 25 anosmic NPS patients (initial OS: 0.7 ± 0.6) did not received the initial systemic steroid treatment. OS increased to 6.3 ± 1.4 after 1 month, to 7.2 ± 1.5 after 3 months and remained stable up to 1 year. In the same conditions, OS for normosmic NPS patients (initial OS: 7.7 ± 0.9) did not change after surgery and slightly increased (8.5 ± 0.8) after 1 year. Clearly, long-lasting correction of NPS related olfactory dysfunction can be achieved by combination of nasalisation technique and low dose of nasal steroids.

249. Electrophysiological Recordings from the Human Nasal Epithelium

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The transformation of a chemical stimulus as an odorant to an electrical signal does occur in the nasal epithelium by receptor neurons. Using specific electrodes, these signals may be recorded from the nasal epithelium. Due to exclusive olfactory stimulation electro-olfactograms (EOG) may be recorded, due to exclusive trigeminal stimulation negative mucosa potentials (NMP) may be recorded. In addition, Monti-Bloch et al. reported recordings of a specific response from the vomeronasal epithelium in response to so called vomeropherins, the vomeronasogram (VNG). EOG recordings were performed in more than 100 volunteers. Stimulation was performed by an olfactometer which delivered stimulants (vanillin, H_2S, CO_2, and androstadienone) of defined concentration and stimulus duration. For recordings, a silver chloride electrode (outer diameter 0.8 mm) was positioned on the epithelium under endoscopical control. The results indicated a certain topographical distribution of olfactory receptor neurons. In addition, as EOG could be recorded even at the insertion of the middle turbinate, the olfactory epithelium is larger than previously thought. EOG to repetitive stimulation did not decrease significantly indicating that olfactory desensitisation seems to be a more central than a peripheral process. With regard to trigeminal stimulation differences in the topographical distribution of the NMP amplitude were observed. This may indicate that trigeminal receptors exhibit differences in density throughout the respiratory epithelium. Finally, in the vicinity of the vomeronasal duct, the NMP could be recorded even after stimulation with androstadienone. Recordings from the vomeronasal duct did not exhibit a specific pattern of responsiveness.

250. Olfactory Evoked Potentials: Usefulness and Limitations

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The development of the modern olfactometer in 1978 by Kobal and Plattig allowed the recording of odorant-evoked potentials in humans and opened up the olfactory system to the same neurophysiological investigation that had been used for many years in vision and hearing. However, owing to the inaccessible location of the receptors, relatively few laboratories have investigated olfactory-evoked potentials (EOG). More commonly, olfactory eventûrelated potentials (OERP) have been recorded on the surface of the scalp. Surprisingly, there is no consensus about the origins of the positive and negative peaks of the OERP and there are inconsistencies in the literature about the existence of the P1 peak and the distinction between P2 and P3. Furthermore, there are disagreements as to whether the amplitude or latency of the OERP is proportional to stimulus strength. Neither have these potentials helped in the understanding of how odours are encoded or discriminated. The small size, the large intersubject variability and inconsistencies between studies have limited the usefulness of the technique. Unlike the other sensory systems, the olfactory system exhibits stimulus inconstancy. However, recording evoked potentials still has the major advantage of high temporal resolution that imaging techniques like fMRI or PET, because they depend upon changes in blood flow, can never match. In this presentation we will; (1) discuss the reproducibility of the OERP both within and between individuals and use correlation analysis to highlight the intrinsic variability, (2) present new approaches to optimise the recording of olfactory-evoked potentials and (3) examine the validity of the new method for studying the human olfactory system by measuring the EOG externally on the bridge of the nose.

251. Specific Agueusia to L-Glutamate. Further Facts

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A population of 1200 human subjects was screened for their relative sensitivity to monosodium L-Glutamate (MSG) and sodium chloride, comparatively. Among them, 23 Ss (6 men and 17 women) were included in an 8-week familiarization experiment including MSG, 5ÆGMP, sucrose and a mixture of MGS and 5ÆGMP as stimuli. Measurements included evaluation of the concentration iso-intense to a NaCl 29 mM reference, finger-span time-intensity profiling, preference evaluation, triangular tests for discrimination and a descriptive test. Three subjects confirmed to be agueusic to MSG and 5ÆGMP, 4 were hypogueusic. These subjects did not exhibit the learning effect of an increased perception with repeated sessions. Some subjects were able to discriminate MSG from NaCl at isomolar concentration but further tests revealed they could not perceive the taste of glutamate. They could discriminate both solutions because they perceived MSG weaker than NaCl at isomolarity. Founded on a statistical criterion for difference, triangular tests show these subjects discriminate MSG from NaCl, leading to categorize them in the group of normal tasters for L-Glu; but, the difference relied on a decreased Na perception. Preference and descriptive tests suggested that these subjects might not taste the umami taste. The most convincing argument was they could not memorize the quality of the taste elicited by MSG and could not recognize, in the triangular tests, the compound they had repetitively tested for 8 weeks. This study suggests that, in some subjects, the glutamate anion inhibits the stimulatory effect of the Na cation when both are present. Similar observations were obtained from 10 hamster chorda tympani out of 25.

252. Toward a Human Olfactory Cleft Mucus Proteome

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We have analyzed for the first time the proteome of the human olfactory cleft mucus (OCM) with two-dimensional gel electrophoresis (2-DE), MALDI-TOF, RPLC chromatography and Edman sequencing complementary approaches. The aim of this work is (i) to have a better knowledge on olfactory cleft mucus (OCM) protein composition (ii) to explain the role of present proteins specifically found in OCM in order to potentially explain olfactory diseases or disorders. Sixty-eight proteins have been identified by 2-DE/MALTI-TOF approach, 8 by RPLC chromatography and Edman sequencing and 7 proteins by both methods. These 83 total proteins have been classified according to their known localization and function using protein databases. The presence of proteins specifically expressed in the olfactory cleft mucus is discussed.

Oral Session 5: Taste Detection

253. EctoATPase in Taste Buds of Fishes: Implications for Evolutionary Origins of Taste Buds

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Taste buds utilize ATP as a crucial neurotransmitter from receptor cells to afferent gustatory nerves. In rodents, Type I taste cells expresses ectoATPase, presumably to break down ATP released by the receptor cells. All jawed vertebrates (including mammals, amphibia, elasmobranchs and teleost fishes) as well as lampreys, possess not only taste buds, but also solitary chemosensory cells distributed within respiratory and other epithelia. Although hagfish (chordates, agnathan vertebrates) have solitary chemosensory cells grouped into specialized endorgans (Schreiner Organs), these chemosensory endorgans are not identical to taste buds based on a variety of cytological criteria. We utilized ectoATPase histochemistry as an index of ATP-utilizing systems in nonmammalian taste buds and other chemosensory epithelial receptors. Taste buds in all teleost fish examined exhibit ectoATPase activity similar to mammalian systems. Neither solitary chemosensory cells on fin rays of sea robins (teleosts) nor Schreiner organs of hagfish exhibit ectoATPase staining. These findings suggest that ATP is not utilized as a neurotransmitter by solitary chemoreceptor cells or Schreiner organs. Thus in the vertebrate lineage, the evolution of taste buds appears to coincide with the utilization of ATP as a neurotransmitter by cutaneous chemoreceptor cells.

254. Exocytosis in Taste Bud Cells

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Taste bud cells can be categorized into four types: I, II, III and IV. Types I and IV are believed to be supporting cells and precursor cells, respectively. Type II cells express G protein coupled bitter,

sweet and umami taste receptors, as well as some putative sour and salty channel receptors, but these cells lack classical synapses with afferent nerve fibers. Type III cells possess synaptic machinery and form synapses with afferent neurons, but do not express taste receptors. ATP and serotonin, as well as other bioactive substances have been identified as taste transmitters or paracrine and autocrine agents. However, it is largely unknown how the release of these agents is regulated in response to taste stimuli. In order to understand the mechanisms underlying the intragemmal cell-to-cell and cell-to-afferent fiber communication, we have attempted to characterize the exocytosis/endocytosis that presumably occur with neurotransmitter release from type II and/or III cells upon stimulation. We used taste papilla slices and fluorescent styryl dyes (FM dyes) to monitor exocytosis and endocytosis in taste cells. We found that a significant number of taste bud cells took up FM dyes in response to high KCl solutions, whereas a smaller number of taste bud cells accumulated dye in response to taste stimuli. Further stimulation of these cells after the removal of the FM dyes resulted in destaining of both type II and III cells. These results indicated that high KCl can depolarize a large number of taste bud cells while specific taste stimuli are able to excite only a fraction of them, that both type II and type III taste bud cells can undergo membrane vesicle recycling, and may release neurotransmitters and/or other bioactive agents when stimulated.

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255. Gustatory Expression Profiles of the Full Complement of Human Bitter Taste Receptor Genes

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Human bitter taste is mediated by 25 members of the hTAS2R family of G protein–coupled receptors. From studies in rodents it is believed that all bitter receptor genes are expressed in the same subset of taste receptor cells. This observation, if applicable to humans, explains why we are not able to distinguish between different bitter substances. Since an intense bitter taste is believed to serve a warning function against the ingestion of poisonous food, it is, perhaps, not necessary to distinguish between individual bitter compounds. There are, however, many open questions: 1) Are human TAS2Rs coexpressed in the same subset of taste receptor cells as observed for rodents, and, if so, are all hTAS2R genes coexpressed. 2) Is the apparent expression level comparable between hTAS2Rs or do we need to take differences in gene expression into account for observed differences in bitter taste sensitivities beyond pharmacological characteristics of the receptors. In the present study we analyzed human fungiform and circumvallate papillae for the expression of TAS2R genes by RT-PCR. Our data demonstrate the presence of all 25 hTAS2R genes in both gustatory structures. By in situ hybridization we detected all hTAS2R mRNAs in taste receptor cells of circumvallate papillae. Careful analyses of the in situ hybridization data revealed that the hTAS2R gene expression differs in the number of positive cells as well as, independent of the cell number, in their apparent expression levels. These observations were confirmed by double-label in situ hybridization to monitor cellular coexpression of selected pairs of hTAS2Rs. Although the expression patterns of hTAS2R genes largely overlap with each other, our data indicate a nonuniform population of human bitter taste receptor cells.

256. Pleasantness Information Facilitates Taste Detection and Interacts with the Time Available for Evaluation

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In three experiments we show that affective context is able to influence taste detection times. In the first experiment with long prime presentation times (1000 ms) and long prime-target intervals (750–1000 ms) we found that detection times of gustatory target stimuli are shortened if the pleasantness information in the prime is congruent with hedonic tone of the taste stimulus (e.g. ''good''-sucrose; ''bad''-caffeine; ''neutral''-demineralised water) as compared to incongruent prime-target combinations ($F(1,14) = 5.253$, $P = 0.038$). In the second experiment using shorter prime presentation times (200 ms), and prime-target intervals (200 ms) this facilitation of detection by an affectively congruent context was replicated ($F(2,24) = 8.787$, $P = 0.001$). In the first experiment congruency facilitation was most prominent for sucrose, in the second experiment for caffeine. We proposed that there may be preferential facilitation of the processing of negative stimuli under time-constraints, and that with more time, positive stimuli might benefit most from congruency facilitation. In a third experiment we investigated this hypothesis by manipulating the time between prime-onset and target (stimulus onset asynchrony or SOA) at three levels: 0, 550 and 1000 ms. Preliminary results indicate that there is an interaction of SOA, prime-target congruency and stimulus valence.

257. Development of a Flavor Descriptive Analysis Procedure for Orange Juices by Two Trained Panels in Spain and USA

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The complex flavor of orange juice makes it difficult to obtain a comprehensive sensory profile. Even though orange juice is a major food product which is traded internationally, few descriptive analysis studies can be found in the literature. The objectives of this study were (1) to set up a specific methodology for the flavor descriptive analysis of orange juices and (2) to identify specific transnational sensory patterns among the major categories of orange juice (hand-squeezed, pasteurized, refrigerated from concentrated and canned from concentrate). A trained descriptive flavor panel ($n = 12$) located in USA, developed the lexicon, standard references, sample preparation, serving and tasting procedures using a wide collection ($n = 35$) of orange juices. Juices selected varied in processing technology and cultivars. Fifteen highly trained flavor panelists in Spain characterized the flavors of the major orange juice sold in Spain ($n = 20$) using

the methodology established by the USA panel. Resulting data indicated that the developed flavor descriptive analysis procedure can be used either in Spain or USA with similar outcomes. Orange juices prepared using similar processes in both countries had comparable flavor characteristics. Thus, hand-squeezed orange juices were described with citrus fruits, green and floral attributes. Pasteurized products were also described as citrusy, green and sweet fruit along with cooked descriptors of low intensity. Refrigerated from concentrate juices had an intense orange peel oil, cooked, caramel, noncitrus fruits and chemical attributes while canned from concentrate juices no longer tasted like orange juice and were characterized by tropical fruits, chemical and cooked attributes.

Poster Session 6

258. Modeling of the Psychophysical Response Curves Using the Grand Canonical Ensemble in Statistical Physics

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This work represents a contribution to the study of the taste mechanism by using the grand canonical ensemble in statistical physics. This study allowed us to better understand this process and to interpret this phenomenon at the microscopic level. We have carried out an experimental part to obtain psychophysical curves relative to four sweet taste sugars (sucrose, fructose, glucose and maltitol). These curves represent the intensity of sugars according to their concentration in solution. For that, a sensory measuring unit for recording flux (SMURF) device was used to carry out the different curves. To model these curves we have established the expressions of some models using a grand canonical ensemble formalism in statistical physics and making some simplifying approaches. The adjustment of the psychophysical data with the suitable model by numerical simulation allowed deducing the different parameters intervening in the expression of the models. We have explained the choice of the appropriate model and we attempt to interpret the behavior of the physico-chemical parameters. These parameters are classified in two categories: the first one is a steric aspect such as the density of taste receptor site and the number of molecules per receptor site and the second was an energetic aspect such as the concentration at half saturation which gives indirectly the interaction sweet molecule-receptor site energy. The evolution of these parameters with the properties of each stimulus is discussed. It's turned out that the calculated interaction energy is linked to the sweetness of the studied molecules.

259. How Do Neurones of the Fish Olfactory Bulb Discriminate between Skin Extracts from Different Species?

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Crude skin extract contains alarm substances which in many fishes initiate antipredator behaviour upon olfactory detection, but in addition a multitude of odorants such as amino acids, bile salts and sex pheromones. This complex mixture of chemicals could help olfactory bulb neurones to detect and discriminate between skin extracts from different fish species. The teleost olfactory system shows a distinct chemotopy; there is clear subdivision of neurones into spatially different regions that each respond specifically to different classes of odorants (Lastein et al. 2006. Chem Senses 31:69). Nervous activity from type I neurones (Hamdani, Døving. 2003. Chem Senses 28:181) in different parts of the olfactory bulb in crucian carp was recorded while stimulating with skin extracts from four different cyprinid species. Preliminary results show that neurones in the lateral bulbar region sensitive to amino acids and polyamines, often respond to all or none of the four different extracts. Neurones in the posterior region of the dorso-medial part of the bulb, sensitive to alarm substances, have more varied response profiles, responding equally often to 1, 2, 3 or 4 extracts. The discriminatory power seems better at lower than higher concentrations. These findings demonstrate that bulbar neurones in different regions are sensitive to skin extracts, but indicate a spatial segregation in the discriminative capacity.

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260. Exploratory Analysis of the Rat Olfactory Bulb Activity

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Olfactory perception and its relation to the olfactory system architecture has been an exciting topic of research in the last decade. This paper explores a database of images of the glomerular activity in the olfactory bulb of rats when exposed to different odorants. The clustering analysis performed reveals the existence of spatial order in this glomerular activity, and the existence of modules that respond in a similar manner to different chemicals. The database can be found at the URL address http://leonlab.bio.uci.edu/. This archive contains the averaged activity maps generated from the glomerular response to selected odorants in rat olfactory bulbs, as assessed by 14C 2-deoxyglucose uptake. At the moment of starting this analysis we downloaded 94 images from 54 different chemicals. Some chemicals appear at different levels of concentration. Images can be either dorsal centered or ventral centered. Several clustering algorithms have been used in order to explore the stability of the results. We have chosen Fuzzy C-means, Gaussian Mixture Models and Fuzzy-ART. First examination of the figures shows the appearance of topologically connected regions that react in a similar manner to the chemicals. Results confirm the existence of spatial order within the bulb, that seems to be invariant among different individuals. On the other hand, this is noteworthy since such an ordering has not been found at the level of the olfactory epithelium. On the other hand, we may observe that several clusters appear in a consistent manner across the different clustering algorithms, while some others appear with slightly different borders.

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261. Relationship between Olfactory Reactivity and Food Rejections in Infants

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The role of hedonic reactions to odours on determining rejections of foods in infants is still debated. To answer this question, the first requirement is to have an instrument to test infants' olfactory reactivity. Indeed, except in neonates, there is a lack of validated method. This study presents results of a new method designed to assess olfactory reactivity of 7- to 8-month-old infants. This method is based on the observation of infants' reactions while they explored different rattles. The rattles were visually identical but differed in their odours (neutral or with a food odour). Two sequences of 6 rattles were presented to each infant. Eight odorants were used. Four odorants were chosen to evoke odours of often rejected foods and four were chosen to evoke odours of often liked foods. The hedonic valence was previously checked with an adult panel. These odours were also equated for intensity and had a very low perceived trigeminal component. Forty infants were tested and videotaped. These videotapes were subjected to frame-by-frame analysis to measure a variety of behaviours supposed to reflect the infants' hedonic reaction (e.g., times spent to mouthing or pushing away the rattle). Duration of these events for each odorized rattle was compared to those observed for the control rattles. It thus allows getting a reactivity index per odorant and over all odorants. Reactivity of infants to these odorants was related to infants' reactions to food during weaning, evaluated by the mother. Methodological considerations concerning the reliability of measures of hedonic reactivity in infants will be discussed. The issue of whether young children experience odours as pleasant or unpleasant will be discussed in relation with their food experiences.

