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Symposium 1: Olfactory Processing

Dynamic Ensemble Odor Coding in the Mammalian Olfactory Bulb: Information at Different Time Scales

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Neural firing discharges are often temporally patterned, but it is often ambiguous as to whether the temporal features of these patterns constitute a useful code. Here we show in the mouse olfactory bulb that ensembles of projection neurons respond with complex odor- and concentration-specific dynamic activity sequences developing below and above sniffing frequency. Based on this activity, almost optimal discrimination of presented odors was possible during single sniffs, consistently with reported behavioral data. Within a sniff cycle, slower features of the dynamics (>100ms resolution, including mean firing rate) were alone sufficient for maximal discrimination. A smaller amount of information was also observed in faster features down to the 20-40 ms resolution. Therefore, mitral cell ensemble activity contains information at different time scales that could be separately or complementarily exploited by downstream brain centers to make odor discriminations. Our results also support novel analogies in the dynamics of odor representations between insects and mammals.

Olfactory Receptor Neurons and Odor Coding

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Odorants are represented as spatio-temporal patterns of spiking in the antennal lobe (insects) and the olfactory bulb (OB, fish, mammals). We combined electrophysiological recordings in the locust with well-constrained computational models to examine how these neural codes for odors are generated. Extracellular recordings from the olfactory receptor neurons (ORNs) that provide input to the antennal lobe showed that the ORNs themselves can respond to odorants with reliable spiking patterns that vary both in strength (firing rate) and time course. A single ORN could respond with diverse firing patterns to different odors, and, a single odorant could evoke differently structured responses in multiple ORNs. Further, odors could elicit responses in some ORNs that greatly outlasted the stimulus duration, and some ORNs showed enduring inhibitory responses that fell well below baseline activity levels, or reliable sequences of inhibition and excitation. Thus, output from ORNs contains temporal structures that vary with the odor. The heterogeneous firing patterns of sensory neurons may, to a greater extent than presently understood, contribute to the production of complex temporal odor coding structures in the antennal lobe.

Our computational model of the first two stages of the olfactory system revealed that several well-described properties of odor codes previously believed to originate within the circuitry of the antennal lobe (odor-elicited spatio-temporal patterning of projection neuron activity, decoupling of odor identity from intensity, formation of fixed-point attractors for long odor pulses) appear to arise within the ORNs. To evaluate the contributions of the antennal lobe circuits, we examined subsequent processing of the ORN responses with a model of the antennal lobe network. The antennal lobe circuitry enabled the transient oscillatory synchronization of groups of projection neurons. Further, we found that the antennal lobe transformed information contained in the temporal dynamics of the ORN response into patterns that were more broadly distributed across groups of projection neurons, and more temporally complex because of GABAergic inhibition from local neurons. And, because of this inhibition, and unlike odor responses in groups of ORNs, responses in groups of projection neurons decorrelated over time, allowing better use of the antennal lobe coding space. Thus, the principle role of the antennal lobe appears to be transforming spatio-temporal patterns in the ORNs into a new coding format, possibly to decouple conflicting odor classification and identification tasks.

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Olfaction as an Active Sense: How Sniffing Shapes Early Odor Coding

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We typically think of sensory systems as passively generating faithful representations of external stimuli at initial, low-level stages of the nervous system and then performing increasingly complex transformations of these representations as information propagates to higher levels. Likewise, the modulation of sensory codes during behavior – for example, as a function of behavioral context or attentional state – is typically thought to occur at higher nervous system levels. This talk will discuss recent findings from our laboratory demonstrating that, in the olfactory system, odor representations in the behaving animal can be transformed at low levels – as early as the primary sensory neurons themselves - via a variety of different mechanisms related to the active acquisition of olfactory information. First, changes in odor sampling behavior (i.e. – 'sniffing')

can dramatically and rapidly alter primary odor representations by changing the strength and temporal structure of sensory input to the olfactory bulb, effectively shaping which features of the olfactory landscape are emphasized and likely altering how information is processed by the olfactory bulb network. Second, neural substrates exist for presynaptically modulating the strength of sensory input to the bulb as a function of behavioral state. The systems most likely to be involved in this modulation – cholinergic and serotonergic centrifugal inputs to the bulb – are linked to attention and arousal effects in other brain areas. Together, sniffing behavior and presynaptic inhibition have the potential to mediate – or at least contribute to – sensory processing phenomena such as figure-ground separation, intensity-invariance, and context-dependent and attentional modulation of response properties. Thus, even low-level representations of olfactory information are actively shaped in the behaving animal as it samples the olfactory world around it.

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Mapping Learning-Induced Modulations of Oscillatory Activity in the Olfactory Memory Network: A new Functional Imagery?

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Current theories put forward that information storage in the brain relies on changes in functional interactions within widely distributed neural areas. This concept of distributed memory suggests in turn the idea that stimuli representations could be achieved through assemblies of simultaneously active neurons. As a consequence, memory should be considered as a dynamical process involving spatio-temporal patterns of reactivation of previously reinforced neural ensembles within and across different brain areas. These assemblies involve both sensory and limbic areas. Synchronous activities in assemblies often occur in a repetitive way and give rise to well-known brain rhythms also called oscillations. They can easily be recorded from multiple intra cerebral inserted electrodes. Using wavelet analysis, oscillatory content can be extracted and quantified from each recorded signal or LFP (local field potential). In the mammalian olfactory system, prominent oscillations in field potential activities have been described: In the absence of any stimulation, slow modulations of local field potentials in the theta range (2–12 Hz) are associated with inhalation cycles. Recordings also show regular spindle bursts of oscillations in the gamma range (30–90 Hz) during each inspiration phase of the respiratory cycle. Whereas gamma and theta activities have been studied for a long time, little attention was paid to an intermediate type of periodic activity in the beta range (15–35 Hz). This activity has now been reported by several authors to be selectively associated with odor sampling not only in the olfactory bulb, but also at higher level of olfactory processing like the perform and lateral entorhinal cortex. Studies pointed out to a more or less prominent increase in the amplitude of this oscillatory activity in response to behaviourally relevant odors or odors experimentally associated with a reward. In this context, I will present a series of experiments we designed on behaving rats chronically implanted for LFP recordings and engaged in different olfactory learning tasks. I then will discuss the idea that emergence of high amplitude beta oscillations in the network could sign for a largely distributed functional assemblies set up through learning.

Processing and Topological Reorganization of Odor-Encoding Activity Patterns in the Brain

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Odor information is first represented in the olfactory bulb (OB) by distributed glomerular activity patterns that contain nested spatial maps of primary and secondary molecular stimulus features. Neuronal circuits in the OB transform these input patterns into spatio-temporal patterns of output activity that are transmitted to higher brain regions by mitral cells. To understand the computations associated with this transformation and the function of chemotopic maps, we measured odor-evoked activity patterns across thousands of individual neurons in the intact brain of zebrafish using electrophysiology, temporally deconvolved 2-photon calcium imaging, and transgenic cell type markers. We found that the OB performs multiple computations including a decorrelation of initially overlapping activity patterns, a multiplexing of complementary information, and gain control. The chemotopic representation of primary molecular features is maintained in OB output activity patterns, while secondary chemotopic maps disappear during the initial phase of an odor response. This reorganization is caused by the local sparsening of MC activity within chemotopic foci and promotes the decorrelation of overlapping input patterns. Computational modelling based on measured connectivity patterns indicates that local sparsening and decorrelation are generic features of circuits with an OB-like architecture and depend on the chemotopy of inputs, even though secondary chemotopy is not maintained in the output. These results indicate that topographic maps configure computational properties of circuits and provide insights into the basic functions of the OB.

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Odotopic Properties of Odorants and the Expression Pattern of Olfactory Receptors

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Olfactory stimuli are represented not only by their odorant/ligand affinity. A chromatographic process in olfaction–a separation of odorants based on their chemical properties and flow dynamics across the nasal cavity has been initially proposed and demonstrated by Mozell et al. Our objective is to correlate the expression pattern of olfactory receptors (ORs) of the dorsal zone of mouse olfactory epithelium with the odotopic properties of their cognate ligands, i.e. volatility, hydrophobicity and water solubility. Our

hypothesis is that olfactory receptors for polar, hydrophilic odorants are present in extreme dorsal regions of olfactory epithelium where the airflow is high, while ORs for non-polar, hydrophobic odorants are absent. To test this hypothesis we combined micro array analysis of RNA expression and micro-transplantation of plasma sheets containing native olfactory receptors into Xenopus oocytes for electrophysiological characterization of ligand selectivity. Left and right hemisections of the dorsal olfactory epithelium are separated into parallel subsections along the anterior-posterior axis. Left hemisections containing native ORs are processed using a ciliary membrane preparation and injected into oocytes. Micro transplanted native ORs from each subsection are tested against 30 odorants using two-electrode voltage clamp and a robotic electrophysiology system (Opus 6000A, Molecular Devices). The pattern of expression of ORs is characterized using micro array analysis of RNA expression in parallel subsections. Correlating the chemical properties of each odorant together with the topographical location of its cognate OR will shed light on the spatial distribution of odorant responses within the olfactory epithelium, thus demonstrating the functional organization of ORs at the periphery.

Calcium Handling in Rodent Olfactory Bulb Granule Cells and Mitral Cells

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In the mammalian olfactory bulb the inhibitory axon less granule cells interconnect mitral cells via large reciprocal spines. There are several types of dendritic granule cell calcium signals all of which show unusually slow decay kinetics when monitored with fluorescent calcium indicators. In acute slices from juvenile rats we imaged granule cell calcium transients in their apical dendrite and its spines in response to somatically evoked action potentials with two-photon laser scanning microscopy, using different concentrations and combinations of fluorescent dyes in order to extrapolate to conditions of zero added buffer (20, 50, 100 μM OGB-1; 100 μM OGB-6F; 100 μM Fluo-5F with 25 μM Alexa 594; n = 10/14/13/9/18).