262. Major Urinary Proteins (MUPs) as a Perspective Model in Genetics of Aggression: Methodological Consideration and Experimental Evidence

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Aggressive behavior represents an important type of social communication system in mammals. Aggression takes place between at least two animals and consists of extremely complicated information exchange between individuals. Thus the unit of genetic analysis involves dynamically changeable and reciprocal interactions of the leading sensory modalities. The model of pheromonally mediated aggression in *Mus musculus* was first applied in behavior genetic analysis 30 years ago. In the given report several genetic models of the pheromonally mediated social behaviors in laboratory mice developed during last decade are reviewed in lights of rapidly growing data in pheromone chemistry and major urinary proteins (MUPs) neurobiology. The obtained results on differential Mup genes expression by steroid induction are described by the formulae "gene → protein → pheromone → behavior" strongly support the idea of fruitfulness of the MUPs model for the fine dissection of the behavioral phenotype in order to visualize the multichain chemosensory pathways from gene(s) to olfactory mediated social behaviors.

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263. Difference of Cognitive Influences on Perceptual Aspects in Typical Odor Pollutants

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It is not easy to control odor pollution because the effects on perception from exposure to odorants were mediated not only by direct chemicals but also by cognitive variable. Last ECRO we reported that the variety of time-intensity curves for 10 min. exposure of triethyl amine was related to the individual perceptual aspects. In the present study we compared the cognitive influence on perceptual aspects of typical odor pollutants. One hundred fifty two healthy women and men were exposed to 1 of 3 odors (ethyl acetate, hydrogen sulfide and ammonia), regulated as the odor pollutants in Japan, after receiving 1 of 3 instructions that the odorant was harmful, healthful, or neutral. Two concentrations were used for ethyl acetate and hydrogen sulfide, and one concentration for ammonia, considering perceived intensity and the security. Participants rated continuously the odor intensity as time-intensity, which was recorded in every 50 ms, during 10 min. exposure of odorant and odorless air before and after odorant. They completed questionnaires on the odor unpleasantness and odor quality after odorant exposure. As the results, cognitive effects on perceptual aspects, such as the unpleasantness, the odor quality, and the total sum of intensity calculated from time-intensity data were not same in 3 odor pollutants; the largest effects in ethyl acetate, the next in hydrogen sulfide, and the smallest in ammonia. The unpleasantness was related to cognitive factors, however more related to the perceived odor quality in ethyl acetate. And the effects were larger for lower concentration in ethyl acetate and hydrogen sulfide. These results suggest several variables to enlarge the cognitive effects in odor pollutants.

264. Psychophysical Tests of the Vibration Theory of Olfaction

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A recent psychophysical study testing predictions of the vibration theory of olfaction found that humans could not discriminate between deuterated and undeuterated versions of acetophenone. Here I present results of a series of double-blind triangle tests in which I test the ability of human smellers to discriminate deuterated and undeuterated versions of acetaldehyde, acetophenone and pyrazine, as well as the two enantiomers of carvone. Contrary to expectation, humans could discriminate between the two versions of acetaldehyde at rates substantially better than chance, and close to the rate at which the carvones could be discriminated. Furthermore, deuterated and undeuterated versions of acetophenone, in this study, could be discriminated at rates better than chance. The ability to discriminate between the versions varies with concentration used. The implications of these results will be discussed.

265. Electrophysiological Properties of Ion Channels Implicated in Olfactory Transduction

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Olfactory transduction is the cellular process that converts the binding of odor molecules to olfactory sensory neurons into electrical

signals. Odor binding to olfactory receptors in the cilia of olfactory sensory neurons leads to an increase in the ciliary concentration of cAMP followed by and increase of intraciliary Ca concentration by Ca entry through cAMP-gated channels. Ca activates a Cl channel that, since olfactory sensory neurons maintain a high ciliary Cl concentration, leads to an efflux of Cl from the cilia, contributing to the amplification of the olfactory sensory neurons depolarization. In this work we used the patch-clamp technique to measure the electrophysiological properties of cAMP-gated channels and calcium-activated chloride channels in inside-out membrane patches excised from dendritic knob/cilia of dissociated mouse olfactory sensory neurons.

266. Enhancement Effect of Aromatherapy on Free Radical Scavenging Activity and Stress

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Free radical/reactive oxygen species are related to many biological phenomena such as inflammation, aging, and carcinogenesis. The body possesses various antioxidative systems (free radical scavenging activity, FRSA) for preventing oxidative stress, and saliva contains such activity. In the present study, we measured the total salivary FRSA induced after the smelling of lavender and rosemary essential oils that are widely used in aromatherapy. Various physiologically active substances in saliva such as cortisol, and secretory IgA were found to be correlated with aroma-induced FRSA. The subjects (22 healthy volunteers) sniffed aroma for 5 minutes and each subject's saliva was collected immediately. FRSA was measured using 1,1-diphenyl-2-picrylhydrazyl. FRSA was significantly increased by lavender stimulation, while no change was observed immediately after stimulation with isovaleric acid as an unpleasant smell. The FRSA values were increased by stimulation with low-concentrations (1000 times dilution) of lavender or by high-concentrations (10 times dilution) of rosemary. In contrast, both lavender- and rosemary-stimulations decreased cortisol levels. A significant inverse correlation (−0.486) was observed between the FRSA values and the cortisol levels with rosemary-stimulation. No significant changes were noted in sIgA. These findings clarify that lavender and rosemary enhance FRSA and decrease the stress hormone, cortisol, which protects the body from oxidative stress.

267. Rhythmical Activities of Individual Cells and LFP Oscillations in the Rat Olfactory Bulb

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By constituting the missing link between unitary- and populational activity, studies on the temporal relationships existing between individual cell discharge and local field potentials (LFPs) could supply an overall view of brain functioning. In the mammal olfactory bulb (OB), such data are very incomplete since most of them have been recorded in vitro where LFPs oscillate in a single frequency band. Therefore, we previously showed that, in the in vivo rat, odor stimulation induced LFPs oscillations in the gamma and beta ranges, these 2 epochs alternating during the respiratory cycle. The aim of this work was to study the temporal relationships existing between mitral/tufted cell (M/T) individual activities and both LFP oscillatory bands in the anesthetized rat. Bulbar activity was recorded as a broadband signal using multichannel probes in the mitral cell layer. M/T cells action potentials and LFPs were extracted from this signal. LFP oscillatory activity was analyzed using wavelet transformation from which frequency and phase were extracted. All analyses were made with regard to the 2 main temporal patterns of M/T cell activities, S+ (excitatory–simple–synchronized) and S− (suppressive–simple–synchronized). We show that: Spike rate of S+ activities increases during both beta and gamma epochs, while S− rate does not change. S+ and S− responses are phase-locked to gamma and beta bursts respectively. During the whole respiratory cycle, M/T cells show rhythmical activity in the gamma range even when gamma oscillations are not present in the LFPs. M/T cells do not show a rhythmical activity in the beta range even when LFPs oscillate in such regime. Our data suggest that rhythmical activity of M/T cells and oscillatory LFP activity could be partially dissociated mechanisms.

268. Odor-Evoked LFP Oscillations in the Olfactory Bulb: A Code for Odorant Molecular Features?

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A central question for chemical senses is understanding the way odorant molecules are represented in the brain. It has been shown that the olfactory system recognizes some particular structural features of the molecules which are represented through a spatio-temporal pattern. 2DG studies showed that glomerular responses are globally chemotopic, so that pairs of odorants with the smallest difference in molecular features evoked the most similar patterns. We wanted to test if the same observation could be made on the temporal structure of bulbar activity. Since local field potentials (LFPs) have been shown to play an important role in information coding, we focused on the relationships between odorant chemical features and LFP oscillation patterns. In the rat olfactory system, the LFPs display oscillatory epochs in two separate frequency bands: beta (10–35 Hz) and gamma (40–80 Hz), these epochs alternating with the respiratory cycle. We recorded the LFPs in the olfactory bulb of urethane anesthetized, freely breathing rats, in response to series of aliphatic odorants varying subtly in their molecular features (carbon chain length and functional group). Our results showed that, regardless to the nature of the odorant, the gamma epochs always occur at the transition between inspiration and expiration. Conversely, the position, duration and amplitude of beta oscillatory epochs in the respiratory cycle depend on both the functional group and the carbon chain length of the molecule. We have also noticed that all odorants are not equally capable of triggering oscillations in beta, gamma or both bands. Our results suggest that the code for odorant quality may be partially contained in the oscillations generated by the system.

269. Functional Endoscopy of the Human Olfactory Mucosa

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Endoscopy of the olfactory mucosa is important for clinical examination of patients with hyposmia and/or anosmia. However,

endoscopy typically provides morphological insights into the status of the epithelium. To record a functional image of the olfactory mucosa with an endoscope, the endoscopic intrinsic signal recording system was developed. A LED (617 nm) light source and a cooled CCD camera were prepared for endoscopy of the olfactory cleft. The light intensity of LED was controlled by the constant current. Image size produced by the cooled CCD camera was 640×480 pixels with a 16 bit resolution gray scale (xx frames/s). The change of light intensity of every pixel per frame was calculated and displayed by the PC. The software of this analysis was coded by LabVIEW7. Phen ethyl alcohol odor was presented with a ''Sniffin' Stick'' for 10 s in front of the nostril; no exposure was used for control. When the subjects sniffed the odor, absorption of 617 nm light on the olfactory mucosa was increased but it did not changed during the control condition. A gradual change of the absorption was observed during both the stimulation and control; these were similar to so-called DC-sift in electro-physiological recordings such as the electro-olfactogram. Because it is well known that activation of the brain induces a decrease of the oxyhemoglobin level in the tissue, the decreasing oxyhemoglobin level in the olfactory mucosa elicited by the odorant was suspected. Decrease of tissue oxyhemoglobin induced an increasing absorption of the 617 nm wave length light so that activated olfactory mucosa could be visualized. It is hoped that functional endoscopy of the olfactory mucosa will become a new technique to study the sense of smell at a peripheral level.

270. Satiation-Related Changes in Olfactory Hedonism: Heterogeneous Effects for Food and Nonfood Odours

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The hedonic value of food odours (FO) decreases after a meal. This hedonic shift is considered specific of food stimuli in general, although no clear evidence with nonfood odours (nFO) is available. Thus, previous studies have not verified whether odorants used as food representatives did effectively evoke food in the subjects. Further, it is not understood whether the meal-related hedonic fluctuation of FO is explained by variations in arousal, liking or wanting. The present study aims: 1) to verify whether the food vs. nonfood categorization of odours matches between subjects' perception and experimenters' assignment; 2) to compare the effect of a meal on arousal, liking, and wanting to FO and nFO. Healthy women (n = 28) came to the lab twice on consecutive days: i) before and ii) after lunch. They had to smell 8 FO and 8 nFO (all stimuli iso-intense, presented in random order), and to rate arousal, liking and, if the stimuli were considered FO, wanting. It came out that: 1) odours were mostly well categorized (FO: 63%, nFO: 77%, n = 224 responses/odour category), the remaining responses being either false or variable as a function of subject's satiety; 2) for odours perceived as FO, the meal reduced arousal, liking and wanting, whereas no decrease in liking was noted for non-FO; 3) the meal-related decrease in liking and wanting of FO occurred only with certain stimuli (i.e., meat, pizza), and not with others (i.e., strawberry, orange). Thus, satiety modulates the affective valuation of food-related odorants, but this effect is uneven according to the nature of the olfactorily presented and/or the cognitively represented food. The psychobiological, developmental and cultural processes that bring about such dissociation in food and nonfood stimuli are being explored.

271. Cognitive Effects on Cerebral Activities under Intermittent Presentation of Short-Duration Odor

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We introduced intermittent presentation method of short-duration odor to evaluate the cognitive effects on odor adaptation/habituation in ECRO 2004. We tried, in the present study, to evaluate these cognitive effects using electrophysiological techniques such as electro-encephalograph (EEG) and magneto-encephalograph (MEG). We measured stimulus-induced cerebral responses following either a hazardous or a healthful instruction. The participants were given intermittent, short-duration odor (anethole) to their right nostril 60 times over two sessions. The odor was presented with a Kobal-olfactometer. The time for 1 session was 20 minutes and 1-h interval (intersession interval) was interposed between the two sessions. We placed an aspirating tube below the nostril to reduce the influence of adaptation at the peripheral (receptor) level so that more cognitive aspects of odor perception would be emphasized. Vanillin was used as a control odor. The participants rated perceived odor intensity from 0 (no smell) to 5 (extremely strong) and odor hedonics from −100 (extremely unpleasant) to +100 (extremely pleasant). We found a difference in odor hedonics according to the hazardous and healthful instructions as we reported in ECRO 2004. We also found remarkable responses in both conditions after averaging EEG, though we have not obtained enough samples yet (4 to 5 samples in each group). We are now in the course of accumulating more samples and more reliable analysis including MEG data, for the cerebral representation of cognitive effects.

272. IMP-Induced Alteration of Pathways for Umami in Brain Stem of Mice

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Umami substances, monosodium L-glutamate (MSG) and monopotassium L-glutamate (MPG) represent considerably different taste qualities from each other in human as well as mouse. However, the taste quality of MPG with inosine 5′-monophosphate (IMP) seems to be close to that of MSG because we found using a conditioned taste aversion (CTA) paradigm that C57BL/6 mice, whose umami sensitivity is relatively similar to that of human, discriminated MSG from MPG, but dose-dependently failed to discriminate MSG from MPG with IMP (MPG + IMP). The distribution pattern of MPG-stimulated Fos-like immunoreactivity (FLI) in the parabrachial nucleus (PBN) and the nucleus of solitary tract (NTS) was altered by addition of IMP to be similar pattern of MSG-stimulated FLI: MSG-stimulated FLI tended to distribute in anteromedial part

of PBN and NTS, but MPG-induced FLI, which dispersedly located in the posterior part of PBN and NTS, shifted to anteromedial part of PBN and NTS with addition of IMP. Furthermore, MPG-stimulated FLI was modulated also toward horizontal and vertical axes in PBN but not in NTS by addition of IMP. These results suggest that IMP-induced taste quality change of MPG is accompanied by the alteration of pathways toward antero-posterior axis within NTS, followed by the alteration of pathways toward three-dimensional axes within the PBN.

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273. Reversed Innervation of Pheromone Specific Antennal Lobe Neurons in the European Corn Borer (*Ostrinia nubilalis*)

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The European Corn Borer is a well known example of pheromone polymorphism and possible speciation through reproductive isolation in the pheromone communication channel. Two strains, the E and the Z strain, do not interbreed freely in sympatry, as they produce and prefer a reversed ratio of the pheromone components E11-14:OAc and Z11-14:OAc. Here we studied how the difference in male preference correlate with differences in wiring of olfactory input and output neurons in the antennal lobe (AL). Intracellular recording and staining techniques were used to establish the structure and function of male AL projection neurons (PN). Physiologically characterized neurons were filled with neurobiotin and their dendritic arborization patterns were reconstructed using confocal microscopy in alpha-synapsin overview stained ALs. In addition, innervation patterns of single olfactory receptor neurons (ORN) were established by neurobiotin staining. Whereas the macroglomerular complex (MGC) of the antennal lobe was morphologically similar in the two strains, they differed substantially from earlier reports. It consists of two major compartments, a large, medial folded around a smaller, lateral one. ORNs displayed a uniglomerular innervation pattern in the MGC. PNs innervating one of the two major interdigitated MGC glomeruli were specifically tuned to either E- or Z11-14:OAc-specific. In the Z-strain Z11-14:OAc-specific PNs arborize in the larger, medial MGC glomerulus, whereas E11-14:OAc-specific neurons innervate the medial, smaller one. In the E strain this topology is reversed. We postulate that the E and Z responding ORNs have exchanged their olfactory receptors, whereas the ORN as well as the PN innervation patterns in the AL has been conserved between the two strains.

274. When Odour Turns into Grasp

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This study used kinematics to investigate the integration between vision and olfaction during grasping movements. Participants were requested to smell an odorant and then grasp an object presented in central vision. The results indicate that if the target was small (e.g., strawberry), the time and amplitude of maximum hand aperture were later and greater, respectively, when the odor evoked a larger object (e.g., orange) than when the odor evoked an object of a similar size as the target or no odor was presented. Conversely, the time and amplitude of maximum hand aperture was earlier and reduced, respectively, when the target was large (e.g., peach) and the odor evoked a smaller sized object (e.g., almond) than when the odor evoked an object of a similar size as the target or no odor was presented. In light of this evidence it can be suggested that conflicts may emerge when the distractor and target objects require different prehensile patterns in order to be grasped or manipulated. Neuronal populations, kinematic planning and functional properties for the task-irrelevant object evoked by the olfactory stimulus are alerted and interfere with neuronal populations, kinematic planning and functional properties activated and executed for the target object. In other words, the objects evoked by the olfactory stimuli automatically activate their motor responses without the participant's intention to act. This may suggest that the representation of olfactory stimuli is already present in memory and implicitly and automatically encoded in terms of the action it evokes.

275. Pheromone which Suppresses Spermatogenesis in Laboratory Mice Is Associated with Urine Protein Fraction

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Thirty days old CBAB6F1 male mice were subjected to volatile chemicals contained in voided urine from mature males of CBA/LacSto strain. It was shown that the significant increase of spermatozoa with abnormal head shape in cauda epididymis after 2 h exposure to urine associated with substances of different polarities presented in protein fraction. The obtained results are discussed in lights of 1) modern views on tight connection between physiological activity of several androgen-dependent pheromones and major urinary proteins (MUPs) and 2) abundant MUP mRNA content in nasal tissues. Taken together these data suggested that MUP artificial analogs could serve as a perspective molecular vector for non-invasive drug targeting into brain and regulation of reproductive function via transnasal delivery.

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276. Monitoring Biosynthesis, Trafficking, and Activation of a Human Olfactory Receptor at a Single Molecule Level

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A novel strategy was explored for gaining insight into olfactory receptor expression and regulation and for studying influences of odorant molecule binding on receptor diffusion, dimerization, and internalization. For enabling such studies in living cells we

employed a differential fluorescence labeling technique for obtaining an optical separation of intracellular and cell surface located receptors. We made use of the posttranslational modification of the E. coli derived acyl carrier protein (ACP) by phosphopantetheine transferase (PPTase), leading to the transfer of 4′ phosphopantetheine from coenzyme A (CoA) to a serine residue of ACP. The human odorant receptor OR17-40 was modified at its amino terminus by the addition of the 77 amino acid encoding ACP sequence preserving the functionality of the receptor as determined by OR-specific calcium signaling in HEK293 cells. By using CoA derivatives modified with fluorescence labels in presence of recombinant PPTase it was possible to selectively visualize functional OR17-40 receptors, which were integrated in the plasma membrane of HEK 293 cells. Furthermore, the covalent attachment of one fluorophore per odorant receptor allowed functional studies at a molecular level using single molecule imaging. These experiments provided first insight into dynamic processes of early events in odorant receptor signaling. By employing a double-tagged and functionally expressed OR17-40 receptor fused at its amino terminus with ACP and C-terminally to EGFP it was possible to temporally and spatially resolve sequential stages in the lifecycle of OR17-40 from intracellular protein synthesis to plasma membrane insertion.

277. Heterogeneous Generation of Periglomerular Cells in the Adult Mouse

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The olfactory system of adult mammals has a continual influx of new neurons. Stem cells in the subventricular zone (SVZ) lining the lateral ventricles give rise to neuroblasts that migrate into the olfactory bulb (OB), via the Rostral Migratory Stream (RMS). In the OB, they differentiate into two populations of interneurons, granule cells and periglomerular (PG) cells. The PG cells, because they represent a small proportion of the new cells, have received relatively little attention. PG cells can be divided into several subtypes, based on morphology or expression of neurotransmitters and calcium-binding proteins. It is not known if all the subtypes continue to be generated in adulthood, or if the adult-generated neurons comprise only one or a few subtypes of PG cell. We have examined this question in mouse by using several techniques. Injection of GFP-expressing retrovirus into the SVZ allows us to assess the morphology of newborn neurons and to study their developmental time course. We have also used BrdU incorporation as a marker of new cells, followed by immunohistochemistry for BrdU and calcium binding proteins, such as calbindin, calretinin, and parvalbumin, or markers of neurotransmitter phenotype, such as tyrosine hydroxylase (TH) and glutamic acid decarboxylase (GAD). We have found that adult-born cells can differentiate into each of the subtypes of PG cell. They are, however, more likely to become GABAergic or dopaminergic. Examining PG cells after longer survival times, we have found that about half of the new PG cells are lost, but that the subtypes are differentially affected. Our data indicate that the generation and survival of PG cells is not uniform and may reflect different functional roles for PG cells or their integration into glomerular circuits.

278. Behavioral, Electrophysiological, and Humoral Studies of the Enhancement of Preference to Sodium in Zinc Deficient Rats

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Several reports demonstrated that zinc deficient (ZnX) rats enhanced their preference to sodium solution, and that zinc deficiency brought about the taste disorder. In the present study, we conducted behavioral, electrophysiological and humoral experiments to investigate the morbid-physiological characteristics of zinc deficient rats. Results were as follows: (1) In the long-term (48 h) two-bottle preference test, the preference percents for 0.1 and 0.3 M NaCl in the ZnX rats were higher than those in the control rats. In the short-term (10 min) test, however, there was no significant difference in the preference percents between ZnX and control rats. (2) When the ZnX rats with dissection of the taste nerves were used for long-term two-bottle preference test, there was no significant difference in the preference percents for 0.1 and 0.3 M NaCl between the ZnX and the sham rats. (3) When ZnX rats were subjected to aversive conditioning to one of the basic taste stimuli, any animals could acquire the conditioned taste aversion, and they never generalized to other stimuli. (4) There was also no significant difference in the neural responses of chorda tympani nerves to some sodium solutions between ZnX and control rats. (5) There was no significant difference in serum concentrations of serum sodium between ZnX and control rats, but statistical variation of ZnX were higher than those of control rats. (6) Serum concentrations of angiotensin II decreased significantly in ZnX group compared with control group. However, concentrations of aldosterone were significantly higher in ZnX than control group. These results suggest that the enhancement of preference for sodium is caused by the disorder of sodium metabolism rather than taste.

279. The Number of Each Cell Type in Mouse Single Fungiform Taste Buds

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Each taste bud, which comprises type I ~ type IV taste bud cells (TBCs), is a functional unit for sensing taste substances and for transmitting taste information to the taste nerves. Recent studies showed that type II cells were sweet, bitter and umami receptor cells, and that only type III cells formed synapses with taste nerves. Quantitative studies on these cell types are essential in understanding the taste transduction mechanisms of taste buds. Using confocal microscopy, we investigated the number of each cell type in single, whole-mount taste buds in peeled lingual epithelia. The maximum taste bud size (MTBS) estimated with DIC images of optical cross-sections was 843.5 ± 217.7 μm^2 (mean ± SD, n = 287). The number of total TBCs was 41.7 ± 12.7 cells a taste bud (n = 72), which highly correlated with MTBS (r = 0.94). Type II cells (IP3R3-immunoreactive cells) occurred in all taste buds examined, and was 10.7 ± 3.4 cells a taste bud (n = 164), and substantially correlated with MTBS (r = 0.74). Type III cells (SNAP-25 immunoreactive cells) occurred in 92% of taste buds examined, and was

2.2 ± 1.3 cells a taste bud (n = 198), and poorly correlated with MTBS (r = 0.49). The sum of type I and IV cells (nonimmunoreactive cells, the difference between total TBC number and the sum of type II and III cells) was 24.9 ± 8.1 cells a taste bud (n = 43), and highly correlated with MTBS (r = 0.95). These results showed that the number of total TBCs and the sum of type I and IV cells were estimated from MTBS. The number of afferent innervating nerves rather than MTAS may be more important in deciding the number of other cell types, typically that of type III cells.

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280. Promotor-Motifs Governing the Spatial Expression Pattern of Olfactory Receptors

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Odorant receptors (ORs) of the OR37 subfamily are only expressed in olfactory sensory neurons (OSNs) which are segregated within a small area in the center of olfactory epithelium. The encoding genes comprise highly conserved DNA motifs immediately upstream of the transcription start site which might be candidate elements for governing the spatial expression pattern. To scrutinize this hypothesis, transgenic mouse lines were generated which carry random integrated DNA constructs with the coding region of OR37C and the 5′-region including the conserved DNA motifs. In 6 out of 7 independent mouse lines, the transgene was found to be expressed in cells segregated in the characteristic clustered pattern. The number of transgene expressing OSNs varied considerably between the different lines. The transgene was expressed in a mutually exclusive manner and only one allele per neuron. The axons of transgene expressing OSNs in all mouse lines projected to the ventral domain of the olfactory bulb; those axons of OSNs located within the OR37 area generally coconverged with the axons of cells expressing the endogenous OR37C gene in the same glomerulus. Ectopically positioned transgene expressing cells formed novel glomeruli. These results demonstrate that the major features of the special OR37 topography are recapitulated by the short transgene; thus, indicating that the conserved DNA elements are indeed involved in controlling the distinct expression pattern of the OR37 receptor types.

281. Can Dogs Sniff Out the Expiration-Odor of Cancer Patient

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Several researchers have reported that dogs may be able to detect mole or tumours on the basis of odour. Recently, it is reported that dogs can be trained to distinguish patients with bladder cancer on the basis of urine odour. Since it is well known that that the patient is scented unique odour, for example, diabetic patients are scented sweet body odour and sweet expiration odour. The ability of dogs to recognize and cross-match many kinds of variety of odours are studied by electrophysiological and behavioral experiments. Here we show that dogs can sniff out the expiration of cancer patients. We are training three black Labradors. The training objective was to enable the dogs to discriminate among the four expirations. There are one cancer patient's expiration and three healthy men's expirations. Dogs were trained to detect (indicate and lying beside the odour box) one expiration sample from a patient with cancer placed among three control specimens.

282. Taste Buds Ultrastructure in the Sturgeon *Acipenser naccarii*

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Fish like other vertebrates use their taste bud cells to sample potential food, either selecting or rejecting substances according to their edibility. Generally, mature fish taste buds possess three types of cells that form the sensory epithelium of the organ: dark cells with many small microvilli, light cells with one large microvilli and basal cells that lie horizontally at the base of the taste bud. The ultrastructure of taste buds in the various groups of fishes and even within the same group depends on the species. Therefore it is difficult to state "model TB" in fishes. The present study provide new insights into the taste buds of sturgeons, specifically *Acipenser naccarii*. In taste buds of *A. naccarii*, two types of light cells can be recognized. They differ in the type of microvilli that they bear at their apical and narrow endings. One type of light cell has a single long and thick microvillus, the other type has a brush like apical ending that consists of between 8 and 12 microvilli that are a little shorter and narrower than the first one. There is only one type of dark cells and they that show a large apical surface and bear many microvilli far shorter and more thinner than those light cells. They also contain electron-dense granules. Basal cells contain electron light synaptic vesicles. Sometimes these vesicles occupy the cytoplasmatic volume entirely. Their most remarkable feature in the presence of spines that are similar to those of taste buds in modern teleost. Usually, basal cells of taste buds in more basic group of fishes have no or only very inconspicuous spines.

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283. Rumen Metabolites Serve Ticks to Exploit Large Mammals

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Hard ticks spend most of their life isolated from passing vertebrates but require a blood meal to proceed to the next life stage (larva, nymph or adult). These opportunist ectoparasites must be capable of anticipating signals that render suitable hosts apparent. Large ungulates that tolerate a high ectoparasite burden are the favoured hosts of adult hard ticks. Ruminants, comprising the majority of ungulate species, must regularly eruct gases from the foregut to relieve excess pressure and maintain a chemical equilibrium. Through eructation from individuals, and particularly herds, ruminants inadvertently signal their presence to hard ticks. We report that all adult hard tick species tested are attracted to cud and we demonstrate that these acarines possess olfactory receptor cells for the

carboxylic acid, phenol and indole end-products of the rumen bioreactor. Compounds from each of these classes of volatiles attract ticks on their own, and mixtures of these volatiles based on rumen composition also attract. Appetence for rumen metabolites represents a fundamental resource-tracking adaptation by hard ticks for large roaming mammals.

284. Human Newborns Differentiate the Odour of Areolar Secretions from Other Species-Specific and Nonspecific Odorants

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Human newborns are known to respond to odorous compounds emitted on the breast surface or in conspecific milk. In a previous study, it was found that the number of areolar (Montgomery's) glands is linked with neonatal suckling behaviour and growth (Schaal et al. 2006. Dev Psychobiol 48:100–110). These glands stain the areolar skin with a milk-sebum blend. Here, we compared the functional activity of distinct odorous secretions from lactating women, and tested them against various odorants. Sleeping newborns (n = 12/8 breast-/bottle-fed; age: 3 days) were recorded for their facial and autonomic responses (respiration rate, RR; inspiratory amplitude, IA) to 8 stimuli (stim. duration: 10 s; interstim. interval: 50 s). Species-specific substrates included 1/nonfamiliar (nf) areolar gland secretion (nf-AG), 2/familiar and 3/nf human milk (nf-HM), 4/nf sebum; Non species-specific substrates included: 5/cow's milk, 6/nf formula milk, 7/vanillin, and 8/distilled water. It came out that, during the 10-s stimulus delivery: 1) more sustained facial activity was triggered by nf-AG odour, relative to all other stimuli; 2) higher mean IAs were noted for nf-AG and nf-HM odours; 3) breast- and bottle-fed newborns were similar in facial and autonomic responses to nf-AG odour. These findings suggest that "pure" AG secretion carries significant activity for newborns, as compared with substrates related to nursing or with arbitrary odorants. Areolar secretions carry salient stimuli for newborns regardless of their direct experience with human milk or breast.

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285. Morphology and Function of Antennal Sensilla in Gall Midges: A Comparative Study

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Sensilla circumfila is a unique type of sensilla reported only in gall midges (Diptera: Cecidomyiidae). Although they exhibit the basical structure of olfactory sensilla—thin walls highly perforated with pores—their function has not yet been demonstrated. We have investigated the morphology and function of sensilla circumfila using electron microscopy and single sensillum recordings in four different species *Contarinia nasturtii* (swede midge), *C. sorghicola* (sorghum midge), *Mayetiola destructor* (Hessian fly) and *Asphondylia ennadii*. In all the studied species, s. circumfila is a looped structure that encircles each antennal segment. It consists of up to 10 individual sensilla fused into one continuous structure. Each individual sensillum is innervated by 1 or 2 sensory cells that split into several branches. In *C. nasturtii* and *C. sorghicola* the s. circumfila exhibit a distinct sexual dimorphism, being highly enlarged in males. In M. destructor and *A. ennadii* the sexual dimorphism is less pronounced. All identified gall midge sex pheromones consist of blends of several compounds, where both ratio and stereochemistry may be important for male attraction. Here we report that s. circumfila in *C. nasturtii* respond to pheromone components as well as to a non-pheromonal stereoisomer. Behavioral experiments have shown that this stereoisomer inhibits male attraction to the pheromone.

286. Local Interneurons and Olfactory Information Processing in the Antennal Lobe of the Desert Locust, *Schistocerca gregaria*

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Physiological and morphological characteristics of projection neurons (PNs) and local interneurons (LNs) in the antennal lobe of gregarious adult desert locust, Schistocerca gregaria, were studied using whole cell patch clamp recording- and staining techniques. In response to odor stimulus, PNs showed excitatory, inhibitory and combined excitatory and inhibitory responses identical to that obtained with the more classical intracellular technique. Several PNs also displayed spikelets, either as part of an excitatory response or within periods of inhibition. These spikelets are assumed to arise intrinsically, be presynaptic neural events or alternatively arise due to electrical coupling through gap junctions between antennal lobe interneurons. In contrast to PNs, LNs produced nonspiking, slow- or long-lasting graded potentials to the presented odours. As for PNs, spikelets were observed frequently, especially during stimulation. Evidence of multipresynaptic input onto LNs was also noted. Based on their response to a set of odorants PNs and LNs were characterized into pheromone-specific, plant-specific and pheromone-plant generalist neurons. Furthermore, the dose-dependent responses of the LNs were studied. Pheromone-plant generalist neurons responded significantly stronger than pheromone generalist neurons to the adult aggregation pheromone blend, the major component of the adult blend, phenylacetonitrile, and the putative solitary sex pheromone, (*E,Z*)-2,6-nonadienal ($P < 0.0005$). Future studies aims at elucidating the interplay between LNs and PNs, in particularly with respect to the modulating function of the LNs and the neurotransmitter GABA.

287. Pheromone Communication in the Sorghum Chafer, *Pachnoda interrupta*

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The sorghum chafer, Pachnoda interrupta (Coleoptera: Scarabidae), is a major pest on sorghum that is an important Ethiopian staple crop. Our objective is to improve a locally developed "lure and kill" trapping method by replacing the current bait (fruit) with

kairomones from host plants combined with aggregation and/or sex pheromones. Trapping experiments in the field show that virgin females attract significantly more males than females, while mated females attract few beetles, with equal numbers of both sexes. This indicates the presence of a female-emitted sex pheromone attractive to males. However, there are indications that this pheromone may also be active in aggregation: virgin females placed on a food substrate (banana), attract high numbers of both males and females (with no significant difference in number of beetles caught of each sex). Mated females or males on a food substrate, in contrast, do not catch more than the food substrate alone. Outside of the mating season, however, trap catch indicates aggregation to both males and females on a food substrate.

288. Instrumental Conditioning and Learning Behavioral Sequences in the Freshwater Angelfish (*Pterophyllum scalare*)

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In aquarium, cichlid fishes exhibit a large variety of learned behaviors. An automatic feeder was constructed, which delivered food reward after the freshwater angelfish (Pterophyllum scalare) pulled the fiber connected to the electric switch operating the feeder. Textile fibers whose colour resembled food—either enchitreid worms or chironomid larvae were used initially to encourage the fish to start operating the feeder. The angelfish learned the operant response in less than 5 days. In final experiments the entire procedure was controlled by a computer using FDS Imaging Solutions software (FDS Research, Slovenia). The fish needed 1 week to master each of the subsequent behavior tasks. They learned to operate the feeder by pulling fibers of different colors and that the feeder was functional only if the conditioning lamp was on. In the following step the angelfish learned to turn on the conditioning lamp. After mastering that food is obtained only if the conditioning lamp is on, the angelfish learned the fiber colors associated with the lamp and the feeder. Finally the angelfish had to turn the conditioning lamp on and, during the short illumination period (6–10 s), trigger the feeder. After approximately 5–8 weeks 30–40% of the angelfish successfully mastered the sequential tasks of operating the spatially separated fibers for the lamp and the feeder. Factor analysis revealed that times between operating the lamp and the feeder fibers primarily depend on age-size, race and character of the angelfish.

We acknowledge the contribution of more than 80 undergraduate students that conducted the experiments during practical works in ethology since 1980.