These data yielded a resting calcium concentration on the order of 50 nM in both dendrites and spines. The extrapolated endogenous granule cell buffer capacities were within a range of 60-100 in the dendrites and even higher in the spines. At zero added buffer and room temperature the time constant of calcium transients in both dendrites and spines was on the order of 250 ms. At physiological temperatures, granule cell calcium transients are thus likely to decay with a time constant of roughly 125 ms. Single action potentials that were evoked by somatic current injection resulted in a calcium elevation to approximately 200 nM. In granule cells from adult mice filled with 100 μM OGB-1 (n = 14) we observed decay kinetics of calcium transients, resting calcium and calcium elevations that were not significantly different from the data in rat. Thus mouse granule cell calcium dynamics are likely to be determined by similar factors. Conversely, the dendrites of juvenile rat mitral cells filled with 100 μM OGB-1 (n = 4) showed calcium dynamics comparable to that of cortical and hippocampal pyramidal cells, consistent with a lower endogenous buffering capacity.

We conclude that the slow calcium dynamics in granule cell apical dendrites and spines are due to both a fairly high endogenous buffer capacity and a slow extrusion of calcium. The slow sequestration of calcium may contribute to asynchronous output from the reciprocal spine and feed into other calcium-dependent mechanisms that play a role in synaptic granule cell signalling.

Symposium 2: An Approach to Clinical Taste Disorders

An Approach to Clinical Taste Disorders – A General Overview

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Gustation is still the least understood of the five senses, although global advances in this understanding are vastly improved. These include taste transduction physiology, functional imaging of the central taste pathways and microanatomy and pathology of the peripheral pathways. Despite these advances, patients with a loss of taste are historically dismissed by clinicians outside of the very few taste (and smell) centers that exist internationally. The impact for the patient includes altered quality of life, health and basic safety, as well as confronting clinicians with no awareness of approaches to these disorders, some of which have been established over decades.

There is an increased need for established clinics for taste disorders, either within health services such as an otolaryngology department, or externally funded independent clinics. Since taste disorders can be associated with a multitude of causes, some of which are serious, established consensus is necessary as to the applied multi-disciplinary approach to the patient. Conflicting reports on the relevance of quantitative electrogustometry, together with concerns regarding chemogustometry costs and application time have not helped patient management in most countries. In those centers where these tests are practiced, differences in psychophysical testing yield conflicting results. A priority is to establish a uniform approach to taste disorders based on current evidence. The roles of electrogustometry and chemogustometry are discussed, together with application of functional magnetic resonance imaging and confocal microscopy. Perhaps most challenging, however, is the role of education in primary care of both patient and clinician in order to advance the outcomes for those suffering from taste disorders.

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Taste Clinics – A Summary: Causative Factors and Treatment of the Patients with Taste Disorder

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Introduction: One of the characteristics of taste disorder is that it occurs most often in aged population, and numbers of patients with taste disorders increase with age. To study the causative factors of taste disorders, we examined 408 patients. We examined therapeutic effects of a zinc agent on taste disorders by taking into account the causative factors of the disorder and age.

Subjects: Patients with the disorder usually came to the ENT Department of Nihon University Itabashi Hospital and complained of a reduction in the sense of taste. Ages of patients ranged from 21 to 84. The therapeutic effects of treatment of taste disorder with a zinc agent were studied in 252 cases.

Methods: The causative factors of the taste disorders were classified as follows: drug-induced, zinc deficiency, systemic diseases, inflammation of the upper respiratory tract, head injuries, glossitis, loss of flavor and idiopathic factors. In the taste disorders patients due to zinc deficiency the triggering mechanisms of the disorder were not known, the serum zinc concentration was low. Serum zinc concentrations lower than 69 μg/dl were considered abnormally low. Taste functions were evaluated with the filter-paper disk method and solutions of sucrose, sodium chloride and tartaric acid were tested. The zinc agent, polaprezinc, was singly given at a daily dose of 150 mg/day (75 mg, b. i. d.). Polaprezinc is a zinc-carnosine complex formulated as white odorless granules whose primary use is treatment of peptic ulcers. 75 mg of polaprezinc contains about 17 mg of zinc.

Results: In the population studied, drug-induced taste disorders accounted for 32% of the causative factors, followed by taste disorders due to idiopathic factors, systemic disorders, and zinc deficiency. Among the 408 patients, 116 cases showed low serum zinc concentration, regardless of the causative factors of the taste disorder. Taste disorders observed in the aged group with a significantly higher incidence of abnormality were caused by drugs and systemic disorders. On the other hand, causative factors that were significantly lower in the aged group were idiopathic factors and inflammations of the upper respiratory tract. Zinc administration was effective in 70% of the whole population studied, and 74% in the aged population. No significant difference was observed in curative effectiveness rate among age groups.

Taste Disorders, Electrogustometry or Solution Tasting, Learning or No Learning

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Some patients are not possibly tested with repeated sessions of solutions tasting. Electrogustometry (EGM) is more rapid, and consists in an iontophoretic application of the saliva cations of the subject onto his/her own receptors. EGM threshold evaluates the smallest current transporting the just necessary number of cations eliciting a tiny taste perception at the limit of detection. EGM will test the whole taste sensory chain from the periphery to the centres, including ionotropic but excluding metabotropic transduction mechanisms (concerned by organic chemicals). Some experiments will validate EGM in adequate cases. A " custom made " electrogustometer including a constant current generator delivered currents to nine tongue loci and eventually 2 soft palate loci. In healthy subjects, EGM threshold de-pended on the loci tested and increased significantly with the number of Dental Deafferentation (DD). The study showed no effect of age on taste sensitivity in *non medicated, non smoking* subjects until 80y.o. for low numbers of DD. Significantly increased thresholds at *anterior* tongue loci were found only in subjects with *anterior* DD and suggested an homologous topy of the projection of both the mandibular (dental) and chorda tympani (taste) nerves in the Nucleus tractus solitarius. Convergence of such somatosensory and taste inputs was demonstrated recording taste neurones in the rat NTS. The cooperation of trigeminal sensitivity to food taste includes dental nerves, and allows hetero-sensory masking, enhancement or compensation. A significant increase of EGM thresholds was shown in cancer patients under chemotherapy. In chronic renal failure (CRF) patients, thresholds decreased during the dialysis but so did the tongue pH: dialysis increased the cation content of the saliva, i.e. the stimulus iontophoretically applied (mainly Na^+, H^+). It was hence necessary to test CRF patients with solutions. In middle ear surgery (stapedectomy), a deficit was noted which progressively recovered with a delay depending on the locus. The study allowed confirmation or demonstration of multiple innervations of some tongue loci by the chorda tympani and glossopharyngeal nerves in the human. Tobacco was shown to induce an increase of thresholds at loci localized on the route of the smoke, which was quantitatively correlated to the dependence. The evaluation of the recovery time-course at quitting tobacco is used as an efficient tool to help motivating subjects.

The EGM test was proven useful for excluding subjects with general taste deficits from panels. Data obtained with CRF patients confirm the taste nature, in our conditions, of the EGM test, for which no effect of learning by repeated exposure (familiarization) to the stimulus was observed, probably because the cations of one self's saliva are familiar. In contrast, data will show the imaging (fMRI) of learning during the repeated exposure to taste solutions.

Confocal Microscopy in Taste Disorders

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Objective: The goal of this study was to characterize features of normal and abnormal taste bud structures using confocal microscopy (CM).

Materials and methods: The human tongue epithelium of 60 healthy subjects with a normal gustatory sensibility, aged 18 to 76 years, was examined in vivo by CM. A combination of the Heidelberg Retina Tomograph HRTII and Rostock Cornea Module was used. The results were compared with those of 28 patients with taste disorders.

Results: Considering age and groups significant differences were found for the parameters "number of cells of taste bud" and "density" ($P < 0.05$).

Conclusions: With CM it is basically possible to reveal the peripherally taste organ. Thus, it may serve as a helpful tool to diagnose patients with taste disorders. Recently, rigid confocal endoscopes have been developed that can obtain confocal images of the taste bud in vivo in near real time. Use of this diagnostic tool provides a high potential to image papillae and following taste bud in a clinically setting.

Gustatory Activation using Psychophysical, Electrophysiological and Imaging Techniques in the Assessment of Patients

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Recently a new test for psychophysical assessment of taste function was introduced[1]. It is based on strips made from filter paper which

were impregnated with different taste solutions (four concentrations each for sweet, sour, salty and bitter). These strips are placed on the tongue and subjects are asked to identify the taste quality. Each subject receives 4 concentrations of each taste in a pseudo-randomized sequence. Results correlate significantly with the results of the well established three-drop-technique (r_{69}=0.67). Repeated measures indicated good reproducibility of the results for the taste strips (r_{69}=0.68). Results suggest the usefulness of this new technique in routine clinical practice. Major advantages are long shelf-life and convenience of administration. On electrophysiological level gustatory event-related potentials appear to be a well-investigated means[2,3] for clinical investigations in specific situations, e.g. medico-legal cases. Finally, functional MRI also can be applied to the investigation of patients with taste loss[4]. In patients with hypogeusia or ageusia clusters of activated voxels were mainly found in orbitofrontal and insular regions of interest. Even those patients who did not perceive any stimuli showed some activation of gustatory centers. Group comparisons revealed higher activation of the insular and orbitofrontal cortices in patients compared to the group of healthy subjects. While further studies are needed, this finding may be interpreted in terms of enhanced neuronal recruitment due to functional impairment in patients with gustatory loss. In summary, the presently available armamentarium of techniques allows to view a taste deficit from various angles. Here the psychophysical techniques appear to be of specific value in the search for therapies of taste disorders[5,6].