Session 6

Symposium 16: Pheromone Reception in Moths

289. Pheromone Receptors of Moths: Identification and Characterisation

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Pheromone sensing of male moths is a remarkable performance mediated by sensory cells housed in sensilla hairs on the antennae. These specialized neurons detect and discriminate female released pheromones with extreme sensitivity and selectivity. The hydrophobic pheromonal compounds are supposed to be ferried by soluble pheromone binding proteins (PBPs) through the sensillum lymph towards the dendritic membrane of the sensory neurons where they interact with G protein–coupled pheromone receptors, which initiate intracellular transduction cascades generating electrical responses of the cells. We have identified a small family of candidate receptors for pheromones in the moths *Heliothis virescens* and *Bombyx mori*. RT-PCR and in situ hybridization studies have shown that several subtypes were selectively expressed in the antennae of male moths. Expression of these receptor types was confined to cells which were surrounded by cells expressing PBP and were located beneath long sensillar hair structures containing pheromone sensitive neurons. Using a receptor-specific antiserum allowed to visualize the receptor protein in sensory dendrites projecting into these sensilla. To assess the ligand specificity of putative pheromone receptors the responsiveness of cell lines stably expressing receptors was analysed using calcium-imaging approaches. These experiments indicated that expression of candidate pheromone receptors rendered HEK cells responsive to low concentrations of pheromones. Moreover, it was found that binding proteins can solubilize the hydrophobic pheromones and may contribute to the remarkable ligand specificity of the system.

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290. DAG, Calcium, and Chloride: Partners Involved in Insect Olfactory Transduction

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The olfactory transduction cascade remains unclear in insects. Both inositol trisphosphate (IP3) and diacylglycerol (DAG) are produced after receptor activation and may serve as second messengers but functional studies mainly focused on IP3. We investigated the role of DAG in moth olfactory transduction. Whole-cell patch-clamp recordings of cultured ORNs of the male moth *Spodoptera littoralis* revealed a novel DAG-activated cation current for insects. This DAG-gated channel is permeable to Ca2+. Its activation does not require Ca2+ or PKC. Decrease of external Ca2+ concentration or the presence of a calmodulin antagonist strongly increased the current amplitude, which demonstrates that this channel is regulated by intracellular Ca2+. An inhibitor of DAG kinase (DGK), a DAG degrading enzyme, produced a sustained activation of a current that shares the properties of the DAG-gated current. The antennal expression of a DGK, which we found in a male *S. littoralis* antennal EST bank, suggests that this enzyme regulates the concentration of endogenously generated DAG. On the basis of these results, we propose that DAG is involved in the chemo-electrical transduction in the moth S. littoralis by activating Ca2+-permeable cationic channels. This would generate an increase in intracellular Ca2+ concentration leading to the amplification of ORN depolarization by the activation of Ca2+-gated conductances such as Cl– currents (see the poster PÚzier and Lucas). The

common properties of the insect DAG-gated channel with TRPC2, a DAG-gated TRP channel recently characterized in mouse vomeronasal neurons, and the existence of Ca2+-gated Cl− channels as in vertebrate ORNs demonstrate the high similarity of olfactory transduction between vertebrates and invertebrates.

291. The Olfactory SNMP/Cd36 Gene Family of Insects

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SNMPs (Sensory Neuron Membrane Proteins) are abundantly located in the receptor membranes of lepidopteran olfactory neurons. In Lepidoptera, they are antennal specific, associate differentially with specific classes of sensilla, and express late in development. Two SNMPs have been identified in *Manduca sexta*; both are antennal specific but are only 40% identical in sequence. These observations suggest that SNMPs play a central role in odor detection. SNMPs are members of a gene family characterized by human CD36 which has diverse functions including transporting cholesterol and interacting with other proteins in cell–cell interactions; CD36 may function in part through interaction with lipid rafts. The *Drosophila melanogaster* genome contains at least 13 SNMP/CD36 homologues; we are characterizing the most likely SNMP orthologue (CG7000). Several of these genes have characterized function, including Nina D (carotenoid transport) and Croquemort (cell–cell recognition of apoptotic cells by macrophage). Expression analysis suggests CG7000 associates with a broad array of chemosensory sensilla both olfactory and non-olfactory, associates with a subset of olfactory sensilla, and expresses in both neurons and support cells. We are currently analyzing the phenotypes of animals in which CG7000 expression is suppressed in order to elucidate the function of this protein. Genomes of several other insects are available, providing an opportunity to examine the evolution of this gene family. We are currently identifying likely orthologues in other fly relatives (including mosquitoes), moths (e.g. *Bombyx mori*), bees and beetles using features of gene structure as characters.

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292. Convergent Projections of Pheromone Receptor Neurons to a Specific Subdivision of Macroglomerular Complex in the Antennal Lobe of the Silkmoth, *Bombyx mori*

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Pheromonal information detected by pheromone receptor neurons in the antennae of male moth is transmitted to the macroglomerular complex (MGC) in the antennal lobe (AL). The *B. mori* MGC consists of three subdivisions the toroid, cumulus and horseshoe. Recently, male-specific olfactory receptors, named BmOR1 and BmOR3, were identified as bombyx sex pheromone receptors. BmOR1 and BmOR3 are mutually exclusively expressed in a pair of neurons in the pheromone sensitive trichodeum sensillum and are fine-tuned to bombykol and bombykal, respectively. In this study, we aimed to relate the MGC structure with the receptor molecular identity and to investigate how the input of pheromonal information is represented in the B. mori MGC. To visualize BmOR1 and BmOR3 expressing neurons separately, we generated two transgenic moth lines that express green fluorescent protein (GFP) under the control of putative promoter sequences of BmOR1 or BmOR3. In the male antennae of the both lines, GFP fluorescence was detected in bipolar neurons in the sensory epithelium with a similar distribution pattern to that of the pheromone sensitive sensilla. The axons of these neurons projected to a bilaterally symmetrical structure at the entrance of the AL where the MGC resides. Confocal microscopic observations combined with anti-GFP antibody staining of the brain revealed that BmOR1 expressing neurons projected only to the toroid, while BmOR3 expressing neurons to the cumulus. These results suggest that the pheromone receptor neurons expressing the same receptor protein, thus having the same ligand specificity, converge their axons on the single subdivision of the MGC. This convergence will allow for the formation of segregated processing pathways between bombykol and bombykal in the male silkmoth AL.

293. Organisation of the Olfactory Pathway in the Moth Brain

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In studies of mechanisms underlying detection and discrimination of odours, herbivorous moths represent suitable models. They have two olfactory systems operating in parallel, devoted to information about insect produced signals and plant odours, respectively. Narrow tuning of receptor neurons to insect produced signals have been demonstrated in many species. In *Heliothis virescens* four functionally described, male-specific receptor neuron types correlate with the four neuron types expressing the identified male-specific receptor proteins (cf. Krieger). Using two-column gas chromatography linked to single cell recordings, twenty types of plant odour receptor neurons, each tuned to one primary odorant, are classified. Comparison between related heliothine species has further enlightened stability as well as changes through evolution of receptor neuron specificity and species-specific sensillum types. The primary olfactory centre, the antennal lobe, exhibit striking similarities and differences across the species. This concerns the functional organisation of the 3–4 glomeruli that constitute the macroglomerular complex dealing with information about insect produced signals. The numerous ordinary glomeruli receiving information about plant odorants, show consistency in the number and position across species. The odour information from the antennal lobe to several protocerebral areas is mediated by antennal lobe projection neurons that follow one of three major tracts and exhibit different projection patterns e.g. in the calyces of the mushroom bodies and the lateral horn. Three-dimensional reconstructions indicate convergence and divergence of the projections. In ongoing studies, the aim is to resolve the functional significance of the neurons belonging to these tracts.

Symposium 17: From Reception to Perception in Primate Sweet and Bitter Taste: Receptors and Communication in the Taste Bud

294. Molecular Mechanisms of Human Sweet and Bitter Perception

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To elucidate the molecular mechanisms of human taste perception we compare the functions of taste receptors in vitro in cell lines with human taste perception in vivo. Our characterization of 12 of the 25 human TAS2R bitter taste receptors revealed that these receptors are sufficient to detect a vast variety of structurally diverse compounds. Moreover, we could demonstrate that sequence variations in bitter taste receptor genes can alter receptor functions and thereby determine individual taste differences to bitter compounds. Variations in taste perception also occur across species. For example, the artificial sweetener neohesperidine dihydrochalcone (NHDC) is sweet to humans but not attractive for rodents. In line with this observation NHDC only activates the human sweet taste receptor TAS1R2 + TAS1R3 but not its rodent counterpart. The functional difference allowed us to identify the NHDC ligand binding site in human TAS1R3 by using rat/human receptor chimeras, site-directed mutagenesis and pharmacophore modelling. Moreover, we observed that high concentrations of the artificial sweetener saccharin allosterically inhibit the human but not the rat sweet taste receptor. This is caused by binding of saccharin to a high affinity agonist and a low affinity inhibitory site which explains why saccharin, albeit intensely sweet at low concentrations, has only a neglectable sweetness at higher concentrations. The preferential removal of saccharin from the low affinity inhibitory site also explains the sweet ôwater tasteö that is elicited when high saccharin concentrations are rinsed away. This shows that in vitro analysis of taste receptors is useful to understand taste perception at the molecular level and may improve the development of new sweeteners and bitter blockers.

295. Molecular Basis of Sweet Taste in Primates

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The ability to perceive sweetness is a common trait in a range of animals but there are differences among species in response to specific sweeteners. Our focus here is the origin of diversity in sweet taste perception among primate species. For primates, sweeteners can be divided into 3 classes, based on patterns of species differences in the gustatory responses: Class I, such as sucrose, elicit a sweet taste in all primates; Class II, such as ampame, induce a sweet taste in Prosimians (e.g., lemurs) and Old World Simians (e.g., chimpanzees) but not in New World Simians (e.g., marmosets); Class III, such as aspartame, are sweet only to Old World Simians. Because studies of mice and cats showed that DNA variation in sweet receptor genes TAS1R2 and TAS1R3 determines species-specific differences in sweet taste perception, we hypothesized that differences in sweet taste perception among primates could be similarly explained. We determined the DNA sequences of the TAS1R2 and TAS1R3 genes in 11 primate species (1 Prosimian, 4 New World and 6 Old World Simians) and found 10 amino acid variants in T1R2 and 33 variants in T1R3 that distinguish the Class III taster from nontaster primate species. The variation in T1R2 is concentrated in the linker region between the N-terminus (VFTD) and the 7TM domain. For T1R3, the variation is evenly spread over the whole protein. The results of receptor modeling suggested that the variants distinguishing Class III taster and nontaster species are more likely to influence allosteric interactions than to affect ligand docking at the putative binding pockets. The identification of variant sites that distinguish Class II taster from nontaster primate species is ongoing.

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296. The Structure of Taste

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Sweet and umami tasting stimuli are detected and transduced by T1R-type G protein–coupled receptors. T1Rs vary in structure, stimulus specificity and breadth of tuning. For example, the heteromeric T1R2:T1R3 sweet taste receptor is responsive to stimuli as diverse as sucrose, saccharin and sweet proteins, yet it discriminates between D- and L-amino acids. In contrast, the T1R1:T1R3 umami receptor displays a more limited selectivity for some L-amino acids and 5Æ-ribonucleotides. Selectivity differences are also observed amongst orthologous receptors. Differences in stimulus selectivity and sensitivity of T1Rs result in large part form the presence of a number of allosteric and orthosteric binding sites for taste stimuli. We have begun to dissect the structural determinants of ligand binding in T1R taste receptors, especially in the context of taste behaviors. Using spectroscopic techniques to quantify ligand binding to the extracellular, N-terminal domain of T1Rs, we find that both subunits of the sweet taste receptor are competent to bind saccharide ligands. Furthermore, each subunit exhibits distinct changes in protein structure upon ligand binding, suggesting that T1R2 and T1R3 make unique contributions to ligand-dependent receptor activation. Such direct measurements of ligand–receptor interactions permit us to decouple the ligand-binding event from downstream mechanisms involved in receptor activation, such as intersubunit interactions or effector activation, and to differentiate the individual contributions of T1R2 and T1R3 to the detection of sweet stimuli.

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297. A Bittersweet Search for Bitter:Sweet Interactions: Cell to Cell Communication in the Taste Bud

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Bitter and sweet taste qualities are often considered polar opposites of gustatory sensation. Hence, it is not surprising that responses to these stimuli demonstrate some degree of segregation at all levels of the neuraxis, from taste buds to the cerebral cortex. Our research suggests that within the taste bud individual taste receptor cells that respond to either bitter and sweet stimuli, as represented by expression of T2R and T1R receptors, may interact with one another via cell to cell communication. Taste receptor cells, once thought to activate only the afferent nerve fiber connecting them to the central nervous system, are now known to employ both neurotransmitters, such as norepinephrine and serotonin, and neuropeptides, such as cholecystokinin (CCK) and neuropeptide Y (NPY), as mechanisms of cell to cell communication. Our research has demonstrated that CCK in the taste bud acts in an excitatory autocrine manner, via CCK-A receptors, to potentiate bitter responses. On the other hand, NPY acts in an inhibitory paracrine manner to inhibit surrounding cells via NPY-1 receptors. Additionally CCK and NPY are colocalized with one another in the same subset of cells. They demonstrate substantial colocalization with gustducin but only marginal colocalization with T1R2. These data suggest the possibility that complex hard wiring patterns of cell to cell communication exist within the taste bud. Release of these peptides could potentially act to augment bitter responses while simultaneously inhibiting sweet T1R-expressing cells. Thus the taste bud is likely to operate as a computational unit, processing and sharpening information contained within the sensory signal prior to initiation of the neural output.

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Symposium 18: Plasticity of Chemoperception in Insects

298. Plasticity of Odour-Evoked Activity in The Honeybee Antennal Lobe: Coupled Aversive Conditioning and Calcium Imaging

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Intense previous work has studied the neurobiological bases of olfactory learning in two insect models, the fruit fly *Drosophila melanogaster* and the honeybee *Apis mellifera*. Until now, however, direct comparison between results in the two species has been difficult because most of the fruit fly work was based on aversive conditioning (reinforcer: electric shock), while the bee work was based on appetitive conditioning (reinforcer: sucrose solution). To allow more direct comparisons between the two models, we have developed a novel aversive conditioning paradigm in the honeybee. The Sting Extension Response (SER) is the reflex extension of the bee sting in response to a mild electric shock. By presenting an odour (CS) in temporal association with the electric shock (US), we show that bees are able to form odourûshock associations. This conditioning is clearly associative, as shown in differential conditioning experiments, in which bees respond to the odour associated to the electric shock (CS+) but not to another nonreinforced odour (CS−). Using a pharmacological approach, we show that SER conditioning depends on the dopamine system in honeybees, as in fruit flies. To study plasticity in the first relay of the insect olfactory pathway—the antennal lobe—related to aversive conditioning, we have developed a coupled SER conditioning/calcium imaging preparation. We can at the same time monitor the behavioural responses of a live bee learning to differentiate between two odours (CS+ and CS−), and record calcium signals evoked by these odours during conditioning. We thus image the honeybee brain while it learns aversive associations, and relate brain activity changes directly to learning success.

299. Evolution of Behavioral Plasticity in *Drosophila*: Costs and Benefits of Learning and Memory

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Learning to associate the odor and the quality of a food resource can be a critical parameter for the survival of a foraging individual. Variations in learning and memory abilities have been observed among closely related species and populations of the same species. This raises the question of the conditions under which learning and memory should evolve. While fitness benefits of learning are relatively well understood, we know little about fitness costs of learning ability, constraints on its evolution, and the nature of heritable variation on which natural selection can act.

For the past 4 years we have been exposing fruit fly populations to conditions that favor aversion learning in the context of oviposition substrate choice. After about 20 generations of such selection these lines evolved higher learning rate and a better memory. The existence of these lines opens an opportunity to study the fitness costs of learning and memory and to understand better why, how, and when learning ability evolves under natural selection.

300. Habituation to Ecdysone in their Diet in *Ostrinia nubilalis* Larvae

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Ecdysteroids in the diet play a dual role for European corn borer larvae. On the one hand, once ingested, they can trigger unwanted molts, possibly interacting with EcR receptors, which are nuclear receptors. On the other hand, they are detected by taste receptors which, when activated, inhibit feeding and in turn increase the locomotor activity. Lastly, European corn borers like many noctuid moths can detoxify ecdysteroids in the diet by excreting them. How plastic is this system? We exposed larvae to different doses of 20-hydroxyecdysone in their diet and monitored their mortality, their feeding and locomotory behavior and taste responses. Our results indicate that these insects adapt to phytoecdysteroids in their diet by a combination of mechanisms: decreasing the quantity of food ingested which limit the quantity of toxin ingested, increasing their detoxication systems and lastly, adapting taste receptors to decrease their sensitivity to 20E. We suggest that these three levels of adaptation should be studied in parallel to get a better understanding of the role of bitter substances in regulating feeding in herbivorous insects.

301. Novel Features of Synaptic Inhibition During Olfactory Perception in *Drosophila*

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Among constituents of the olfactory pathway, local interneurons play an inhibitory role through the generalized innervation of olfactory glomeruli in the antennal lobe. In these glomeruli, 3 types of neurons converge: inhibitory local interneurons, excitatory sensory neurons and excitatory projection neurons. We investigated the behavioral effects on the perception of several odorants by genetically manipulating the number of synapses (N) in specific groups of olfactory neurons. We modified N in 30% of inhibitory local interneurons to the extent of a total reduction of about 10%. Conversely, similar manipulations could elicit a total increase of the same type of synapses up to 90%. Behavioral studies with the same odorants were carried out in animals in which excitatory sensory neurons had increased N by up to 200%. Based of these quantitative modifications, the behavioral effects of altering inhibitory versus excitatory synapses seem to be quite different. Inhibition seems to be an optimized value that causes no effect if N is increased but has profound effects if it is reduced, even at 10%. In that case, odorant perception increased transforming all responses into repulsive reactions. Interestingly, the relative proportion between strength of the behavioral response and magnitude of the stimulus is maintained. Then, the higher the odorant concentration is, the more aversive the response is. Changes in the excitatory input, however, do change the olfactory perception when a) attractive responses are elicited at lower odorant concentrations than in the sibling controls, and b) the attractive and repulsive responses become more robust that controls. That is, sensitivity of perception and strength of the behavioral response are increased when excitatory N is increased.

302. Overlapping Processing of Sex Pheromone and Bitterness in *Drosophila*

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In many animal species, survival and reproduction largely rely on the perception of chemicals which indicate the quality of food and the status of potential mates. However, food flavors and sex pheromones radically differ both for their chemical structure and for behaviours elicited. This suggests that these molecules are processed by divergent neural circuits. We found that a particular set of *Drosophila* taste neurons involved in the gustation of bitter substances, is also crucial to taste a major male pheromone (Z-7-tricosene = 7-T). Genetic and surgical manipulation of these neurons revealed their effect on the physiological and behavioural responses to pure 7-T and to various bitter molecules. Increased amounts of 7-T and of bitterness tended to inhibit appetitive and courtship responses, and each type of substance affected both behaviours. Moreover, the same taste neuron showed a dose–response to increasing amounts of 7-T, of caffeine, and showed an additive and a cross-reaction effect of the two substances indicating that they produced an equivalent sensory representation in the male fly. Given that 7-T and bitter molecules are processed at the periphery by the same neurons and can be equally learned by the fly, it is possible that their association takes place in the same neural centers.

303. Memory Is a Smelly Business—Neuronal Constraints in Specialist Olfaction

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Odours play a central role in finding food for fruit flies. With a new operant conditioning paradigm we can easily train the generalist *Drosophila melanogaster* to change preferences for a number of odours and especially esters signalling attractive fruits. We can either enhance attractiveness, or decrease it unto repellence. However, trying to establish similar shifts in the specialist *D. sechellia* shows that the preferences can be changed to a slight degree only for less important odours. The positive assignment of the most important odours signalling their host plant seems to be almost 'hardwired'.

With immuno-histology and electrophysiology we trace changes in the neuro-anatomy possibly explaining these behaviour patterns and probably emerging as an example for the neuronal constraint hypothesis.

Oral Session 6: Olfactory Receptors

304. Polymorphism and Functional Studies of Canine and Rat Olfactory Receptor Genes

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Why dogs or rats are so good at detecting odorant molecules?

Why some dog breeds are better than other at sensing odorants?

To approach these two questions and many others we first made the inventory of the dog and rat olfactory receptor gene repertoires by in vitro and in silico cloning and compared it with that of human and mouse. A number of dog OR genes (>100) were then selected as representative of many families and subfamilies identified through amino acid sequence comparison. These genes were cloned and transiently expressed in HEK293 cells (human embryonic kidney cells). Binding properties of these OR toward a series of ligands were investigated by the measure of Ca2+ intracellular concentration. Results will be discussed in light of the odorant recognition combinatorial code which supposes that an OR is able to recognize a small number of odorant molecules whereas an odorant is recognized by a small number of OR. In parallel to this functional aspect,

we have analyzed the OR nucleotide/amino acid sequence polymorphism of more than 150 OR genes. This study confirmed and extended our previous preliminary results that indicated a very high level of polymorphism with the existence of numerous alleles able to affect the binding properties of the corresponding OR. We also turned our attention to the catalogue of rat OR genes that are expressed under various life conditions. Total RNA were extracted from nasal epithelium of rats exposed or not to a given odorant for various period of time and analyzed either by qPCR and/or hybridization onto dedicated OR long oligonucleotide microarrays after fluorescent labeling.

305. Beta-Arrestin2 Mediated Desensitization of Mammalian Odorant Receptors

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Odorant receptors (ORs) comprise the biggest subfamily of G-protein–coupled receptors. Although the endocytic mechanisms of other G-protein–coupled receptors have been characterized extensively, almost nothing is known about the intracellular trafficking of ORs. In the present study we provide the first report on the endocytic mechanism of ORs, which bind beta-arrestin2 with high affinity and are internalized via a clathrin-dependent mechanism. After prolonged odorant exposure receptors are not targeted to lysosomal degradation but accumulate in recycling endosomes. Odorant-induced OR desensitization is promoted by PKA phosphorylation and is dependent on serine and threonine residues within the third intracellular loop of the receptor. Moreover, beta-arrestin2 is redistributed into the dendritic knobs of mouse olfactory sensory neurons after treatment with a complex odorant mixture. Prolonged odorant exposure resulted in accumulation of beta-arrestin2 in intracellular vesicles, concomitant with the phosphorylation of cytosolic MAPK in the olfactory epithelium.

306. A Single Lysyl Residue Is the Major Determinant for Governing the Binding Specificity of a Human Odorant-Binding Protein

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Odorant-binding proteins (OBPs) are small abundant soluble proteins belonging to the lipocalin superfamily, which are thought to carry hydrophobic odorants through aqueous mucus towards olfactory receptors. Previously, we demonstrated that human variant hOBP-2A binds numerous odorants of different chemical classes with a higher affinity for aldehydes and large fatty acids. We proposed that highest affinity for aldehydes could result from an interaction between aldehyde function and lateral chain of a lysyl residue, stabilizing odorant docking. A computed three-dimensional model of hOBP-2A revealed that three lysyl residues of the binding pocket (K62, K82 and K112) may account for the increased affinity for aldehydes. In order to identify the lysyl residue involved in the higher affinity of hOBP-2A for aldehydes, we independently substituted these residues for alanine using site-directed mutagenesis, generating K62A, K82A and K112A mutants. By measuring the displacement of fluorescent probes by odorants, we showed that only the mutation K112A led to a dramatic reduction of binding affinity for aldehydes and small aliphatic acids (from 9- to 12- carbons), whereas binding of larger fatty acid (14- and 16-carbon length) were not affected by any mutation. To our knowledge, this study provides the first evidence of a single residue governing the binding specificity of a vertebrate OBP.

307. *Drosophila* Odorant Receptors and Novel Signal Transduction Genes

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Odorant receptors (Ors) in *Drosophila melanogaster* are a highly divergent family of 62 seven trans-membrane domain proteins that show no sequence similarity to other odorant receptors or to any known proteins. The extreme diversity of the Or gene family in *D. melanogaster* (average level of identity ~20%) poses a considerable challenge in identifying the functional domains of these proteins and the signal transduction pathway they activate. We have undertaken a comparative genomics approach to identify domains in *Drosophila* Ors that may interact with ligands or with downstream signalling proteins. The evolutionary dynamics and patterns of selective pressure on orthologues of 10 Or genes from eight *Drosophila* species have been analysed and show that these genes are under strong purifying selection indicating overall functional constraint. However, we have identified sites of positive selection that may assist in identifying ligand binding regions. To identify candidate olfactory signal transduction genes we used an approach to identify novel *Drosophila* genes expressed in the olfactory organs. We screened 500 "gene trap" lines that contain a P element insertion within the coding sequence of a gene, thus generating a mutant in that gene whilst still allowing detection of the expression patterns of the trapped gene. We have identified 16 lines with expression in olfactory receptor neurons and are performing molecular genetic experiments to identify the genes that are "trapped" in these lines as well as characterizing their olfactory phenotype. (Lukacsovich T, et al. 2001. Dual-tagging gene trap of novel genes in *Drosophila melanogaster*. Genetics 157:727–742.)

308. Hamsters and Cycloheximide: A Lineage-Specific Detection of Bacterial Toxin Contamination

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Cycloheximide, a toxic, protein-synthesis inhibitor generated by the actinomycete *Streptomyces griseus*, has emerged as a primary focus of studies on mammalian bitter taste. Rats and mice avoid it at concentrations well below the thresholds for most bitter stimuli. A G-protein–coupled T2 receptor specific for cycloheximide with appropriate sensitivity and its gene, CyX, have been identified. Like mouse and rat, golden hamsters, *Mesocricetus auratus*, detected <1 μM cycloheximide. However, hamsters' aversion for cycloheximide increased dramatically after ingestion of 1.5 ml of 500 μM cycloheximide, an amount near the rat LD50, during an initial 1-h

presentation ($P < 0.000001$). Such plasticity is reported for neither rat nor mouse. Furthermore, unlike hamsters' averse reactions to other bitter stimuli, the induced cycloheximide aversion did not readily generalize, but led to a weak suppression of ionic and non-ionic bitter stimuli alike ($P < 0.0001$). A clade of 5 T2R genes, including CyX in mouse and rat, probably also occurs in hamsters, but only a single orthologous T2R10 gene was found in each of the human and rabbit genomic databases. T2R species divergence and multiplicity make functional relationships of T2 receptor proteins across species uncertain. The rodent cycloheximide–T2R interaction is likely one of many lineage-specific, stimulus–receptor interactions reflecting a response to a toxin present in the environment.

309. Few Small Regenerated Olfactory Lamellae of Catfish Enable Olfactory Discrimination

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Olfactory bulb (OB) connections of olfactory receptor neurons (ORNs) in small regenerated olfactory organ of black bullhead catfish (*Ameiurus melas*) that contain 2–9 olfactory lamellae ($N = 11$) were investigated. Six of eleven regenerated tissues were not functional [no electroolfactogram (EOG) could be recorded]. Taste alone does not allow olfactory discrimination and these catfish could not be conditioned to the amino acid stimuli. Anosmic catfish responded behaviorally to taste stimuli L-Ala, L-Arg, L-Cys more than to the repeatedly rewarded L-nVal. Catfish with small regenerated olfactory lamellae (>two lamellae) ($N = 5$) rapidly learned to discriminate amino acids. The L-nVal conditioned catfish responded nearly twice as intense to the conditioned stimulus than to other amino acids: L-Ala, L-Arg, L-Cys, L-Pro, L-Met, L-Val, L-nLeu, L-Leu and L-Lys. To visualize ORNs connections to the OB, ORNs were retrogradely labeled with the fluorescent marker DiI, which was inserted with entomological needle into antero- and latero-ventral areas of the OB. Axonal connection of the fluorescent ORNs with the OB were studied 21 days after the DiI crystal insertion. As in intact olfactory organs the tall ORNs were connected to the anterior ventral surface of the OB and the intermediate ORNs were connected to the lateral ventral surface of the OB. In the small regenerated olfactory organs relative EOG amplitudes of tested amino acids did not differ from the relative EOG amplitudes in intact olfactory organs. Small numbers of ORNs that are connected to the appropriate areas of the OB in at least one of the paired olfactory organs enable effective olfactory discrimination of amino acids.

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Session 7

Symposium 19: Evolution of Olfactory Detection

310. Toxic Fruit Tunes Fly Smell

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Drosophila melanogaster is a cosmopolitan generalist species. However, several of its sibling species are specialists, offering an excellent opportunity to study the evolutionary and ecological dynamics of olfactory systems. We compared the structure and function of the generalist *D. melanogaster* with that of specialist *D. sechellia*, which oviposits exclusively on morinda fruit. Our analyses show that the fruit's major volatiles, hexanoic acid and esters, even when presented pure, were highly attractive to *D. sechellia*. Using GC-EAD we found that *D. sechellia* exhibited an extraordinary strong response to methyl hexanoate (MeHex). Single sensillum recordings revealed that this response was caused by a 2.5–3× overrepresentation of ab3 sensilla housing MeHex neurons on the antenna, on the cost of others (ab1 and ab2 reduced by 65% and 95–100% respectively). In addition, the MeHex neuron (ab3A) exhibited an extreme sensitivity down to femtograms of its ligand. Ab3A and B neurons projected to two enlarged glomeruli, of which the voluminar increase matched the numerical increase of ab3 sensilla (2.9×). However, an increased sensitivity would likely decrease attraction to high concentration, whereas *D. sechellia* was attracted even to pure odor. Using odor mixtures and ectopic OR expression in *D. melanogaster* we investigate the possibility that the projection neurons, originally arborizing neighboring 'ab2 glomeruli,' now project into the two enlarged glomeruli. Finally, we found no evidence for that *D. sechellia*'s increased attraction to acids is peripherally mediated. These findings are a demonstration of evolution acting at several levels in the olfactory circuitry in mediating a fruit fly's unique preference for fruit toxic to its sibling species.

311. The Scent of Speciation: Chemoreception and Sympatric Speciation in *Rhagoletis* Flies

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The *Rhagoletis pomonella* species complex has been a cornerstone of the sympatric speciation debate over much of the last half-century. Members of the *R. pomonella* group are believed to be undergoing sympatric speciation via host shifts from one plant species to another. We have shown that flies from sympatric populations infesting hawthorn, apple and flowering dogwood fruit can distinguish among unique volatile blends identified from each host. This odor discrimination acts as a premating barrier to gene flow between flies infesting each fruit, and variation in olfactory preference provides a potential source for the host shifting process. We have also discovered significantly reduced olfactory preference for host blends in *R. pomonella* hybrids, as well as a segregation of preference in F2 individuals implying the existence of discrete genetic loci affecting fruit odor preference. We have investigated the source of this divergence in olfactory preference by comparing olfactory receptor neuron (ORN) response characteristics from the three populations of R. pomonella as well as F1 and F2 hybrids and backcrosses between the populations. Dose–response trials revealed similar chemical specificity in all parent populations, but unique response profiles

in hybrid offspring as well as significant variability in ORN sensitivity and temporal firing pattern among all populations. These peripheral alterations in ORN response profiles could result from mis-expression of multiple receptors in hybrid neurons as a function of genomic incompatibilities in receptor–gene pathways in parent populations. Our results provide evidence for a chemosensory correlation to host preference, host shifts, and potential speciation in phytophagous insects.

312. Comparative Genomics of Insect Olfaction

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Insects use their sense of smell to locate food, avoid environmental dangers, recognise kin and identify potential mates. Despite a wealth of data on the neuroanatomy and physiology of their olfactory circuits, remarkably little is known about the molecular basis of insect olfaction. We previously found that the insect odorant receptor gene family is structurally unrelated to vertebrate odorant receptors. Given this unique evolutionary solution to the problem of odour recognition, we reasoned that other components of olfactory detection pathways might also be specific to insects. To identify such molecules, we have therefore taken a comparative genomic approach. Using data from the *Drosophila melanogaster*, *Anopheles gambiae*, and vertebrate genome projects, we have performed an in silico screen for insect-specific orthologues that have highly tissue-specific expression. This recovered all known classes of olfactory genes—including the odorant receptors, the odorant binding proteins and the odour degrading enzymes—and has identified approximately 50 novel genes expressed in olfactory tissue. We will describe this screening strategy and present our progress towards functional characterisation of some of the identified genes. Such insect-specific molecules represent excellent candidate targets for custom-designed insect repellents to inhibit the olfactory-driven behaviours of agricultural pests and disease vectors.

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313. The Mammalian Nose: 200 Million Years in the Making

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Our present understanding of the mammalian olfactory receptor (OR) family, regarding, e.g. its size and organization, is based on only a few species, all stemming from the sister clades laurasiatheria (which houses, e.g. carnivores, such as the dog) and euarchontoglires (e.g. primates and rodents). Accordingly, the prevailing picture of mammalian ORs may not be the norm, but rather specific features for the species of these two clades, perhaps resulting from adaptations to specific conditions in their ancestral home on the supercontinent laurasia. Hence, we should be careful when we extend the established generalizations regarding the mammalian olfactory subgenome towards placental mammals of the two other clades (Afrotheria and xenarthra) and most definitively towards Marsupials and Monotremes, which have a highly different evolutionary history. We have mined the recently sequenced genomes of the rabbit (euarchontoglires), cow and dog (laurasiatheria), tenrec and elephant (afrotheria), armadillo (xenarthra), opposum (marsupialia) and platypus (monotremata) for potentially functional ORs in order to establish size and structure of the mammalian olfactory subgenome. We have identified the complete functional OR repertoires from each of the listed species and in total identified over 4900 ORs with an intact open reading frame. Our phylogenetic analysis, which also incorporates the previously published olfactory repertoires of human and mouse, reveals that the mammalian OR family has been conserved to a remarkable degree.

314. Blocking the Ip3 Pathway Permits Typical Sugar Receptor Cell Firing in Response to Na-Saccharin in the Blowfly, *Phormia regina*

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Since blowflies give no behavioral (PER) and little æsugar cellÆ responses to artificial sweeteners, it has been thought that they lack receptor mechanisms for these stimuli. Yet the æfifth cellÆ responds to Na-saccharin (Na-S), and there is reciprocal inhibition for Na-S and sugar. We studied sugar cell responses to Na-S during inhibition of IP3-mediated adaptation with 1-(5-isoquinolinesulfonyl)-2-methylpiperazine and Na-deoxycholate (H7). Action potential responses to a concentration series of Na-S with (+) and without (−) H7, and of sucrose, were tip-recorded from single sensilla in isolated proboscises. Sugar cell firing to sucrose and to Na-S +H7 gave typical concentration response curves, but firing rates to Na-S −H7 were low and atypical. At most concentrations, firing to Na-S +H7 was greater than firing to Na-S −H7 ($P < 0.05$, Mann–Whitney). Sugar cell adaptation rates decreased as sucrose concentration increased, but increased as Na-S +H7 concentrations increased, and did not change with Na-S −H7 concentration. Fifth cell firing gave similar response curves for Na-S + and Na-S −H7. Flies that gave a PER to sucrose 50 mM did not to Na-S 7.4 mM +H7, and while the highest firing rates to Na-S +H7 were to Na-S 7.4 mM +H7, they remained lower than those to sucrose 50 mM ($P = 0.01$, Kramer). The results indicate an excitatory receptor mechanism for Na-S in the sugar cell and suggest that sugar cell firing to Na-S is suppressed by a rapid onset of IP3-mediated adaptation. It seems unlikely that IP3 is either a transduction or adaptation mechanism in the fifth cell. Whether the lack of PER resulted from inadequate sugar cell firing or inhibition via fifth cell firing is under investigation.