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Symposium 3: Conditioned Taste Aversion: A Multidisciplinary Approach

Brain Networks and Molecular Mechanisms Involved in Taste Recognition Memory Formation

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In our laboratory we are investigating the molecular mechanism underlying recognition memory formation and evocation. Recognition memory; the ability to discriminate events or stimulus previously experienced, is a form of declarative memory that has been linked to a network of media temporal cortical regions, including the perirhinal, parahippocampal, entorhinal cortices and the hippocampus. One of the most critical survival skills that animals have developed throughout evolution is taste-recognition memory; that is the ability to discriminate gustative stimuli previously encounter. When an animal encounters a new taste, it hesitates to eat it, showing a neophobic response. Depending on its gastric consequences the taste becomes recognized as either aversive signal (reducing its consumption, called Conditioned Taste Aversion; CTA), or safe signal (increasing its consumption, Attenuation of Neophobia; AN). Both CTA and AN are models of taste recognition memory, and it has been demonstrated that their consolidation depends on protein synthesis. Thus, in the present study, we addressed the role of protein synthesis in consolidation of CTA and AN in perirhinal cortex, hippocampus, basolateral amygdala and central amygdala by means of post-trial intracerebral microinjections of anisomycin, an inhibitor of translation. We found that perirhinal cortex and hippocampus are involved in consolidation of safe but not aversive taste recognition memory, whereas central amygdala is involved in aversive but not safe memory. In conclusion, our data show that medial temporal lobe structures play different roles in safe or aversive taste memory consolidation. Currently, we are identifying the putative neurotransmitters released and the intracellular molecular mechanism involved in the establishment of recognition memory traces in different brain networks.

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Conditioned Taste Aversion: A Developmental Approach

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Conditioned taste aversion (CTA) is most suitable for a developmental approach to learning and memory in rats, given its late ontogenetic emergence and its resistance to the deleterious effects of aging. We have previously demonstrated that the time of day may act as a context in CTA. Moreover, depending on subtle changes on the behavioural protocol a shift of the temporal context between preexposure, conditioning and testing allows us to explore either the context dependency of latent inhibition or the context dependence of the learned aversion. Data obtained in infantile, adolescent and aged Wistar rats have shown that each context-dependent learning phenomenon not only follows a different ontogenetic development but interact in peculiar ways depending of the developmental stage. The temporal context dependency of latent inhibition shows a late emergence at the end of adolescence and is not evident in old rats. However, the time-of-day learned an aversion is facilitated during the adolescence and in aged rats with dorsal hippocampal NMDA lesions. The results may be discussed in terms of different brain circuits' organization along early development and

aging leading to peculiar learning abilities and support a hippocampal role in the temporal context dependency of CTA latent inhibition.

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Learned Aversions to Flavours Signalling Reduction in a Nutrient

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Series of experiments in which hungry rats were given maltodextrin (M) solutions yielded inhibitory flavour learning, i.e. acquired avoidance of a flavour such as almond or saline that signals the absence of otherwise expected high concentration nutrient (20%M). Flavour aversions were acquired in Experiments 1 and 2 as a result of *explicitly unpaired* training in which sessions in which rats were given unflavoured 20%M alternated with sessions in which they were given the flavour in 2%M. Subsequently these aversions were transformed into preferences by giving further sessions in which the flavour was now mixed with 20%M. The training given in Experiment 3 consisted of *differential conditioning* in which one flavour, CS+ e.g. almond, was mixed with 20%M and a second flavour, CS- e.g. saline, was mixed with 2%M. This training produced both a conditioned preference for the CS+ flavour and aversion to the CS- flavour, relative to a *non-differential* control group that received both flavours in 2%M. These experiments appear to provide the first demonstration of learned aversions based on inhibitory learning. Its properties will be compared to those of extensively studied types of taste aversion learning. The present effects suggest an animal model of how individual humans come to dislike particular foods or flavours.

Neural Circuitry for the Formation and Maintenance of Conditioned Taste Aversion

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In a typical conditioned taste aversion (CTA) paradigm, when ingestion of a novel tastant (conditioned stimulus, CS) is followed by an internal distress caused by an intraperitoneal injection of nausea-inducing lithium chloride (unconditioned stimulus, US), animals detect and avoid the ingestion of the potentially harmful CS, and reject it when taken into the mouth with aversive behaviors. Neural mechanisms of CTA, thus, should at least be elucidated in terms of the following 3 aspects: 1) increased perceived intensity which may be helpful in detecting the harmful CS at lower concentrations, 2) aversion-formation to the CS, i.e., a hedonic shift of the potentially preferred CS from positive to negative, and 3) cautious access to the CS, i.e., a kind of contextual fear learning. Our previous and on-going studies on rats suggest that different brain circuitries are involved corresponding to each of the above 3 aspects. Among several areas where the CS is associated with the US and elicits long-term potentiated responses to the CS, the essential areas are the amygdala and the insular gustatory cortex (GC). Our previous studies showed that neurons responsible for taste- quality coding in the GC and the pontine parabrachial nucleus (PBN) showed enhanced responses to the CS after the acquisition of CTA. We suggested that the enhanced responses in the PBN were induced in part by descending influence from the central nucleus of the amygdala. The reward system consisting of the ventral tegmental area, nucleus accumbens (NAcb), ventral pallidum (VP) and the hypothalamus is suggested to play an important role in CTA formation. Our studies have suggested that the enhanced GABAergic input from the NAcb to the VP after CTA plays a role in the hedonic shift from appetitive to aversive when saccharin is used as the CS. Activation of the NAcb may be induced by inputs from the basolateral nucleus of the amygdala which shows enhanced activation to the CS after CTA. Other regions including the supramammillary and thalamic paraventricular nuclei are activated in the retrieval phase of CTA, which may be related to psychological stress and anxiety. Thus, although CTA is often referred to as one of the classical conditioning, the brain mechanism is not so simple, but involves different neural circuitries corresponding to each aspect of CTA.

Taste-Immune Associative Learning

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Pavlovian conditioning can be understood as learning about the temporal/causal relationships between external and internal stimuli to allow for the appropriate preparatory set of responses before biologically significant events occurs. In this regard, the capacity to associate a certain immune response/status with a specific stimulus seems to be of high adaptive value, and might be acquired as an evolutionary strategy in order to protect the organism and/or prepare it for danger. We have developed several conditioning protocols exploiting the naturalistic association of food/drink ingestion with its possible immune consequences. On the experimental bench, acquisition phase involves contingent pairings of a taste (i.e. conditioned stimulus) with a stimulus inducing unconditionally immune consequences (e.g. an immunomodulating drug, cytokines or antigens). At evocation time, subjects exposed just to the conditioned stimulus, display a complex repertoire of physiological responses, often affecting immune parameters in the same direction as the unconditioned stimulus normally does (i.e. conditioned immune response). Regularly, conditioned taste aversion/avoidance is displayed just after a single acquisition trial; however the conditioned effects on the immune system might not be evident until several rehearsals are applied. Current data indicate that both innate and adaptive immune responses can be modulated by Pavlovian conditioning. Recently, we have revealed fundamental principles and mechanisms underlying this particular kind of associative learning. To conclude, we propose that such behavioral approach might have clinical applications as

a supportive therapy with the aim to maximize pharmacologic therapeutic benefits, or to prevent undesired anticipatory effects.

ERKII-Dependent PSD-95 Induction in the Gustatory Cortex is Necessary for Taste Learning but Not Retrieval

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The biological process underlying the formation of long-term memories in the neocortex of the mammalian brain is poorly understood. Both screening and direct methods confirmed that induction of a synaptic protein - PSD-95 - paralleled taste learning. This learning-related induction of PSD-95, which occurred in the gustatory cortex, was temporally restricted. Induction of PSD-95 was coupled to learning of a novel taste, but not of a familiar one, it was controlled by MAP-kinase. To examine whether PSD-95 induction in the cortex was necessary for taste-memory formation, we attenuated PSD-95 expression in the gustatory cortex in vivo, by temporally and spatially restricted lentiviral RNAi knockdown. Enhanced PSD-95 levels in the gustatory cortex proved necessary for learning novel tastes, but not for recollection of known ones.

Symposium 4: Olfactory Coding in *Drosophila melanogaster* – From Receptors to Behavior I

Introductory Notes

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Research in olfactory coding in Drosophila has progressed enormously in recent years. This is due to the wealth of tools that are available in this species, be it molecular, genetic, and increasingly physiological. In addition, Drosophilids form a divergent group of species that is used for comparative evolutionary and ecological studies. Thus, research with Drosophila is shedding new light on the development, function and evolution of olfactory systems. In this symposium, we will cover the breadth of Drosophila olfaction, starting from signal transduction, including novel techniques of physiological analysis, neuroanatomy and behavior, and leading into ecology and evolution.

Function of Drosophila Olfactory Receptors

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Insect odorant receptor (OR) proteins form a unique family of 7-transmembrane proteins. They show weak sequence similarity to other GPCRs and have an inverted orientation in the plasma membrane. Insect ORs are heterodimers composed of an odour-sensitive OR protein (e.g. Or22a in *Drosophila*) and a ubiquitously expressed chaperone protein (as Or83b in *Drosophila*). Odour stimulation of Or22a and Or83b coexpressed in mammalian cells produces nonselective cation currents activated via an ionotropic and a metabotropic pathway. The activation of currents via the ionotropic pathway requires considerable higher odorant concentration than via the metabotropic pathway (>100x). Expression of Or83b alone leads to odour-insensitive ion channels that are activated by cAMP or cGMP. On the other hand, odour-stimulation of Or22a expressed alone leads to cAMP production. Thus, insect ORs act as ligand-gated channels as well as complexes of odorant sensing units and cyclic nucleotide-activated nonselective cation channels. This design provides rapid and transient as well as sensitive and prolonged odorant signaling.

Olfactory Information Processing in the *Drosophila* Antennal Lobe: Anything Goes?