Symposium 20: Molecular and Genetic Approaches to Umami Reception and Preference

315. Multiple Receptors, Transduction Pathways and Fiber Types Underlie Umami Taste in Mice

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Umami, first described by Ikeda, is the characteristic taste elicited by glutamate (MSG) and 5Æ-ribonucleotides such as IMP and

GMP. Recent molecular studies proposed that various receptors, such as a truncated type 4 metabotropic glutamate receptor (taste mGluR4), heterodimers of T1R1 and T1R3, taste mGluR1, and brain-type mGluR4, might underlie umami taste. To date, however, the roles in umami taste of each of these receptors and their downstream signaling molecules have not been made clear. Apparently contradictory data was obtained from two T1R3 knockout (KO) mouse models: Zhao et al. showed that umami detection and perception was abolished in their T1R3 KO, while we found that responses to umami compounds were diminished in our T1R3 KO mice. In the present study, we further examined responses to umami compounds at single nerve fiber levels in wild type, T1R3-KO and TRPM5-KO mice. The results indicated that umami-responsive single fibers of the chorda tympani nerve in wild type mice could be classified into more than two types, with one type showing large a synergistic effect on responses to MSG when IMP was added. In the KO mice, single fiber responses showing this large synergism were absent, but other umami-responsive types with slight or no synergism still remained. This provides additional evidence for the existence of multiple receptors, transduction pathways and nerve fiber types underlying umami taste in mice.

316. Umami Transduction in Taste Cells: More than One Receptor, More than One Site

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A number of gustatory receptors have been proposed to underlie umami, the taste of L-glutamate, and certain other amino acids and nucleotides. However, the response profiles of these cloned receptors have not been validated against responses recorded from taste receptor cells that are the native detectors of umami taste. We have investigated umami taste responses in mouse circumvallate taste buds in an intact slice preparation, using confocal calcium imaging. Only a few taste cells responded to L-glutamate when it was focally applied to the apical chemosensitive tips of receptor cells. The concentration–response range for L-glutamate fell approximately within the physiologically relevant range for taste behavior in mice, namely 10 mM and above. Inosine monophosphate enhanced taste cell responses to L-glutamate, a characteristic feature of umami taste. Using pharmacological agents, ion substitution, and immunostaining, we have shown that intracellular pathways downstream of receptor activation involve phospholipase C beta2. Each of the above features matches those predicted by studies of cloned and expressed receptors. However, the ligand specificity of the umami receptors that have been proposed to date did not appear to explain the taste responses observed in mouse taste cells. Furthermore, umami responses were still observed in mutant mice lacking T1R3, one of the GPCRs believed to comprise umami taste receptors. We propose that a full explanation of umami taste transduction may involve novel combinations of the proposed receptors and/or as-yet-undiscovered taste receptors.

317. Genetic Factors Influencing Ingestive Responses to Umami Compounds

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Our goal is to identify specific genes influencing ingestive responses to umami compounds (monosodium glutamate [MSG] and inosine monophosphate [IMP]) using inbred mouse strains. These genes may be involved in peripheral recognition mechanisms (e.g. taste) and/or postingestive mechanisms (e.g. metabolism). We found that whereas there was great variation in intake of MSG during 48-h 2-bottle preference tests among 28 different inbred strains, there was remarkably little variation in response to IMP. In the latter case, virtually all strains exhibited a peak preference greater than 60% at 10 mM IMP. In contrast, MSG preference and intake varied from high in some strains to avoidance of almost all concentrations in others. Responses to MSG may reflect a combination of sensory and metabolic responses to the sodium and the glutamate. In other studies, we have concentrated on evaluation of genetic differences in two selected inbred strains, C57Bl/6J (B6) and 129P3/J (129). B6 mice exhibit a greater preference for, and at high concentrations consume much greater amounts of, MSG than do 129 mice; a similar tendency is evident for IMP. Several lines of evidence indicate that these strain differences in response to MSG are unrelated to strain differences in response to sweet or salty substances; indeed they are probably not related to taste per se but more likely to postingestive consequences of consuming glutamate. Ongoing genetic mapping studies indicate that loci on several chromosomes may be involved with a particularly prominent locus evident on chromosome 9. Future studies are aimed at identifying the gene(s) responsible and at understanding the mechanisms underlying differential responses to glutamate and other nutrients.

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318. Monosodium Glutamate and Related Pharmacological Agents Affect the Taste Response Differentially at Apical or Basal Part of the Taste Bud

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Antagonists of brain glutamate receptors reduced the firing rate of intrapapillar single units recorded in the taste pore and the whole chorda tympani (CT) response to Monosodium Glutamate (MSG) or 5ÆGuanosine monophosphate (GMP) but not to NaCl. CNQX (anti ampa-kainate) and MSOP (anti mGluR group III) produced, at 20 ÁM, a limited but significant inhibition of 6% and 10%, respectively. No effect of DAP5 (anti NMDA) was observed. Increased CT responses were also observed. When applied at the basal part of the taste bud (TB) using systemic injection in the common carotid, a mode of application that stimulates the basal part of the taste bud and eventually afferent fibres, agonists and antagonists of brain glutamate receptors, including CNQX and LAP4, an mGluR group III agonist, decreased both the firing rate of intra papillar single units and the CT responses to MSG but not to NaCl; ACPD (and maybe NMDA) decreased the NaCl response. At the apex of the TB, comparatively, MSG produced 1/3 potentialisations and 2/3 inhibitions. Glutamate seems able to stimulate the taste system through more than one receptor, including the quantitatively modest but significant contribution of brain like glutamate receptors antagonised by MSOP and CNQX; the responses to glutamate by activation do not use the same signalling pathway. At the basal part of the TB, the glutamate neuromodulatory effect also acts

through brain glutamate receptors, which are partly different from those of the apex.

319. The Role of Mglur1 in Rat Taste and Stomach Tissue

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L-Glutamate confers cognitive discrimination for umami taste and also conveys nutrient information to the brain. It is well accepted that the cognitive differentiation of a taste stimulus drives ingestive behavior according to their hedonic properties. However this is not the only role for taste sensation since it is also implicated in other physiological functions such as cephalic phase response and reflex-like consummatory behavior. Most of the dietary L-glutamate is significantly oxidized in the intestinal mucosa where is used as a substrate for energy in different processes such as motility. For that reason may not be coincidental that foods with abundant naturally occurring free L-glutamate taste delicious. Additionally the copious presence in human breast milk corroborates that L-glutamate is an essential metabolic linker in many tissues. Directly into the stomach, L-glutamate also provokes a highly specific reflex activating the afferent gastric branches of the vagus nerve. Overall, these reports support the existence of specific L-glutamate sensors not only in the tongue but also in the lumen of the gastric wall that can evoke brain relays to ensure efficient digestion and metabolism. The sensory input for dietary L-glutamate in peripheral tissues is proposed to begin by the binding of the amino acid in one or more G-protein–coupled receptors (GPCR). Immunohistochemistry and molecular data implicates mGluR1 variants in the chemoperception of L-glutamate in taste and stomach mucosa. We will discuss the possible regulatory mechanisms by which L-glutamate influences gastric function through mGluR1.

Symposium 21: Pre- and Postnatal Chemosensory Learning: Human and Animal Research

320. Olfaction in the Preterm Infant: Functional Characteristics and Clinical Application

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As the in utero testing of human foetuses appears difficult, we further explored the early development of nasochemosensory function in premature infants. In a first experiment, breathing responses were recorded in 25 premature newborns (born at 28–32 gestational weeks, GW) exposed to 2 hedonic contrasted odorants (vanilla, V; butyric acid, B; diluted to match the intensity of the odour of amniotic fluid) and a control (water). Stimulations were presented on cotton swabs for 10 s. Systematic changes in respiration to either odorant as compared to baseline and control indicated reliable odour detection. The respiratory variations triggered by either odorant followed opposite direction, an effect that was interpreted to be indicative of odour discrimination. While the pleasant odour of V elicited respiratory acceleration, the unpleasant odour of B produced a decrease in respiratory rate. In a second experiment, we asked whether the premature infant's sensitivity to odorants can be used to stimulate respiration in the case of apnoeic infants. Fourteen premature infants (26–30 GW) presenting recurrent apnoeas despite pharmacological therapies were exposed to the stimulating odour of V diffused during 24 h in the incubator. Apnoeas associated with hypoxemia or bradycardias diminished significantly during odorization of the incubator (44% and 45%, respectively), an effect found in all subjects. These data demonstrate the readiness of the olfactory system to detect and discriminate olfactory qualities 2 months before gestational term and reveal that the introduction in the incubator of chemosensory agents that stimulate respiration may be of therapeutic value at least for immature infants suffering from respiratory instability.

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321. Early Exposure to Ethanol Induces a Conditioned Preference for the Drug's Flavour

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During the last days of gestation the fetus can perceive and recognize the chemosensory properties of substances present in the amniotic fluid. Frequently, prenatal exposure to different tastes and odors induces a higher acceptance for them. This same effect has been found in several studies in which fetuses were exposed to moderate ethanol doses during the last days of the gestation. Specifically, the administration of 1 or 2 g/kg of ethanol during gestational days (GD) 17–20 induces higher ethanol intake both in infants and in adolescents. Could this effect be explained by the same mechanisms underlying preferences to other chemosensory stimuli acquired during the prenatal period? Studies in the last 15 years indicate that fetuses can perceive the ethanolÆs chemosensory properties, and associate them with the rewarding properties of the drug, the last ones mediated by the activation of the opioid system. Interestingly, fetuses and infant rats during the first postnatal week can learn a conditioned preference for ethanolÆs flavor, even after intoxication with doses that induce taste aversion later in ontogeny. This paradoxical effect could be explained if considering an early sensitive period described by Sullivan and collaborators, in which moderately aversive stimuli exert appetitive effects, and also when the opioid system plays a key role in the acquisition of conditioned preferences.

322. Learning about Alcohol Early in Life: Mechanisms and Effects upon Alcohol Seeking and Intake Behaviors

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Alcohol exposure during fetal and infantile stages in development not only poses the threat of physical and behavioral impairments but also is likely to generate specific memories related with the drugÆs sensory and toxic effects. Epidemiological and experimental research strongly endorse the capability of near term fetuses and lactating infants to process alcoholÆs chemosensory cues present in the amniotic fluid and in maternal milk, respectively. In turn,

physiological and behavioral effects of alcohol affecting the perinate and/or the dam are likely to be associated with the perception of alcoholÆs sensory cues and give rise to conditioned responses that are manifested later in life. Alcohol-related early experiences sensitize the organism to the reinforcing effects of the drug yielding heightened predisposition to seek and ingest this pharmacological agent. Recent studies endorse the possibility of synergistic effects of fetal and infantile alcohol-related experiences. These studies indicate that sequential ontogenetic alcohol experiences may not only reactivate original alcohol-related memories but also strengthen the predisposition to ingest alcohol and to socially interact with intoxicated counterparts which in turn provide additional experience related with alcoholÆs sensory and toxic attributes. The above described effects have been observed in humans and subprimates, pre- or postnatally exposed to low to moderate alcohol doses that fail to substantially affect physical and neurobehavioral developmental patterns.

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323. Fetal and Neonatal Flavor Programming in Humans

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How preferences for foods and flavors develop is a mystery, despite much speculation. During the past few decades, experimental research has demonstrated that, like other mammals, the flavor of human milk and amniotic fluid reflects the flavor of the mothersÆ diet. Memories form as a result of these early experiences that, in turn, modifies the infantsÆ acceptances of similarly flavored foods. Thus, the development of preferences for culture-specific flavors has its beginnings during gestation and breastfeeding. This is the first, but not the only, way in which children learn about what foods are acceptable and preferred by their mothers. In addition to demonstrating that experience with flavors in amniotic fluid and mothersÆ milk contribute to individual differences in flavor preferences, we have identified a convenient and powerful model system to study how the amount and timing of exposure modifies subsequent flavor preferences. Using as a model system a class of infant formulas that are hydrolyzed-protein based and have distinctive flavors that are unpalatable to older-aged infants and adults, we found that early exposure to this type of formula resulted in a complete shift in hedonic tone to the flavor of this formula from one of absolute distaste to eager acceptance. The effects of early exposure were particularly long-lived, leading to heightened preferences for sour tastes, as well as for the taste and aroma of the formula and similarly flavored foods, during infancy and several years after the childÆs last exposure. In other words, the characteristic flavor of the formula experienced in early life is ôimprintedö and remains preferred for a considerable time thereafter.

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324. Olfactory Learning and Infant Attachment: Unique Role of the Locus Coeruleus and Amygdala Supporting Learning

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Infant attachment is expressed by caregiver approach and tolerating abuse to maintain caregiver contact. To explore the neural basis of attachment learning, we used an infant rat mammalian imprinting model, where pups exhibit an increased ability to acquire odor preferences and a decreased ability to acquire odor aversions during a sensitive period. The increased ability to acquire odor preferences is depends on the neonate's prolonged locus coeruleus (LC) activity causing copious NE release into the olfactory bulb. The decreased ability to acquire odor aversions depends on amygdala nonparticipation and low corticosterone (CORT). Specifically odor −.5 mA shock conditioning in sensitive period pups (PN8) results in an odor preference. However, systemic or intra-amygdala CORT infusions cause precocious odor aversion learning and amygdala activation (14C-2DG). Similarly, CORT depletion in older postsensitive period pups (PN12) that normally learn to avoid odors paired with shock, can have sensitive period learning reinstated. Next, since maternal presence can block postsensitive period pups' stress (i.e. shock) induced CORT release, we used the mother to decrease postsensitive periods CORT and assessed learning and amygdala activity. Our naturalistic decrease of CORT also produced the odor preference without amygdala activity. Overriding maternal suppression of CORT through intra-amygdala or systemic infusions permits fear conditioning and amygdala activation. Thus, two learning systems may coexist during the pups' preweanling period and may represent pups continued dependence on the mother, while preparing for extra-nest life by learning to avoid situations with negative contingencies.

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Oral Session 7: Olfactory Receptors II

325. Selective Inactivation of Olfactory Receptors Modifies the Quality of Odorants

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Odorant molecules are perceived by sensory cells in the olfactory epithelium (OE), transformed there into electrical signals and sent to the olfactory bulb (OB). In the OB each odorant elicits a specific neuronal activation pattern. It is, therefore, believed that detection of odors requires a combinatorial strategy. There is direct evidence that receptor proteins for odorants are glycosylated. Lectins—carbohydrate-binding proteins that bind glycoproteins via their sugar-specific sites—inactivate one or more types of olfactory receptors (receptor molecules) (glycoproteins) which normally respond to certain odorants or functional groups of an odorant. To explain how different types of sensory cells are involved in the coding of olfactory information we developed an experimental approach combining behavioral studies with an immunhistochemical method (c-fos method). We selectively inactivated receptors or at least parts of a receptor by lectin application to the OE to ''modify'' the receiving station for further analysis of the effects of these manipulations on odor coding and subsequent behaviors. The behavioral studies demonstrated that an animal trained to respond to a specific odor will not respond anymore for several hours to that specific odor after a lectin has applied to the OE; the c-fos analysis revealed a different neuronal activation pattern in the OB. These results indicate to us that if a receptor possesses several types of lectin-specific sugars or if different receptor types are involved in

odor identification the odor will obtain a different quality when only one type is inactivated—leaving others free to still interact with other functional groups of the odorant molecule.

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326. Olfactory Epithelium and Olfaction in Zinc-Treated Mice

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That intranasal administration of zinc sulfate is known to produce transient degenerative changes in the olfactory epithelium (OE) has raised concerns about the safety of zinc gluconate (ZG) compounds in nonprescription nasal formulations. We used mice to assess the effects of 2, 8 or 50 μl nasal administration of 1.6% zinc gluconate Zicam nasal spray (doses equivalent to ~ 4×, 16× and 94× of the recommended dose for humans) on axonal transport of horseradish peroxidase (HRP) from OE to olfactory bulb (OB) and on ability to detect and discriminate odors using precision olfactometry. Anatomically, effects of 2 μl of ZG were indistinguishable from saline-treated controls and treated mice had excellent retention of odor detection/discrimination tasks. Anatomical results for 8 μl ZG were highly variable; behaviorally, most treated mice had a transient hyposmia with essentially full recovery of function in 3–8 days. Fifty microliters of ZG produced severe disruption of OB inputs after 2 days but there was nearly complete recovery within 3–4 weeks. 50 μl ZG-treated mice were hyposmic when tested 2–3 days after treatment but full or nearly full recovery of function was observed within 3 weeks. In sharp contrast, similar volumes of 5% zinc sulfate (ZS) produced much more severe effects at each dose level and those given 50 μl tested as anosmic for 8–20 days. No ZG-treated mouse was anosmic and all ZS had marked recovery in OB input and in olfaction within 3–4 weeks. These data support the contention that ZG containing nasal sprays, used as directed, should be without effect on the olfactory system and that even massive doses of the product fail to produce anosmia.

327. Aspects of Quality and Concentration Coding in Olfactory Receptor Neurons of Catfish (*Ameiurus melas*)

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Electrophysiological responses of the olfactory organ of bullhead catfish to amino acids are similar in dechlorinated tap and in highly purified (R > million Ωcm) water. In highly purified water most of the olfactory receptor neurons (ORNs) responding to amino acid are spontaneously inactive prior to stimulation. High correlations between numbers of responding ORNs and amplitude of EOG indicate that receptor potentials of ORNs add up into EOG amplitude. Nearly 37% of ORNs responded to the two amino acids, L-Met and L-nVal, the most effective stimuli as determined with EOG amplitude. L-nVal is not a natural amino acid therefore these ORNs can be considered L-Met specialist cells. Five L-nVal only and four L-Met only cells were also observed. Based on responses to 10 amino acids 60% of ORNs were specialist neurons. Of 46 ORNs, 5 cells responded to two, 3 cells to three and two cells to 4, 5 and 6 amino acids. Response thresholds for L-nVal were between 10^{-7} M and 10^{-4} M. At concentrations that were 10 times above the response thresholds action potential frequencies attained maximum values for short periods of time (<250 ms), responses to higher amino acid concentrations were longer. Numbers of ORNs responding to different L-nVal concentrations correlate highly with logarithm of its concentration. The dose-dependent durations of ORNs responses to amino acids have differential slopes. Theoretical EOG amplitudes plotted from numbers of responding ORNs for each concentration are similar in shape to the EOG amplitude at the same concentration. This indicates that amino acid concentrations are coded by ORNs recruitment rather than their action potential frequencies.

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328. Ligand Binding Study Revealing a Human Olfactory Receptor Involved in Waxy-Floral Odor Perception

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Olfactory perception results from a combinatorial code, in which one olfactory receptor (OR) recognizes multiple odorants and different odorants are recognized by different combinations of ORs. About 350 ORs provide a basis for the ability of humans to recognize and discriminate a large number of odorants. In a recent study, Sanz et al. reported the odorant repertoire of a human class II OR (OR1G1), including both agonists and antagonists. In the present study, we attempted to decipher the odotope of OR1G1 agonists and antagonists in order to understand the role of OR1G1 in olfactory perception. For this purpose, we used calcium imaging data obtained from the work of Sanz et al. to perform a 3D-QSAR study of OR1G1 ligands. We obtained a double-alignment model which satisfactorily explained the biological activities. This model was experimentally validated using functional expression of OR1G1 based on calcium imaging and air phase odorant stimulation at physiological doses. Thereafter, we evaluated the statistical link between OR1G1 response to odorants, 3D-QSAR categorization of OR1G1 ligands and their olfactory description. Taking together, our results showed that OR1G1 recognizes two distinct groups of odorants which share both 3D structural and odorous characteristics. Indeed, OR1G1 could be involved in the olfactory coding and human perception of waxy and floral-rose odor. These results thus constitute a step forward in the understanding of the link between olfactory reception and perception. (Sanz G, Schlegel C, Pernollet J-C, Briand L. 2005. Comparison of odorant specificity of two human olfactory receptors from different phylogenetic classes and evidence for antagonism. Chem Senses 30:69–80.)

329. Single-Channel Recordings of Olfactory Cilia Transduction Channels

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Odor transduction takes place in the chemosensory cilia of olfactory receptor neurons (ORNs). Odorants bind to receptors proteins, triggering a cyclic-AMP cascade that leads to the activation of nonselective cationic cyclic nucleotide gated (CNG) channels. Ca2+ entering the cilia through such channels in turn activates Ca2+-dependent Cl− (ClCa) channels. Both channels contribute to the excitatory odor response. Neither the CNG nor the Cl− channel had been previously recorded at the unitary level directly from the cilia of intact ORNs. We succeeded on recording both channel types in excised ciliary membrane patches. In divalent-free conditions the CNG channel exhibited a conductance of ~38 pS and much longer open times than under divalents, in agreement with previously published studies. However, the single-channel recordings revealed that the ciliary ClCa channel has two unitary conductances, of approximately 11 and 22 pS, considerably higher than previously estimated by noise analysis (sub-pS values), although its K0.5 for Ca2+ agrees with published macroscopic data. Estimates of the number of ClCa channels per ORN (3-5000) suggest that this channel is sufficient to account for the whole Cl− transduction current. A third transduction conductance present in the ciliary membrane is K+ selective and Ca2+ activated (KCa), which in isolated ORNs underlies inhibitory odor responses. Four classes of KCa channels have been identified, with conductances between 14 and 210 pS (Delgado et al. 2003. J Neurophysiol 90:2022–2028). These results indicate that for a full understanding of odor transduction direct single-channel recordings from olfactory cilia are crucial.

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330. Calcium Activates a Chloride Current in Moth Olfactory Receptor Neurons

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Responses of olfactory receptor neurons (ORNs) involve a two-step transduction pathway in vertebrates. Second-messengers activate Ca2+-permeable channels, leading to an increase in intracellular [Ca2+]. Ca2+ then gates channels that depolarize the neuron. In insects, the olfactory transduction involves the production of inositol trisphosphate (IP3) and diacylglycerol (DAG). Both activate Ca2+-permeable channels and induce an ORN depolarization. However the intermediate step of current activation by Ca2+ is uncertain. We studied Ca2+-activated currents with the whole-cell patch-clamp technique on cultured ORNs of the moth *Spodoptera littoralis*. The Ca2+-activated current was inwardly rectified in low external Cl− and outwardly rectified in low internal Cl−. In external high Cl− and low Na+ a current with a slight outward rectification and a reversal potential around 0 mV was recorded. Application of Cl− channel blockers, NPPB and flufenamic acid, reversibly blocked Ca2+-activated currents. These findings support the hypothesis of the expression of Ca2+-activated Cl− channels in insect ORNs. Our results allow us to predict that a Ca2+ influx through IP3- and DAG-activated channels activates a Cl− conductance. Equilibrium potential for Cl− remains to be determined to know whether this Ca2+-activated Cl− current is involved in ORN depolarization.

Closing Plenary Lecture

331. Reconstructing Taste

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Taste memories are uniquely robust. In mammals, the brain circuits that reconstruct them are distributed from the brainstem to the cerebral cortex. A key role in taste memory is played by the central gustatory area (CGA) in the insular cortex. The CGA is involved in the identification of taste familiarity, and in the encoding, consolidation, persistence, retrieval and extinction of taste associations. These processes engage partially overlapping yet distinct sets of neurotransmitter and neuromodulatory systems and their downstream intracellular signal transduction cascades. A critical rite of passage in the biography of a specific taste association occurs in the first minutes after its retrieval. At that point in time, multiple, sometimes conflicting associations of the taste compete for the control of behavior. The outcome of this implicit competition, which probably involves shared plasticity resources in cortex, determines whether the association is to be left intact, strengthened, or, alternatively, weakened or even erased. Analysis of the dynamic reconstruction of long-term taste memories casts light not only on the representation of taste experience in our brain but also on general processes that control the fate of many types of long-term memories after their retrieval. This has both theoretical and clinical implications.

Session 8

Symposium 22: Homeostasy of the Olfactory Epithelium

332. Gain Control in the Olfactory Epithelium

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Appetitive, reproductive, arousal, emotional and other states alter both the levels of neuro-active molecules in the olfactory mucosa as well as the sensitivity of their molecular targets. Recent studies in several labs suggest that hormones, neuropeptides, nucleotides and neurotransmitters are released into the olfactory epithelium or olfactory mucus. Functional receptors found in olfactory sensory neurons (OSNs), supporting cells, and ensheathing cells suggest that cells of the olfactory epithelium are under dynamic modulatory controls. This talk will focus on the modulation of OSNs by three classes of signaling molecules: neurotransmitters (dopamine), nucleotides (ATP) and neuropeptides (PACAP). Dopamine, acting through D2 receptors, reduces excitability of OSNs by reducing both Ih and calcium channels. ATP also reduces odorant sensitivity but via increases intracellular calcium. PACAP has neuroprotective effects associated with reducing K channel expression. A role for these modulatory molecules in gain control, or alterations in sensitivity and excitability of the OSNs at the level of the periphery, will be explored. Recognizing that gain control and feedback mechanisms are present in the olfactory bulb, the expression of these

modulatory pathways in the olfactory epithelium may appear redundant. However, peripheral sensory systems are designed to turn their sensitivity up or down to match the animalÆs present physiological state. The olfactory system is no exception. Advancing our understanding of peripheral gain control at the level of the olfactory epithelium provides novel therapeutic or hedonic avenues for modulating odor sensitivity.

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333. Microvillar Cells and the Regeneration of the Olfactory

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The olfactory epithelium of mammals consists of three major cell types: 1) Chemosensory neurons, 2) supporting cells, and 3) basal stem cells. The principle function of these cells is now well understood. Chemosensory neurons detect volatile odour molecules and transmit this information to the brain. Supporting cells seal the epithelium to protect the chemosensory neurons and contribute to regulating the composition of the mucus. Basal cells represent olfactory stem cells, which maintain the regenerative capacity to the olfactory epithelium. Other cells, such as olfactory microvillar cells, have so far attracted only limited attention. Despite their relative abundance, the microvillar cells remain poorly characterized and their function is elusive. We have recently characterized a specific population of olfactory microvillar cells1,2, which is defined by the expression of a set of transduction molecules. Their molecular repertoire suggests that they might be involved in transmitting signals from the mucus across the epithelium. However, the adequate stimulus of these cells and the exact nature and effect of their response remain unknown. Neuropeptide Y stimulates the proliferation of stem cells in the olfactory epithelium, leading to the generation of chemosensory neurons and other cells3. Reportedly, neuropeptide Y is expressed by an unclassified population of olfactory cells in adult animals. We have now shown that these cells, which are responsible for the production of neuropeptide Y, are identical to those microvillar cells that we previously characterized4. Our data suggest that a specific population of olfactory microvillar cells is involved in controlling neurogenesis in the adult olfactory epithelium of mammals.

334. Regulation of the Olfactory Neuron Terminal Maturation

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Neurons within the olfactory system undergo functional turnover throughout life. This postnatal regeneration is a complex process that requires the coordination of neuronal precursor proliferation, differentiation, maturation and survival to maintain the mature neuronal population. Using in vivo and in vitro experiments, we have investigated the function of several candidates such as transcription factor, neurotrophins, or signaling molecules. The transcription repressor factor, MeCP2, has a specific action on terminal maturation. Its expression is neuronal specific and correlated with olfactory neuron maturation. Cellular analyses of the MeCP2 mutant mice revealed that MeCP2 is not involved in neurogenesis, differentiation or neuron survival but is necessary for the proper maturation and synaptic organization of the olfactory neurons. Finally by a proteomic differential display analysis we found that MeCP2 controls the expression of several proteins implicated in the cytoskeleton assembly, cell signaling, energy metabolism and chromatin rearrangements. The cytokine LIF is also involved in the terminal maturation of olfactory neurons. Expressed in differentiated and mature neurons, LIF activates the phosphorylation of the transcription factor, STAT3, which in turn controls the expression of genes characteristic from the maturational stage. The neurotrophin, NT-3 maintains the homeostasis of the olfactory neuron lineage. Secreted by the sustentacular cells, NT-3 increases neuronal survival and decrease both neuronal precursor proliferation and differentiation. Finally, the neuronal nitric oxide synthase (nNOS) is involved in the regulation of neurogenesis during prenatal age, while in the adult nNOS regulates the dendritic arborization of the mitral cells.

335. Expression of the Long and Short Isoforms of the Ret Receptor Tyrosine Kinase in the Olfactory System

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Members of the GDNF family of trophic factors signal through the Ret receptor tyrosine kinase. The gene is alternatively spliced to yield two main isoforms, Ret9 and Ret51, differing in their carboxyl termini, regions involved in intracellular signalling. Each isoform appears to play a distinct role in development: the Ret9 null mouse is nonviable due to renal dysgenesis, whereas the Ret51 null develops normally. We showed that the two ligands, GDNF and neurturin, their accessory proteins and Ret, were detectable in olfactory sensory neurons (OSNs) and bulb. This study aimed to determine which isoforms were expressed in the olfactory system. Adult Sprague–Dawley rats were cardiac perfused and neonates immersion fixed with 4% PFA and paraffin sections examined by immunofluorescence. Antibodies used included a rabbit pAb to Ret9 (C-19) and a goat pAb specific for Ret51 (C-20) (Santa Cruz). Ret isoforms were found to be differentially expressed with Ret9 being predominant in OSNs, although a significant subpopulation of OSNs coexpressed both isoforms. In the neonate, Ret51 was expressed in a subset of OSNs but only in colocalisation with Ret9. Ret51 was avidly expressed on dendrites and axons. In the adult bulb, double-labelling showed coexpression in a subpopulation of periglomerular (PG) cells, although single-labelled PG cells existed for each isoform. Both isoforms labelled both tyrosine hydroxylase-+ve and −ve PG cells. Further studies are needed to determine the exact relationship of Ret to the pre- and postsynaptic compartments of glomeruli. This data suggests Ret functioning via its various ligands is important in the support of some PG cells and we predict both isoforms of Ret will have specific functions in this system.

336. Evidences for a Role of Endothelin on Rat Olfactory Receptors Neurons

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Besides its sensory function, the olfactory mucosa (OM) undergoes regulatory and homeostatic controls, finely tuned by the physiological status of the animal. In search of regulatory factors, we identified several peptides and their receptors in the OM, like orexin, leptin, AVP and endothelin. To characterize their transduction pathways and possible modulatory roles of the olfactory signal, we have developed a primary culture of rat OM cells, and used molecular and cellular approaches, such as immunocytochemistry, RT-PCR and real-time measurement of intracellular Ca2+ concentrations. Among others, endothelin (ET) produced major effects on the different OM cell types and was chosen for further studies. The endothelin converting enzyme, both ETA and ETB receptors and ET1 have been identified in the OM. Additionally, ET triggered a strong, dose-dependent, intracellular Ca2+ response in primary cultured OM cells. Neurons and supporting cells exhibited a large Ca2+ peak, initiated through ETB and ETA receptors, respectively. Both responses were initiated via the PLC pathway and dependant on intracellular Ca2+ stores. However, supporting cells displayed not only a peak but also a plateau phase depending upon extracellular Ca2+ influx through cyclic-nucleotide–gated channels. Our results indicate possible roles of ET1 either as a neuromodulator of the olfactory signal or as a differentiation/survival factor, which remain to be explored.

337. Metamorphosis of an Olfactory System: Hormonal Regulation of Growth and Patterning in the Antennal Imaginal Disc of the Moth *Manduca sexta*

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Peripheral olfactory systems of insects undergo metamorphosis, transforming from a simple larval antenna to the highly complex adult antenna mediating diverse chemosensory behaviors. Adult antennae derive from imaginal discs which grow during the larval stage, and undergo neurogenesis and morphogenesis during the pupal stage. We are characterizing patterns of morphogenic activities in the imaginal disc and early developing antenna to identify hormonally regulated events which lead to the patterning of the adult antenna. This study focuses on development the antennal disc in *M. sexta*. Disc growth occurs throughout most of the fifth larval instar. The antennal imaginal disc grows inward from an epithelial ring surrounding the base of the larval antenna. We have quantified DNA content during disc growth as an indicator of cell number, observing a sharp decline in DNA content just prior to disc eversion. We have subsequently identified apoptotic activity in a spatial pattern which is reflected in the spatial organization of the adult antenna. We have explored the role of ecdysteroids regulating disc growth. Prior to pupation the imaginal discs elongates and everts; we have demonstrated ecdysteroid sensitivity of disc eversion, and are currently exploring the role of ecdysteroids in regulating the post eversion apoptotic events. These studies are establishing a foundation for identifying the hormonal regulation of growth and patterning that will give rise to the selection of specific chemosensory phenotypes of adult olfactory sensilla.

Symposium 23: Modelling Olfactory Perception

338. Structural Design of Insect Olfaction for Pattern Recognition

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Natural neural systems still have an important edge in pattern recognition respect to what the artificial ones can do. There are many aspects of natural systems that are questionably transferable to artificial technology but there are others that can have critical impact in engineering applications. Insect olfaction is a model system that presents big advantages due to the substantial existing anatomical and physiological data, the simplicity of the layered structure, and successful genetic manipulations that provide means to determine the function of each processing layer. We intend to discuss the aspects of insect olfaction design that can have important implications into insect olfaction. Specifically we will focus on the Antennal Lobe, which is a neural center that can be useful for feature extraction methods.

339. Neuromorphic Implementation of Olfactory Systems

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The biological olfactory pathway still outperforms technological solutions to chemosensing in many aspects. For instance many biological olfactory systems respond to a vast range of chemical stimuli, yet maintain extreme sensitivity to certain compounds. This impressive detection capability is also combined with an ability to segment complex odour mixtures into discrete olfactory percepts, e.g. coffee or orange juice. In this talk I will discuss a number of neuromorphic implementations that are based upon our understanding of neural information processing occurring during early olfaction. Technologies to be discussed include an analog-VLSI olfaction chip and field programmable gate array neuromorphic implementation of the mammalian olfactory bulb, as well as robotic implementations for chemical search. In future such implementations promise performance advantages over existing engineered chemical sensing and chemical search hardware.

340. Ratio Coding of Odour Mixtures in Olfactory Receptor Neurons

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Identification of natural odours poses several difficult problems. First, odours exist in nature as mixtures of tens, or even hundreds,

of different chemical compounds with concentrations spanning at least 10 orders of magnitude. Thus, the population of olfactory receptor neurons (ORNs) must be sensitive to a broad range of stimuli and concentrations, whilst avoiding overload in its response. Second, animals must identify odours independently of their concentration over a large range. How these problems are solved is not clear. Here we describe how fundamental biophysical mechanisms of competition between ligands for olfactory receptors account for recent experimental data reporting highly nonlinear interactions between mixture compounds in ORNs responses. Additionally, we demonstrate how this competition leads naturally to neuronal responses which depend upon the relative concentrations of the mixture components, which we term ratio coding. We show how extracting behaviourally relevant ratio based features provides an efficient solution to the aforementioned problems of natural odour identification. Taken together, this has important consequences for understanding how the olfactory pathway processes highly complex chemical information. We conclude that, surprisingly, such nonlinear interactions might be crucial to forming robust coding of complex stimuli.