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When an animal smells an odor, olfactory sensory neurons (OSN) generate an activity pattern across olfactory glomeruli of the first sensory neuropil, the insect antennal lobe (AL) or the vertebrate olfactory bulb. Here, several networks of local neurons (LN) interact with OSNs and with output neurons - insect projection neurons (PN), or vertebrate mitral/tufted cells. The extent and form of information processing taking place in these local networks has been subject of controversy. In order to investigate the role of LNs in odor information processing we have used the calcium sensor G-CaMP to perform *in vivo* recordings of odor-evoked spatio-temporal activity patterns in five genetically defined neuron populations of the antennal lobe of the fly *Drosophila melanogaster*: two distinct GABAergic LN populations, one population of cholinergic LNs, OSNs and PNs. Odor-specific and concentration dependent spatio-temporal response patterns varied between neuron populations. Activity transfer differed along the olfactory pathway for different glomerulus-odor combinations: we found cases of broadening, of linear transfer and of complex interactions. Moreover, the information content also varied across neuron populations and was maximal in PNs. Discriminatory power increased with higher odor concentrations over a wide dynamic range, but decreased at the highest concentration. These results show the complexity and diversity of odor information processing mechanisms across olfactory glomeruli in the fly AL.

Odor Elicited Oscillations in *Drosophila*

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Stimulus-evoked neural oscillatory synchronization is commonly observed in a wide and diverse range of species. Here we show that common odors at natural concentrations evoke neural oscillations in Drosophila. Upon odor stimulation, oscillations are generated by neural circuits in the antennal lobe (AL), and are transmitted downstream to the mushroom bodies (MBs), where oscillating local field potentials (LFP) can be recorded. Paired intracellular recordings from local and projection neurons within the AL revealed odor-elicited spikes and subthreshold membrane potential oscillations that were phase-locked to LFP oscillations recorded in the MBs. The oscillations were reversibly blocked by application of the GABAa receptor antagonist picrotoxin. By conditionally and reversibly blocking the chemical transmission from genetically targeted populations of AL local neurons, we identified a specific class

of widely-branching GABAergic neuron necessary for producing the oscillations.

Supported by a JSPS grant to NKT and an intramural grant from NIH-NICHD to MS

Olfactory Coding and Processing in *Drosophila*

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Most organisms rely on their olfactory system to detect and analyze chemical cues in the environment, cues which are subsequently utilized in the context of behavior. The basic layout of the first olfactory processing centers, the olfactory bulb in vertebrates and the antennal lobe in insects, is remarkably similar. Odors are encoded by specific ensembles of activated glomeruli (the structural and functional units of the bulb-lobe) in a combinatorial manner. However, a comparison of the transformation of odor representations between input to the antennal lobe and output to higher brain centers yields a complex and contradictory picture. The question of how odors are processed is accordingly open. A central problem regarding our present understanding of olfactory processing is that virtually nothing is known regarding the inhibitory components. The inhibitory processes are assumed to be as important as the well-studied excitatory pathways, however, the necessary tools to study the former processes in imaging studies have so far been lacking. In order to visualize inhibitory responses, we used a newly described fluorescent protein, named *Clomeleon*, which functions as an indicator for chloride ions — the main mediator of synaptic inhibitions in mature neurons. Using the standard GAL4-UAS system in *Drosophila melanogaster*, we genetically expressed Clomeleon in subpopulations of olfactory neurons to measure and characterize neuronal inhibitions at different processing levels in the *Drosophila* olfactory system.

How does Smell Turn into Behaviour?

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We are interested in understanding the circuit basis of olfactory perception in *Drosophila*. The strategy is to combine targeted *in vivo* physiology with high-resolution neuroanatomy in order to build up a single cell resolution model of the circuit. The organisation of the sensory periphery and its projection into the brain is already quite well described. We are therefore focussing on the central olfactory neurons. These include second order projection neurons and third order neurons of the lateral horn and mushroom body. Ultimately we hope to understand at the level of individual neurons how raw sensory information is transformed into behavioural output.

How Precise is the Peripheral Olfactory Code?

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The exact nature of the olfactory signals that arrive in the brain from the periphery, and their reproducibility, remain largely unknown. In most organisms the sheer number of olfactory sensory neurons (OSNs) makes it impossible to measure the individual responses of the whole population. We measured the individual *in situ* electrophysiological activity of OSNs in *Drosophila* larvae, in response to stimulation with 10 aliphatic odors (alcohols and esters). We studied control larvae (a total of 296 OSNs) and larvae with a single functional OSN, created using the Gal4-UAS system. Most OSNs showed consistent, precise responses, including both excitation and inhibition, in response to a given odor. Some OSNs also showed qualitatively variable responses – ''fuzzy coding''. This robust variability was an intrinsic property of these neurons: it was not due to odor type, concentration, stimulus duration, genotype or inter-individual differences, and was seen in control larvae and in larvae with one and two functional OSNs. We conclude that in *Drosophila* larvae the peripheral code combines precise coding with fuzzy, stochastic responses in which neurons show qualitative variability in their responses to a given odor. We hypothesise that fuzzy coding occurs in other organisms, is translated into differing degrees of activation of the glomeruli, and forms a key component of response variability in the first stages of olfactory processing.

Specialized Noses in the Artropod Lineage

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The olfactory system directly interfaces with the external world. Changes in the chemical makeup of the environment should accordingly also affect the olfactory system. Specialization towards a single type of resource is a potent way in which the odor landscape is changed and where we can expect the olfactory system to have been adjusted over evolutionary time. I will outline a number of ongoing projects that concern specialized olfactory systems in insects and crustaceans. The Drosophila lineage holds many interesting examples of species with rather unlikely associations and preferences. E.g. D. sechellia which solely feeds on a single species of fruit, which is highly toxic to all other drosophilids and D. endobranchia which is solely found on (and inside) gecarnoid land crabs. Both species also shows altered olfactory systems vis-à-vis their closest relatives. The land crabs themselves represents another highly advanced form of specialization, where the shift from sea to land has caused all encompassing adaptations of the olfactory system in order to operate in the radically different environment. Ongoing work in the group aims at elucidating the molecular, morphological, physiological and behavioral adaptations in the olfactory system of several species of anomuran land crabs.

Chemotaxis in *Drosophila*: From Genes to Behavior

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We are interested in understanding the integrated function of the neural circuits underpinning olfactory perception in *Drosophila* larvae. The larval olfactory system is composed of 21 peripheral olfactory sensory neurons (OSNs) expressing one, or occasionally two, specific odorant receptors along with the Or83b co-receptor. Accordingly, individual OSNs can be viewed as distinct information channels to the olfactory system. Combining genetic reengineering tools with behavioral analysis and *in vivo* electrophysiology, we have undertaken to disentangle the contribution of single, and combinations, of OSNs to the odor code.

Using calcium-imaging techniques, we studied the encoding of odor concentration at different levels of the larval olfactory circuit. After identifying subsets of OSNs that are activated by the same odor, we found physiological and behavioral evidence that different OSNs can be tuned to different concentration ranges. Our results indicate that local inter-neurons might act as a sensory filter which allows the larval brain to integrate odor intensities across a wide range of ethologically relevant concentrations. We will discuss how these recent findings clarify the mechanisms underlying the integration of multiple OSN channels within the antennal lobe.

Symposium 5: Chemoreception in the Gastrointestinal Tract

Amino Acid Consumption, Postingestive Effects, and Internal Chemoreception

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Amino acids are essential nutrients for living organisms. There are genetic differences in voluntary consumption of amino acids among mouse strains. In two-bottle preference tests, C57BL/6ByJ (B6) mice consume more L-glutamate (presented as monosodium glutamate, MSG) and glycine solutions than do 129P3/J (129) mice. These strain differences in intake could be due to differential taste responsiveness to these amino acids or due to differences in their postingestive handling. However, our analyses of gustatory neural responses and qualitative taste perception did not detect major strain differences in taste responses to these amino acids. We therefore examined next whether B6 and 129 mice differ in postingestive metabolism of L-glutamate and glycine. We found that after intragastric administration of MSG or glycine, B6 mice preferentially metabolized them through the gluconeogenesis, while a predominant process for 129 mice was the thermogenesis. This suggests that postingestive effects of amino acids affect their consumption, which must involve internal chemoreception. Our data indicate that this internal chemoreception may involve detection of amino acids themselves, their metabolites, and effects of their metabolism (e.g., temperature changes or cellular energy status), and the sites of detection could involve the intestine, liver and other internal organs.

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The EC Cell is Gut Chemo- and Mechanosensor Regulating Serotonin Release and Gastrointestinal Function

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The enterochromaffin (EC) cells of the diffuse neuroendocrine cell system are ubiquitous throughout the gastrointestinal (GI) mucosa. They exhibit apical luminal processes that function as luminal- and mechano-sensors which activate baso-lateral secretion of EC secretory products serotonin (5HT), guanylin and Subst P all of which are potent regulators of gut secretion, motility and visceral pain. Altered EC cell secretion may therefore be a key in secretory and motility alterations associated with GI function in irritable bowel syndrome, inflammatory bowel disease, as well as food allergy and intestinal infections. The agents and mechanisms by which luminal content activate EC cells is unknown. We evaluated whether EC cells act as chemo-sensors for nutrients, investigated the relationship to mechanical force and defined the EC secretory mechanisms using pure (98-99%) human EC cells and neoplastic EC cells (KRJ-I). RT-PCR identified transcripts for T2R1 (bitter), OR1G1 (class II olfactory) and trace amine (TAR1) GPCRs, and transporters for glutamine (SNAT1/2), glucose (GLUT1/3/SGLT1) and bile salts (ABST). Glutamine and Na^+-deoxycholate stimulated 5HT release (EC_{50}=0.002-0.2μM; 2-fold release) but were 10-100x more potent in KRJ-I which also secreted 6-13x more 5HT. Tastants (caffeine, tyramine, octopamine) and olfactants (thymol/eugenol) stimulated normal and neoplastic EC cell 5HT secretion (EC_{50}=1.2nM-2.1μMand 0.05nM-0.1μM release) as did 2-DG and the artificial sweetener, sucralose (EC_{50}=9.2nM and 0.38nM). 5HT secretion was associated with ERK phosphorylation (1.5-fold, $p<0.02$) and calcium influx and could be inhibited by a somatostatin analog (IC_{50}:10^{-12}M). Application of cyclic strain (computer controlled flexible-bottom well vacuum system - 10cpm, 10% radial strain) caused a time-dependent increase in 5HT release (2.5-fold, $p<0.03$) reversed by MRS1754 (0.1μM) (ADORA2B antagonist). We conclude that EC cells are gut chemosensors responsive to luminal factors including tastants, olfactants and bile salts as well as mechanical forces and regulate 5HT secretion through mechanisms that include Ca^{2+} influx and ERK phosphorylation. Luminal factors that influence EC cell neuropeptide/amine secretion may be key elements in normal gastrointestinal physiology and gastrointestinal disease.