341. Olfactory Processing with Only 21 Neurons: Smell in Maggots

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Drosophila larvae have only 21 olfactory receptor neurons (ORNs), yet they respond to virtually every odour they are presented with. Most odours are attractive, but some induce a strong repulsive response. Our laboratory uses behavioural, neurogenetic and electrophysiological studies to investigate larval olfaction. We show how the larva processes signals peripherally, where sensory adaptation plays a key role in tuning the response, with evidence from electrophysiology to highlight the role of specific olfactory receptors (ORs) in shaping the olfactory response. Neurogenetic studies enable us to alter the activity of specific cells in both the peripheral olfactory organ and in the larval brain, and to observe the effects of this manipulation on olfactory acuity. The effects of other physiological systems, such as hunger and the larval circadian clock, on olfactory responses are also described. Finally, a preliminary sketch of the various stages of olfactory processing in this simple model system, from peripheral detection, through central integration, to behavioural output, will be presented.

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342. Studying Multimodal Sensory Integration Processes by Presenting Odours and Textures in Concert and at High Temporal Resolution

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From studies on chemosensation, audition and vision, it is known that, for different stimulus modalities to merge into one consistent stimulus percept, impressions from different modalities should: (1) have naturally matching qualities (2) cooccur within a sufficiently small time window (3) be presented through natural presentation paths. In order to study the temporal limits for the integration of texture and odour stimuli into one percept, presented textures and odours matched the qualities of a creamy milk product. A computer-controlled system combining remote-controlled liquid pumps and an olfactometer allowed for the exact presentation of odour pulses during (1) presentation of the milk, (2) oral processing of the milk or (3) swallowing of the milk, thus allowing for the study of the effects of stimulus timing on stimulus integration. Furthermore, odour presentation was varied between the orthonasal and retronasal path. Results showed that odour stimuli increased perceived thickness and creaminess, but only when odours were presented retronasally, i.e. as if the odour would have originated from the milk. Furthermore, this enhancement was most pronounced when odours coincided with swallowing, less pronounced when odours coincided with oral processing and absent when presented during mouth filling. Results suggest that cross-modal interactions are the rule rather than an exception, provided that multimodal sensory integration has occurred.

343. Computer Modelling Strategies to Investigate the Molecular Basis of Odour Discrimination

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The initial step of odour perception occurs in the olfactory epithelium of the nose through olfactory receptors (ORs) which are located on olfactory sensory neurons. ORs belong to the large superfamily of G protein–coupled receptors and provide a remarkable ability to recognize and discriminate numerous chemically diverse odorants. To address the molecular basis of this discrimination power, three distantly related olfactory receptors were studied: OR3A1, OR1D2 and OLFR43 which recognise some identical ligands with distinct affinities and specificities. This knowledge provides us with the unique opportunity to deal with the question of odorant discrimination on the receptor level. First we generated rhodopsin-based homology models followed by molecular dynamics simulations to take the considerable flexibility of the loops especially on the intracellular side into account. Ligand docking simulations were performed to define the odorant-binding site of each receptor. Our results indicate that the binding site is formed by mainly hydrophobic amino acids, located in a pocket created by TM3, TM5 and TM6. Of critical importance is a serine in TM3. Consistently recent studies based on computational and experimental findings showed that this residue serves as hydrogen bond donor in other olfactory receptors. However, Ser is replaced by a cysteine in OR3A1 and OLFR43. Interestingly mutation studies of this Ser to Cys in mOR-EG led to altered, but not abolished responsiveness of odorants. Another interesting feature of our studies is the finding of partially common binding-site-patterns for identical ligands, despite the low sequence identity between OR1D2, OR3A1 and OLFR43 (approx. 40%). However experimental studies are needed to validate these results.

Symposium 24: From Reception to Perception in Primate Sweet and Bitter Taste: From Taste Bud to Cortex (II)

344. From Taste Buds to Nerve Fibers: The Role of ATP in Transmission of Taste Information

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Sweet and bitter tastes are transduced respectively by T1R and T2R family GPCRs located in a distinct type of taste cell (Type II). Despite differences in receptor ligand specificity, all TRs appear to impact on similar downstream signaling cascades involving PLC?2 and TrpM5. Curiously, the Type II cells, which possess both the taste receptor molecules and downstream transduction components, do not form classical synaptic contacts with the gustatory nerve fibers within the taste bud despite close apposition of the cells and the nerve fibers. Rather, synapses onto gustatory nerve fibers arise from Type III cells which possess none of the known taste receptors or downstream elements. The gustatory nerve fibers express P2X2 and P2X3 receptors for ATP so release of ATP from any of the taste cells could activate these nerve fibers. Genetic ablation of both P2X2 and P2X3 receptors eliminates all taste-evoked responses from the gustatory nerves. These findings indicate that ATP is an essential transmitter linking the taste buds to the gustatory nerve fibers. Consonant with this is the observation that ectoATPase is expressed by the Type I cells which surround the contacts between Type II cells and taste nerves. Despite the total loss of gustatory neural activity and absence of sweet-associated appetitive behaviors, the P2X KO mice still exhibit aversive responses to many bitter tastants albeit at higher concentrations than normal. These residual behavioral responses to bitter in the absence of input from the gustatory nerves suggest that a nontaste chemoreceptor system, perhaps in the larynx or GI tract, can arrest intake of classical bitter substances.

345. Coding of Sweet and Bitter Taste in Peripheral Primate Nerves

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The relationship between what humans call ''the sweet and bitter taste qualities'' and the organization of the taste fibers is not resolved. Taste nerve recordings from three simian primates: the common marmoset, *Callithrix jacchus jacchus*, rhesus monkey, *Macaca mulatta*, and chimpanzee, *Pan troglodytes* suggest that sweet and bitter taste is mediated in different sets of taste fiber, in a sense ''hard wired''. This conclusion is based on behavioral and electrophysiological data. First, hierarchical cluster analysis and multidimensional scaling consistently identify two clusters of taste fibers. The nerve fibers in one cluster, here called Q fibers, respond to compounds that are rejected. The fibers in the other cluster, called S fibers, respond to compounds, which are preferred. The S and Q clusters donÆt overlap, i.e. Q fibers responding to aversive compounds do not respond to preferred compounds and vice versa. Second, after miraculin, which adds a sweet taste to sour compounds and changes the behavior from rejection to liking, S fibers show a response to acids, not present before miraculin. No other taste fibers were affected. Third, gymnemic acid which abolishes sweet taste and taste nerve responses to sweet compounds in humans, abolished the S fiber response to sweet. No other fiber type was affected. These results show that in primates the sweet and bitter taste qualities are caused by two separate sets of taste fibers from the tongue.

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346. Cortical Representation of Sweet and Bitter Taste

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In the primate primary taste cortex in the rostral insula and frontal operculum, taste identity is represented by a distributed code, with each neuron tuned to a different set of tastants. In the secondary taste cortex in the orbitofrontal cortex there is also a distributed code for taste, but the tuning of the neurons may be more fine. In the secondary but not in the primary taste cortex neurons represent the reward value of taste, in that the neuronal responses decrease to zero in a sensory-specific way in the orbitofrontal cortex when the macaque is fed to satiety, but there is no modulation by hunger in the primary taste cortex. Neurons in the primary taste cortex and also in the orbitofrontal cortex may be unimodal for taste, but may also in some cases represent also oral texture and temperature. In human functional imaging studies, it has been shown that the primary taste cortex, the amygdala, and the orbitofrontal cortex, are activated by sweet taste and also by other tastes. In these human functional neuroimaging studies, it has also been shown that activation of the orbitofrontal cortex (OFC) and adjoining anterior cingulate cortex by liquid food is hunger-dependent, and indeed the pleasantness of the food is correlated with the degree of activation found. (Kadohisa M, Rolls ET, Verhagen JV. 2005. Neuronal representations of stimuli in the mouth: the primate insular taste cortex, orbitofrontal cortex, and amygdala. Chem Senses 30:401-419. Some papers are available at www.cns.ox.ac.uk.)

347. fMRI of Sweet and Bitter in the Human CNS: Response to Reward Value

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We have employed fMRI to investigate cortical activation in response to taste stimuli that have very high reward value (sucrose, sweet) and to taste stimuli that constitute a negative outcome (caffeine, bitter). Subjects rated the pleasantness of stimuli using the General Labeled Magnitude Scale (gLMS) while stimuli were presented to the mouth as 3 μl boluses in a continuous stream of distilled water. Psychophysical assessment confirmed positive and negative valences for these stimuli. Imaging was conducted on a 3T GE scanner using a standard gradient echo EPI pulse sequence to acquire T_2*-weighted functional images [(24 axial slices, FOV = 19 cm, matrix size = 64×64, spatial resolution = $2.97 \times 2.97 \times 3$ mm^3, flip angle = 90°, echo time (TE) = 30 ms, repetition time (TR) = 2 s)]. An

event-related paradigm allowed us to examine specific neural events in response to sweet and bitter stimuli. Image analysis was conducted using AFNI. For each region, an average fit coefficient was obtained for each subject, and those fit coefficients were subjected to ANOVA to compare the degree of activation in ROIs. There was a dramatic difference between activation in response to sweet and bitter when subjects were judging relative pleasantness, supporting differential cortical activation in response to positive and negative reward value. The differential brain activation observed in response to sweet and bitter supports the existence of differential brain patterns in response to differential reward value and suggests that these findings may have implications for situations with differential reward value in other domains (e.g., economics).

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Oral Session 8: Olfactory Perception

348. Chemosensory Inputs to the Basal Ganglia

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Amygdalo-striatal projections have been largely studied. Chemosensory inputs to the basal ganglia, however, have been partially neglected. In snakes, which posses a hypertrophied vomeronasal system, the nucleus sphericus, the main vomeronasal-recipient structure in the telencephalon, mainly projects to the olfactostriatum. The olfactostriatum is a basal ganglia vomeronasal area that closely resembles the mammalian shell of the nucleus accumbens. The present work tries to investigate whether this projection is actually present in mammals as well as to analyze to what extent the mammalian basal ganglia receive vomeronasal and olfactory afferents. Adult rats received iontophoretic injections of dextran-amines in different nuclei of the vomeronasal and olfactory amygdalae. Results indicate that vomeronasal-recipient areas such as the posteromedial cortical amygdaloid nucleus projects to shell of the nucleus accumbens. On the other hand, olfactory-recipient areas such as the posterolateral cortical amygdaloid nucleus projects to the core of the nucleus accumbens as well as to the ventral pallidum. The present data could help to understand the neural basis of reward circuits of chemosensory sensation.

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349. Predictions of Human Odor Descriptors and Odorant Chemical Classes from Glomerular Response Patterns in the Rat Olfactory Bulb

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Odorants bind to receptors on the surface of olfactory sensory neurons in the nasal cavity, and the differential activity of these neurons is reorganized into distinct spatial patterns in the olfactory bulb. Such patterns have been described for hundreds of odorants using the [14C]2-deoxyglucose uptake technique. Although previous studies have detected relationships between these activity patterns and odorant chemistry, the considerable overlap in patterns for odorants of different chemistry has complicated our understanding of how perceptions are extracted from these activity patterns. We now have determined both the aspects of the glomerular patterns that actually predict chemical class and the combinations of glomerular activity that predict perceived odor. Using a linear classifier (SVM with linear kernel functions), we extracted predictive profiles (decision images) for various human odor descriptors as well as for various odorant chemical classes. We find that both chemical classes and most of the human odor descriptors sort the rat glomerular patterns in a statistically meaningful way, suggesting an overall similarity in olfactory coding in rats and humans. Decision images regarding chemical class differed greatly from simple averages of activity evoked by all odorants of that chemical class. Surprisingly, we find that the regions predicting the odorant chemical class are spatially segregated from the regions predicting the perceptual class for the same odorant. Our findings suggest that the spatial organization of the glomerular activity patterns may represent both molecular features and odor quality.

350. The Vomeronasal Organ of the Tammar Wallaby (*Macropus eugenii*)

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The vomeronasal organ (VNO) is the primary olfactory organ that detects pheromones in mammals. We have investigated the anatomy of the VNO of the tammar wallaby (*Macropus eugenii*), a small macropodid marsupial, where we believe pheromones are important for activation of the hypothalamo-pituitary axis of males at the start of the breeding season. The gross anatomy, connection to the brain and receptor abundance in the VNO was examined by light and electron microscopy in adult males and females, and by immunohistochemistry for receptors of the VNO receptor family. The VNO is well developed in adult males and females, with the typical structure of receptor and nonreceptor epithelium. It connects via the nasopalatine ducts to the mouth and nose cavity. At the rostral end the lumen is half-moon shaped, changing to a narrow oval shape distally. Glandular tissue in the walls increases towards the blind end. There are microvilli on the surface of both epithelia and cilia on the surface of the nonreceptor epithelia. The nonreceptor epithelial cells seem to secrete mucus into the VNO lumen. Goa-protein–coupled receptors were found in the deeper cell layers of the receptor epithelium as was previously found in mice and in the short-tailed opossum. The degree of VNO development makes it likely that pheromones are also important in reproduction in the tammar as they are in other mammals.

351. Fast Adaptation in Mouse Olfactory Sensory Neurons Does Not Require the Activity of Phosphodiesterase

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Vertebrate olfactory sensory neurons rapidly adapt to repetitive odorant stimuli. Previous studies have shown that the principal molecular mechanisms for odorant adaptation take place after the odorant-induced production of cAMP, and that one important mechanism is the negative feedback modulation by Ca^{2+}-calmodulin (Ca^{2+}-CaM) of the cyclic nucleotide-gated (CNG) channel. However, the physiological role of the Ca^{2+}-dependent activity of phosphodiesterase (PDE) in adaptation has not been investigated yet. We used the whole-cell voltage-clamp technique to record currents in mouse olfactory sensory neurons elicited by photorelease of 8-Br-cAMP, an analogue of cAMP commonly used as a hydrolysis-resistant compound. We measured responses to repetitive photoreleases of cAMP or of 8-Br-cAMP and we observed similar adaptation in response to the second stimulus. Control experiments were conducted in the presence of the PDE inhibitor IBMX. Since the total current activated by 8-Br-cAMP, as well as that physiologically induced by odorants, is composed not only of current carried by Na+ and Ca^{2+} through CNG channels, but also by a Ca^{2+}-activated Cl− current, we performed control experiments in which the reversal potential of Cl− was set, by ion substitution, at the same value of the holding potential, −50 mV. Adaptation was measured also in these conditions of diminished Ca^{2+}-activated Cl− current. Furthermore, producing repetitive increases of ciliary's Ca^{2+} with flash photolysis of caged Ca^{2+}, we showed that Ca^{2+}-activated Cl− channels do not show adaptation and that there is no Cl− depletion in the cilia. All together, these results indicate that the activity of ciliary's PDE is not required for fast adaptation to repetitive stimuli in mouse olfactory sensory neurons.

352. Development of an Odor Awareness Scale

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The extent to which people are aware of odors in their environment may affect how they perceive odors and how they process odor information. To measure this degree of explicit odor awareness we developed a new questionnaire instrument: The Odor Awareness Scale. The scale consists of items that describe situations and common products people tend to encounter in everyday situations, and asks about their reactions towards these odors. The questionnaire was administered to 332 students. Principal Components Analyses on 34 items indicated that several items (n = 12) loaded high on more than one component. After removal of these items, a 4-component solution emerged that explained 39% of total variance. The first component was a general odor awareness component: People who score high on this component attach importance to the sense of smell, have an increased tendency to pay attention to odors in their environment or notice sudden smells. The second component was characterized as body odors: People who score high on this component attach importance to how people smell, and are easily attracted or put off by body odors. The third component was an alarm component, which was related to the warning or signal value that off flavors from stale foods can have. Finally, there was a product purchase component: People who value the scent of shower gel, all-purpose cleaner and deodorant over price, packaging, and product effectiveness scored high on this component. We are currently testing whether odor awareness is related to olfactory abilities and information processing speed for odor-related information.

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353. Genetic Component of Perceiving Odours: A Study with Finnish Families

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We studied genetic effects on olfactory-related traits by combining methods of psychophysics and quantitative genetics. A total of 146 genome-wide scanned Finnish adults (100 females, 46 males, aged 18–78 years, mean 49 ± 15 years) from 26 families performed the Brief Smell Identification Test™ (B-SIT), that includes 12 odours in scratch-and-sniff format (cinnamon, turpentine, lemon, smoke, chocolate, rose, paint thinner, banana, pineapple, gasoline, soap, onion). In this study, pleasantness and intensity ratings were also given on each odour (5-point category scales, data standardized prior to genetic analyses). Heritability analysis and multipoint linkage analysis for loci harbouring potential underlying genes were performed using program Merlin. Effects of age, sex, smoking, migraine status, and identification of the odour were examined and taken into account in genetic analyses when appropriate. Heritability (h2) of the score of correct identifications was low (11%), but when odours were analyzed separately, several traits showed heritability over 30%. Suggestive evidence for linkage was found between pleasantness of cinnamon odour (h2 = 61%) and a locus on chromosome 4q32.3 (multipoint LOD score 3.01) as well as between intensity of paint thinner odour (h2 = 31%) and a locus on chromosome 2p14 (multipoint LOD score 2.55). These chromosomal regions are not known to contain potential odorant receptor genes. However, they might harbour genes influencing the signal transmission or central processing.

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