Fat Sensing in the Gut

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Utilizing the human genome database and the receptor internalization assay based GPCR-deorphanizing strategy, we found that an

orphan GPCR, GPR120, which is abundantly expressed in intestine and adipose tissue, functions as a receptor for unsaturated long-chain free fatty acids (FFAs). We next examined the enteroendocrine cell line STC-1, which express GPR120, and showed that FFAs stimulation promotes GLP-1 secretion, ERK activation and intracellular Ca^{2+} rise in STC-1 cells. Furthermore, we found GPR120 and GLP-1 are co-localized in colon and that the stimulation of GPR120 by FFAs promotes the secretion of GLP-1 *in vivo*. These data indicate that GPR120 functions as a sensor molecule for unsaturated long-chain FFAs and can potently regulate the secretion of incretin hormone GLP-1 from the gastrointestinal tract.

Recently, GPCRs of the GPR40-43 family have been also shown to be activated by FFAs. As GPR120 and GPR40 are activated by similar properties of FFAs, and GPR40 directly and GPR120 indirectly promotes glucose-stimulated insulin secretion, both GPR120 and GPR40 will be important for assessing the mechanism of FFA-mediated nutrition regulation. Moreover, given the significance of GLP-1 in appetite and feeding control, GPR120 represents a promising new target for the treatment of obesity and other eating disorders, such as bulimia, that arise from a lack of control over-eating.

Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, Sugimoto Y, Miyazaki S, Tsujimoto G. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med. 11:90-4, 2005

Characterization of Mouse Intestinal Cells Expressing TRPM5

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The chemical nature of food in the gastrointestinal (GI) tract plays an important role in regulating the GI physiological functions, but the cells and receptors that allow the GI tract to chemically analyse its contents are largely unknown. It has been suggested that intestinal solitary cells expressing taste receptors and signalling proteins act as chemosensory cells. We labelled a population of intestinal cells that express Trpm5 with enhanced green fluorescent protein (eGFP) in transgenic mice and studied them using microarray analysis, immunohistochemistry and quantitative PCR. The findings of the study are: 1) most eGFP+ cells look like and express markers of brush cells. 2) Most proteins involved in taste signal transduction are expressed in eGFP+ cells, although some isoforms are different between the tongue and the gut (e.g. PLCβ2 in taste tissue and PLCγ2 in the intestine). 3) A subset of eGFP+ cells express synaptic markers and show close contacts with nerves. 4) Several genes that play a role in inflammation, including COX-1, COX-2 and interleukin 17 receptor B are expressed specifically in eGFP+ cells. Furthermore, these cells express the entire biosynthesis pathway of leucotriene C4, an eicosanoid also involved in modulation of intestinal smooth muscle contraction and secretion and uptake of electrolytes. 5) Angiotensinogen is plays an important role in the Trpm5 expressing cells.

Amino Acid Receptors in the Gastrointestinal Tract

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The quantity and quality of food chemicals are detected in the tongue through specialized taste receptors cells (TRCs). The processes by which we decide whether to ingest or reject a particular food depend on taste, texture, appearance, familiarity, odor, temperature and post ingestive effects. Chemosensory detection of macronutrients in the lumen of the gut seems to relay on cells and receptors similar to those described in the tongue. Thus, gustatory and gastrointestinal (GI) information converges in the brain denoting food palatability. Umami taste is a basic taste that regulates different metabolic and digestive functions. Its prototype molecule, L-glutamate (Glu), binds in the tongue to the heterodimer T1R1 and T1R3 and the metabotropic glutamate receptors (mGluRs) that are expressed also in the stomach. Amino acid sensors are widely found in the GI and especially Glu seems to modulate food digestion since directly activates gastric branch afferents of the vagus nerve. We will discuss our newest understanding of amino acid receptors in GI and their regulation of gastric function.

Symposium 6: Transduction in Chemosensory Systems of the Nasal Cavity

A Dawn for the Nano-olfactory Technology; From Theories to Applications

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Olfactory energy conversion is carried out at the sensory cilia that display nano-tube structure. Despite of its small size, this structure has so many functions involving olfaction, including odorant recognition, signal transduction, adaptation, signal amplification and possibly an olfactory masking. However, quantitative understanding of these functions is limited, mostly because of the technical difficulties accompanying nano-level biological system. A recent work in our laboratory employed a novel experimental system combining fluorescent visualization with LSM, local manipulation with ROI & caged compound and simultaneous recordings with patch clamp method. It was found that CNG channels that play a central role for the olfactory signal transduction distributed broadly to increase the quantum efficiency in the olfactory cilia. Furthermore, Cl (Ca) channels that involve in the signal amplification and persistence for ion strength changes were distributed broadly as well. Therefore, these functions of Cl (Ca) channel would be conducted homogeneously in the ORC, since transduction channels are equally situated in the cilia. By targeting CNG channels, furthermore, modulation of the system could be achieved efficiently and homogeneously along entire cilia. The local responses are amplified by the dense ion channels, displaying >100 pA current with 1 micron length cilium. This value is surprising in that only 1 pA is sufficient for ORCS to generate action potential that transmit olfactory information to the brain. And individual responses sum

independently, presumably due to the hindered diffusion of cytoplasmic soluble factors. Understanding of biophysical nature of the nanotube cilia will serve variety of possibilities for the clinical and industrial applications of the olfactory system. Furthermore, the present findings may serve possible molecular architectures to design effective agents, targeting olfactory manipulation at the nano-scale ciliary membrane.

Calcium-Activated Chloride Channels in Olfactory Transduction

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The initial steps of olfaction occur in olfactory sensory neurons (OSNs), located in the olfactory epithelium (OE) of the nasal cavity. OSNs are responsible for the detection of odorant molecules present in the environment and the generation of the neural signal that is transmitted to the brain. Odorant molecules bind to olfactory receptor proteins and this interaction triggers an increase in the ciliary concentration of cAMP, through the activation of receptor coupled G-protein and adenylate cyclase. Cyclic nucleotide gated (CNG) channels located in the ciliary membrane are directly activated by cAMP, causing a depolarizing influx of Na^+ and Ca^{2+} ions. It is well known that Ca^{2+}-activated Cl^- channels are present in the ciliary membrane and that the increase in Ca concentration inside the cilia activates a Cl^- current. OSNs maintain an unusually high internal concentration of Cl^- that is in the same range of the Cl^- concentration present in the mucus at the external side of the cilia. In physiological conditions the opening of Ca^{2+}ctivated Cl^- channels in the ciliary membrane causes an efflux of Cl^- ions from the cilia, corresponding to an inward current that further contributes to the depolarization of OSNs. However the molecular identity of the Ca^{2+}-activated Cl^- channel involved in olfactory transduction is still obscure. We investigated here the electrophysiological properties of Ca^{2+}-activated Cl^- in mouse OSNs by using inside-out membrane patches from dendritic knobs/cilia. Moreover we analyzed the temporal activation of CNG and Ca^{2+}-activated Cl^- current performing recording in intact mouse OSN in response to fast photolysis of caged cyclic nucleotides and caged calcium. These functional data will be useful for a complete molecular identification of the channel protein and its possible modulators.

Transduction in Solitary Chemoreceptor Cells of the Nasal Epithelium

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The anterior nasal epithelium contains a population of solitary chemoreceptor cells (SCCs) innervated by the trigeminal nerve. SCCs express several components of the bitter taste transduction cascade, including the T2R bitter receptors, Gα-gustducin, and the downstream signaling effectors PLCβ2 and TRPM5 (Finger et al., 2003; Lin et al., 2008). In the tongue, bitter taste transduction is used to detect potentially noxious chemicals in the oral cavity. Bitter stimuli bind T2Rs, causing activation of PLCβ2 and IP_3-mediated release of Ca^{2+} from intracellular stores, leading to activation of gustatory afferent neurons. We asked whether SCCs might serve a similar role in the nasal cavity. In this case, SCC stimulation would lead to activation of the trigeminal nerve, and initiate protective airway reflexes. To test this hypothesis, we used SCCs isolated from the nasal epithelium of transgenic mice expressing GFP from the α-gustducin promoter (Gulbransen et al., 2008). GFP-labeled SCCs were loaded with fura-2 and imaged for changes in intracellular Ca^{2+} in response to a variety of bitter compounds and trigeminal irritants. Stimulation with denatonium, an intensely bitter compound, elicited a rapid increase of intracellular Ca^{2+} in approximately 75% of the gustducin-GFP SCCs. Responses were concentration-dependent, with an EC_{50} of approximately 5 mM. Denatonium responses were inhibited by the PLC inhibitor U73122, indicating that they are mediated by activation of PLCβ2, similar to the mechanism in taste receptor cells. Interestingly, other bitter compounds, as well as several trigeminal irritants were generally ineffective in activating SCCs. To determine if activation of SCCs leads to protective airway reflexes, similar to those evoked by trigeminal irritants (Silver et al. 1990), we perfused the nasal cavity and nasopharynx of anesthetized mice with denatonium and monitored respiratory rate. Denatonium evoked respiratory apnea at the same concentration that evoked increases in intracellular Ca^{2+} in SCCs, suggesting that activation of SCCs is mediating the protective airway reflexes.

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Phosphatidyl-Inositide Signaling Proteins in a Class of Microvillar Cells

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The olfactory neuroepithelium of mammals is a highly dynamical organ. Olfactory neurons periodically degenerate by apoptosis and because of chemical or physical damage. To compensate for this loss of cells, the olfactory epithelium maintains a lifelong ability to regenerate from a pool of resident multipotent stem cells. To assure functional continuity and histological integrity of the olfactory epithelium over a period of many decades, apoptosis and regeneration require being precisely coordinated. Among the factors, which have been implicated in mediating this regulation is the neuropeptide Y. Knockout-mice that lack functional expression of this neurogenic peptide show defects in embryonic development of the olfactory epithelium and in its ability to regenerate in the adult. In postnatal olfactory epithelia, neuropeptide Y is exclusively expressed by a specific population of microvillar cells that we have recently characterized in the olfactory epithelium. These cells also co-express multiple elements of an $InsP_3$-mediated signal transduction cascade, including phospholipase C b_2 (PLC b2), type 3 inositol-1,4,5-trisphosphate receptors ($InsP_3R$-III), and type 6 transient receptor potential channels (TRPC6). Our findings allow suggesting that microvillar cells are involved in connecting apoptosis to neuronal regeneration by stimulus induced release of neurogenetic molecules.

Chemoresponsive but Non-Olfactory Microvillous Cells in the Olfactory Epithelium of Mice

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In the past, several authors reported the existence of microvillous cells within the main olfactory epithelium of rodents. These cells are scattered between the regular ciliated olfactory neurons and the supporting cells. Only some of the microvillous cells seem to project an axon to the olfactory bulb. In addition, several types of microvillous cells exist that most probably lack an axon. The function of these cells is unknown. The aim of the present study was to characterize the various types of microvillous cells in the main olfactory epithelium of mice. We utilized immunocytochemical and electron microscopic methods on wild-type mice as well as on transgenic mice. In particular, we examined the olfactory epithelium of a mouse where tau-GFP replaced the IP3R3 coding region and a mouse where GFP is driven by the TrpM5 promoter. Our results show several different types of microvillous cells that can be distinguished by morphology, ultrastructure, and expression of cell markers. Antisera against espin, a marker for microvilli, react with all microvillous cells whereas other cell markers are limited to a certain type of microvillous cell. Even within the group of either TrpM5-GFP- or IP3R3-GFP-positive cells, subpopulations exist. Neuronal markers like PGP9.5 or Hu-D do not label either of these populations. Substance P-positive nerve fibers contact some but not all IP3R3-GFP-positive cells indicating that at least some of the cells are innervated by the trigeminal nerve. We tested antisera against members of chemosensory transduction pathways. While the G-protein subunit G-alpha-q/11 is expressed in all IP3R3-GFP cells, phosphodiesterase 2 is present only in few IP3R3-GFP-positive cells. We conclude that IP3R3-GFP-positive as well as TrpM5-GFP-positive cells lack axons, i.e. are non-olfactory, but still express some markers of chemoresponsive cells.

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Sensory Processing in the Vomeronasal Organ

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The traditional distinction that the mammalian main olfactory system recognizes general odor molecules and the accessory olfactory system detects pheromones is no longer valid. The emerging view is that both systems have considerable overlap in terms of the chemical signals they detect and the effects that they mediate. A combination of large scale Ca^{2+} imaging and electrophysiological recording in wild type and gene targeted mice has afforded a comprehensive analysis of the signaling properties of vomeronasal sensory neurons. This analysis has revealed a basic framework of the neuronal processing capabilities of vomeronasal neurons. Here, I will summarize recent advances that led to our current understanding of mammalian vomeronasal sensory function.

Symposium 7: Aquatic Chemosenses

Olfactory Receptor Gene Repertoires in Fish

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The olfactory sense of vertebrates can perceive and discriminate thousands of structurally diverse odor molecules. In mammals detection of odors is accomplished by at least four different families of olfactory receptors, the OR, V1R, V2R, and TAAR genes. We have investigated the corresponding teleost families in an attempt to understand the evolutionary changes accompanying the transition from teleosts to tetrapods, i.e. from water to air environment.

The V2R-related family in teleost fish segregates in two subfamilies with opposing characteristics concerning expression pattern and phylogenetic properties. Overall family size is comparable to that of mammalian species.

A small family of six V1R-related olfactory receptors in teleosts, the *ora* genes, is highly conserved among teleost and even cartilaginous fish species, in contrast to all other known families of olfactory receptors. These genes show a peculiar pair wise arrangement in the genome and an unusual intron/exon structure. Orthologs are already found in lamprey, which makes *ora* genes the oldest recognizable olfactory receptor genes. Upon transition to tetrapods massive local gene duplication is observed, and all above-mentioned characteristics of the *ora* family are lost, consistent with a major change in function from *ora* to *v1r* genes.

Another family of olfactory receptor genes, the TAAR family, is much younger, as it appears to have originated in the common ancestor of bony and cartilaginous fishes (present in tetrapods, teleosts and shark, but absent in lamprey). TAAR1 orthologs are found in shark, but all other extant teleost *taar* genes have emerged much later, after the split between basal teleosts (zebrafish) and *neoteleostei* (stickleback, medaka, pufferfish). Teleost *taar* gene families are much larger than their mammalian counterparts and teleost *taar* genes evolve more rapidly, as evidenced by notable intron dynamics as well as extensive positive selection in some teleost *taar* genes.

Thus, very different evolutionary strategies become apparent in two olfactory receptor gene families upon the teleost/tetrapod transition.

Genetic Dissection of Olfactory Neural Circuits in Zebrafish

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Zebrafish is now becoming one of the most useful model organisms in neurobiology. In addition to its general advantageous properties (external fertilization, rapid development, transparency of embryos, etc.), the zebrafish is amenable to various genetic engineering technologies such as transgenesis, mutagenesis, gene knockdown, and transposon-mediated gene transfer. With respect to the

olfactory system, the numbers of odorant receptor genes and olfactory bulb glomeruli in zebrafish are smaller than those in mice by an order of magnitude. Thus, we can reduce the complexity of olfactory neural circuitry by using zebrafish and easily exploit the benefits of this animal model for studies of odor information reception, coding, processing, and olfactory-related behaviors. To decipher the molecular and cellular mechanisms underlying functional organization of zebrafish olfactory system, my laboratory has been applying several molecular genetic strategies including transgenesis, mutant analysis, and transposon-mediated gene trap screening. We previously succeeded in visualizing two distinct types of olfactory sensory neurons (ciliated vs. microvillous) with spectrally different fluorescent proteins in transgenic zebrafish and dissected the mutually exclusive glomerular innervations by the two types of sensory neurons (J. Neurosci. 2005). In addition, we demonstrated in BAC transgenic zebrafish that the two basic principles identified in mice, so-called ''one neuron-one receptor rule'' and ''axon convergence to target glomeruli'', are basically preserved also in the zebrafish (J. Neurosci. 2007). Furthermore, we found a crucial role of Cxcl12/Cxcr4 chemokine signaling and Robo2/Slit axon guidance system in the formation of initial olfactory axon scaffold and the establishment of a precise glomerular map (Development 2005; 2007). In this talk, I will present our new data on (1) Tol2 transposon-mediated gene trap approach to visualization and manipulation of different subpopulations of the olfactory sensory neurons and (2) visualization of mitral cell axon projection from the olfactory bulb to the higher olfactory centers in transgenic zebrafish.

Seasonal Variation in Sensitivity to Sex Pheromones in Crucian Carp

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The number of crypt cells in the olfactory epithelium of crucian carp is reduced in the winter months October-March (Hamdani *et al.,* Chem. Senses 33: 119–123, 2008). As these sensory neurones are believed to respond to sex pheromones, it is of interest to investigate if there are seasonal variations in neural responses towards sex pheromones that match the appearance of crypt cells. To this end, we performed EOG recordings in crucian carp at different times of the year upon exposure of the epithelium to L-serine (L-ser, standard), 2 preovulatory [17α,20β-dihydroxy-4-pregnen-3-one (17,20 P) and 17α,20β-dihydroxy-4-pregnen-3-one-20-sulfate (17,20 PS)] and 2 postovulatory hormonal pheromones [prostaglandin $PGF_{2\alpha}$ (PGF), and 15-keto prostaglandin $F_{2\alpha}$ (15 PGF)]. All EOG amplitudes were expressed relative to the responses to L-serine. As seen from the enclosed table (mean ± one standard deviation, n=6), all substances gave the largest responses and displayed marked peaks in EOG amplitudes compared to serine in May. However, the prostaglandins gave responses that were significantly lower than those to serine in November and March.

Substance, M	March	May	August	November
L-ser, 10^{-5}	100	100	100	100
17,20 P, 10^{-9}	146 ± 44	407 ± 92	118 ± 11	175 ± 42
17,20 PS, 10^{-9}	98 ± 9	242 ± 85	88 ± 13	202 ± 103
PGF, 10^{-6}	36 ± 5	319 ± 57	75 ± 28	61 ± 14
15 PGF, 10^{-7}	35 ± 13	135 ± 49	84 ± 7	68 ± 11

These preliminary results indicate that crypt cells respond to prostaglandins, but additional types of sensory neurones probably respond to the steroid pheromones used in the present study.

Fish Chemosenses; Olfaction, Taste, and Ligand-Specific Endocytosis

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In our present study on the fish olfactory organ and taste buds, we have developed a new method on how the olfactory receptor neurons (ORNs) and receptor cells can be visualized by ligand-specific endocytosis. The different types of ORNs are widely dispersed in the fish olfactory epithelium, yet their axons project to distinct regions in the olfactory bulb. The chemotopy of the axonal projection of sensory neurones has been demonstrated in several species, and is also reflected in the anatomical and functional division of the olfactory tracts. The olfactory neurones have a prominent endocytic activity (Bannister and Dodson, Microsc. Res. Tech. 1992, 23:128-141), and our studies on the crucian carp have demonstrated a ligand-specific induction of endocytosis. Exposing the olfactory cavity with an odorant, taurolithocholate, together with a fluorescent dye (FM1-43) give staining of a discrete number of ciliated cells with long dendrites. The process is fast and dependent upon intact microtubules as pre-exposure to nocodazole prevents staining. The sex pheromone prostaglandin stains crypt cells, and the polyamine spermidine stains microvillous cells. These studies demonstrate that it is possible to visualize the different types, the distribution, and the relative number of the sensory neurones activated by a specific odorant.

Studies of the oral taste buds in brown trout reveal ligand-specific induction of endocytosis in a few receptor cells. Stimulants and deterrents induced staining in different receptor cells.

The method applied in the present study opens for a variety of experimental avenues for studies of the organization and function of chemosensory organs.

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Identity and Function of a Potent Multi-Component Migratory Pheromone in the Sea Lamprey

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The sea lamprey, *Petromyzon marinus*, is an ancient cartilagenous vertebrate that begins its life in freshwater streams which it

eventually leaves to parasitize oceanic fishes before eventually re-entering streams to spawn and die. Individual lamprey often migrate hundreds of kilometers before locating and entering a few select streams (<5% of the streams in the Great Lakes). Nearly two decades of lab and field studies have discovered that lampreys use a larval-derived pheromone to locate these streams. The pheromone is now known to be comprised of at least three novel sulfated steroids, one of which, petromyzonamine disulfate, is derived from antimicrobial products produced in the liver and is detected at concentrations ranging below 10^{-13}Molar. Initial field work demonstrated that adult lamprey lacking a functional olfactory system are unable to locate spawning streams. Behavior studies in the lab next found the odor of spawning streams to be more attractive than that of non-spawning streams and to be distinguished by the presence of conspecific larvae. A single 1-gram larva was found capable of activating over 300 l of flowing stream water. Bioassay guided fractionation using liquid chromatography- mass spectromtry, electro-olfactorgram recording and behavioral assays of larval holding water eventually permitted isolation of three components at ~mg quantities. Nuclear magnetic resonsance spectrometry and chemical synthesis completed structure elucidation. Finally, behavioral studies in the lab and field has confimed pheromonal activity and demonstrated that this signal is perceived within a complex of stream odors. Present work focuses on developing structural analogues and suggest that all aspects of these large molecules are discerned by olfactory receptors. It will be interesting to determine if other migratory fishes employ related sulfated amino sterols and the cellular and molecular basis of their discrimination.

Specificities of Olfactory Receptor Neurons to Amino Acids in the Black Bullhead Catfish *(Ameiurus melas)*

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In vivo investigations of single teleost olfactory receptor neurons (ORNs) were previously limited to studying responses of spontaneously active cells whose activity prior to stimulation facilitated positioning of the recording electrode. The olfactory organ, however, also contains ORNs that lack spontaneous activity which have not previously been investigated. In present report we showed that their electrophysiological activity is readily observed while bathing the olfactory organ with highly purified water (HPW). The low ion concentration of the HPW reduced shunting of electrical signals thereby producing favorable environment for detecting ORN responses and, at the same time, not deleteriously affecting their function. Amino acids were used to characterize responses of the spontaneously inactive ORNs. The ORNs exhibited two types of activities: either phasic-tonic or tonic only. For the ORNs that evoked phasic-tonic responses the phasic activity was dose-dependent and contained transient frequencies up to 108 Hz lasting < 450ms. The tonic activities that followed the phasic response and tonic activities in the tonic only neurons saturated at action potential frequencies of ~18 Hz. For all spontaneously inactive ORNs, the duration of the tonic response was dose-dependent as the duration closely followed that of the suprathreshold stimulation. Response specificity of 44 spontaneously inactive ORNs was investigated with ten amino acids tested at 10^{-4}M concentration. Thirteen ORNs were excited by only one amino acid: L-norvaline, L-methionine or L-alanine. Twenty-two additional ORNs were excited by two amino acids whereas nine ORNs were excited by >2 amino acids. In 29 of 31 ORNs responding to >1 amino acid the duration of the responses to the most stimulatory amino acid (L-norvaline, L-methionine, L-valine or L-proline) was at least twice longer than responses to the second best amino acid, suggesting their narrow tuning. The cumulative number of activated spontaneously inactive cells mirrored the amplitude of EOG during stimulation with specific amino acid.

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Zebrafish and Catfish Olfactory Discrimination Capabilities Compared

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The fish taste system is narrowly tuned to few amino acids and it does not enable their discrimination. Fishes with intact olfactory systems can be conditioned to search for food after presentation of a single amino acid stimulus. To obtain stable and intense conditioned food searching responses in an entire group of fish more than 30 conditioning session are needed. During conditioning and tests sessions a small volume of amino acid solution is injected into the aquarium that is diluted with aquarium water within 5 ms 300-3000 times. Later eddies containing the amino acid are carried around aquarium in currents created by the aerations system, in ~90 seconds the amino acids contained in the eddies are diluted to the background amino acid concentration. Fishes encounter the amino acid containing eddies intermittently and consequently increase their klinokinetic food searching activity. During the conditioning sessions food rewards are given 90 seconds after the stimulus delivery. The larger swimming activity increases the chances of encountering food items. Non-conditioned amino acids also release swimming activity, however the speed of searching behavior is smaller by at least 50% in catfish and by ~ 30% in zebrafish than the conditioned swimming activities. Based on differential swimming activities fish olfactory discrimination capabilities were established. In catfish the non-conditioned amino acids provoke lesser swimming activities than the non-conditioned amino acids that are similar to the conditioned stimulus. Catfish (*Ameiurus melas* and *Ictalurus punctatus*) discriminated between most amino acids with the exception of L-isoleucine and L-valine, some catfish also did not discriminate L-alanine from L-serine and glycine. Zebrafish (*Danio rerio*) also discriminated most amino acids. The bulbar chemotopic representations for L-isoleucine and L-valine are in many individual zebrafish nearly the same (Friedrich and Korsching, 1997 and Friedrich and Laurent, 2001) which predicted that zebrafish are not able to discriminate these two amino acid stimuli. As expected from their nearly identical chemotopic representation patterns for L-phenylalanine and L-tyrosine that remain stable 2200 ms of observation time the zebrafish were not capable of discriminating these two amino acids irrespective of which of the two amino acids was the conditioned stimulus.

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Symposium 8: Neural Correlates of Learning in the Gustatory System

Synchronization of the Taste – Reward System Improves Discrimination in a Taste Guided Task

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Animals learn which foods to ingest and which to avoid. Despite much study, the electrophysiological correlates underlying this behavior at the circuit level are not well understood. To shed light on this problem, neuronal ensembles in the orbitofrontal cortex, insular cortex, amygdala, and nucleus accumbens were simultaneously recorded while rats licked for taste-cues and learned to perform a taste discrimination go/no-go task. We found that rhythmic licking at theta frequency, entrains the activity of multiple brain regions, thus demonstrating that the animal's licking acts as a ''clock signal'' against which single spikes in multiple neuronal populations can be timed and synchronized. Moreover, neurons that fired in synchrony with licking exhibited greater cue-discrimination than non-synchronized neurons and this effect significantly increased as animals learned the task. These results show that throughout the taste reward circuit, appetitive and aversive associative learning can be mediated by licking induced synchronous interactions.

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Taste Recognition Memory Induces Dendritic Accumulation of ARC Protein in the Insular Cortex

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When an animal encounters a new taste, it hesitates to eat it, showing a neophobic response. If the gastric consequences of the taste are positive (lack of illness), it becomes recognized as safe signal (increasing its consumption, Attenuation of Neophobia; AN). Arc (Activity regulated cytoskeletal associated protein) is known to accumulate in soma and dendrites of activated neurons in a robust and highly replicable way. It represents a powerful methodological tool for the studying of recently activated neuronal networks. We sought to determine whether taste-learning experience modulates the expression of Arc protein. We found a singular evolution of the expression of Arc protein on the different days of the taste familiarization process in the insular cortex. As expected, Arc was strongly expressed in the soma of pyramidal neurons of the fourth layer of the gustatory cortex 60 minutes after novel presentation of saccharin. Surprisingly, when familiar saccharin was presented for the second time, we observed a pronounced increment of dendritic localization of Arc protein as compared to the first presentation. In summary, the present results suggest that selective activation of cortical dendritic translational machinery occurs during the familiarization process of taste recognition memory.

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In Vivo Imaging of Learning Induced Plasticity in the Rat Gustatory Cortex

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Among the five senses, gustation has been largely under-studied. Yet, it is of great interest to understand how the brain processes taste stimuli, which play a key role in feeding and survival. Recently, molecular biology studies have sparked new interest in the taste field through the cloning of taste receptors. However, the neural processing occurring in the brain and especially at the cortical level is still largely unknown and subjected to debate. Using genetic tracing, it has been shown that sweet and bitter taste are processed through segregated neuronal circuitries along the gustatory pathway up to the cortical level. This is in disagreement with the evidence that gustatory cortex (GC) neurons recorded in both anaesthetized and behaving animals responded to multiple taste modalities (including sweet and bitter). To investigate the functional architecture of the GC in regard to taste modalities we used *in vivo* intrinsic optical imaging, a technique that has been successfully applied to explore the organization of other neocortical regions. We will present how the sweet modality is represented in the GC and we will compare to the bitter modality representation. We will show that the two taste modalities are represented by distinctive spatial patterns but with common territories. Interestingly, these representations are plastic. We used a conditioned taste aversion paradigm (CTA), a learning paradigm whereby one learns to avoid a taste stimulus (here a sweet taste) previously associated with visceral malaise. We showed that an internal state of malaise induces topographical plasticity of the sweet taste representation in the GC that is associated to behavioral shift of the stimulus hedonic value. We propose that general changes in internal body may be the source of some food intake disorders.

Neural Activities in the Primate Gustatory Cortices in Taste Discrimination GO/NOGO Task

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In primates, several gustatory cortices are noted. Primary gustatory cortices (PGC) receiving direct thalamic projections are areas G, 3 and probably 1-2, whereas higher-order cortices (HGC) are precentral operculum (PrCO), orbitofrontal operculum (OFO) and orbitofrontal cortex (OFC or area 12). In alert, but non-tasking monkeys, gustatory neurons can be recorded from these cortices, but they do not show features specific to cortices. We have recorded task related cortical neurons from PGC and HGC of monkeys engaged in a NaCl-water discrimination GO/NOGO task, and

found task-related neurons specific to PGC and HGC, especially in its delayed version and task reversal. In the latter, monkey presses lever during LED illumination period in response to 0.1 M NaCl (GO cue), but withholds to do so in response to water (NOGO cue), If the task is correct, he receives 0. 3 M sucrose as reward. During the task, relation between cues and behavioral responses was changed without warning (task reversal).

Two main cue responses were noted; one kept the same response pattern to a given taste cue in spite of task reversal (gustatory nature-dependent, Gr I) mainly found in PGC, and the other changed the response pattern according to consequent behavior (behavioral context-dependent, Gr III) mainly found in HGC. Among the latter, three types were noted, phasic (cue perception), sustaining (working memory like), and phasic followed by phasic responses around lever pressing. In the reward phase, responses were noted in correct trials, error trials and both. The former two types were found in PGC and HGC. Among the latter, onset type of expectation which yielded excitatory responses in both trials in spite of reward missing in incorrect trials were frequently found in HGC, and those signaling the rewarded trials or not (excitation in correct and suppression incorrect trials) were rather frequent in PGC.

Thus, PGC and HGC are concerned with different levels of the task.

Symposium 9: Talking about Taste: Linking the Views of the Terminologist, the Sensory Analyst and the Food Scientist

Language of Food Perception: A Sensory Perspective to Build a Bridge Between Semantics and Taste

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We eat and drink every day and try to express our perception about the food that we consume. We describe foods analytically and hedonically. In the former case descriptors like *sweet* or *fruity* are used. This type of language is also used to characterize food products by sensory panels, i.e. trained tasters. To clarify meanings of descriptors in panels, definitions are created and references like products or solution of single compounds (e.g. sucrose for *sweet*) are presented. Intensities of product attributes can be measured by line scales for example. Hedonic descriptions in everyday language include *tastes good* or *it is delicious*. However, for some words like *fresh* or *creamy* the meaning is often not clear and definitions are difficult to find. What exactly do we mean when we use this type of terms? That was a key question we faced in a sensory project on *freshness* of fruits and vegetables. As food scientists we approached linguists and initiated collaboration to investigate the consumer understanding of *freshness*. The combined approach from a sensory and a linguistic perspective revealed that *fresh* is a polysemous, i.e. plurivalent word that has no clear limits. It was also shown that *fresh* was often used in advertisement in the fifties and sixties of the 20th century, when processed food has become common. As an example, the vegetables pieces in powered soup were advertised as *garden-fresh* to indicate the quality of the product. When consumers were asked to write down words related to the freshness of fruits and vegetables, sensory and non-sensory words were mentioned. The sensory category was larger including mostly terms related to appearance, e.g. *good appearance*, and texture. The non-sensory category contained terms related to location, e.g. *from the garden* or temporal aspects, e.g. *harvested on the same day*. It was also shown that the terms mentioned depended on age and the access to a garden. Recently, we started a common project entitled 'Sensory Language and the Semantics of Taste' (www.sensorysemantics.com). The main objectives of the project are to investigate complex processes of taste perception verbalization and the development of a gustatory database in the German language.

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Dimensions of Meaning in Gustatory Terms

Macher Daniela, M. A.

Project SenS: "Sensory Language and the Semantics of Taste", Deutsches Seminar, University of Zurich

In German, gustatory sensations can be expressed in adjectives such as *süss*, *fruchtig*, *köstlich*, *würzig*. This contribution analyzes the dimensions of meaning of such gustatory expressions based on data gathered from a linguistic corpus of texts written in standard German, focusing on sensory and biological parameters, the relationships between adjectives and the impact of contexts in which such words are used.

The Way to the Lexical Field of Taste is Through Field Questionnaires but not only

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Language plays an essential role in the conceptualization of taste. Therefore, the linguist has something to contribute to the research on taste by exploring how language expresses its concepts. Lexical analysis, which points at the conceptualization of taste, can be backed up by questionnaires aimed at determining to what extent language influences the way speakers experience taste. Lexical analysis in French, German and English shows that taste is conceptualized differently in each language. In French, *goût* expresses an intrinsic property of the tastant that the taster only must submit to; in English, *taste* expresses the reaction of a subject to an item that is representative of an object, through which the subject can get to know the object as a whole; in German, *Geschmack* is an event, a physical interaction between taster and tastant. This sheds a new light on the conceptualization of taste: speakers may be influenced in their experience of taste by the structures of their particular language. Can taste then be seen as universal and objective by taste scientists or are they unconsciously influenced by their mother tongue? For instance, the long popular idea that there are four simple tastes rests more on the existence of four current taste descriptors, *sweet*, *bitter*, *sour* and *salt* than

on a biological basis? Can biology ignore linguistic facts? Questionnaires can help answer these questions, naive speakers and taste scientists being asked to express themselves about how they see taste. By comparing the results of linguistic analyses and field questionnaires, the linguist can determine whether and how language influences the conceptualization of taste. The linguist establishing questionnaires has to take linguistic and sociocultural aspects into account. For instance, *taste* does not only refer to a chemical sense but also, in English, to an aesthetic judgement or an inclination (a taste for art) among other things. The questionnaire has to consider this polysemy to ensure that the object is not unduly restricted to physiological taste, potentially biasing the study. Moreover, speakers of different languages experience taste in different ways, as taste has a social and cultural signification. This must also be taken into account in the questionnaires. However, if the questionnaires consider such criteria, they can be helpful to improve the results of lexical analysis. To conclude, we believe that the collaboration of linguists and physiologists could be precious and fruitful to help develop a better understanding of taste addressing all its aspects.

English and French Taste Descriptors used Metaphorically

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The English say: *have tasted blood, give someone a taste of their own medicine, have a sweet tooth*··· In French, *on fait des yeux doux, la déception est amère, la lutte est âpre*··· We look for equivalents for these expressions and define taste using a written corpus of literary and non literary quotations. A lexical comparison of the derived nouns *taster* vs *goûteur*, *tasting session* vs *séance de dégustation* and *tastant* vs *substance sapide* shows a more direct approach to taste in English, where the nouns are directly derived from the verb *taste*. The syntactic structures of *taste*, verb or noun, are different from those of *goût* and *goûter*. English is more direct when talking about food: *taste* is followed by a direct complement. In French, *sentir* is often used instead of *goûter* as a euphemism, which suggests that French-speakers are more reluctant to refer to taste directly. *Taste* collocates with *can*, like the other verbs referring to the senses. This confirms our hypothesis that *taste* is one of the prisms through which reality can be apprehended and knowledge achieved via direct experience of the world. If the focus is on the tastant, the reversible English verb *taste* is prepositional, whereas in French the noun *goût* is used in collocation with the verb *avoir*. So, English focuses on taste as a subjective experience, which is present in the transitive verb *taste* linking the taster and the tastant. On the contrary, French aims to describe the property of the food objectively, often avoiding using *goût* or *goûter* and without a direct reference to a taster (cf. Farge 2007). *Taste* can be used to refer to food, but also - and, in a literary context, mainly - to non-food. In English, adverbs in *-ly* (*sweetly, bitterly*, etc.) are directly derived from taste adjectives and more closely linked to them than the corresponding analytical expressions in French, which implies a greater distance from the central concept <goût>. According to the language spoken, inductive thought (cf. Bacon) as opposed to deductive thought (cf. Descartes), filters the perception of reality and frames the mind: *taste* draws from experience, *goût* is based on analysis. So, the concept <taste/goût> might be universal because of a common biological substrate in humans, but its conceptualisation emphasises different semantic traits: pragmatic experience leading to knowledge through senses and emotions in English *vs* intellectual analysis and judgement leading to hedonistic appreciation in French.

Communicating Taste

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Words will never be able fully to describe how an orange tastes or a clove smells. The difficulties begin when we need to be precise – in description of food sensory experiences, for example. Indeed, it is hard for a language to convey the sensation of taste. The question of how language and taste relate to each other is still not researched.

This is the reason behind "Sensory Language and the Semantics of Taste", an interdisciplinary project in which the universities of Zurich and Basle, the ETH Zurich and the Zurich University of Applied Sciences are all involved. Linguists, food sensory analysts and cognitive scientists are engaged in investigating the lexis of taste in the German language. The project, sponsored by the Gebert Rüf Stiftung, conducts basic research at the interface of sensory experiences and language and is application-oriented, working together with experts from the food industry to compile an online lexicon of taste. SenS brings together linguists, sensory analysts and psychologists to provide a broad portfolio of skills in the fields of language, sensory experiences and food marketing (www.sensorysemantics.ch).

In the Zurich University project team, we are engaged in investigating the complex processes by which language is used to convey sensations of taste. We intend to ascertain how people talk about how they experience taste. From a linguistic perspective, taste is not limited to the chemical processes involved in experiencing the taste of something. It encompasses all the various sensory perceptions involved in the ingestion of food. These include smell, aroma, trigeminal and tactile perceptions, and temperature. Based on data from focus groups with participants of different social backgrounds, the contribution focuses on ways of putting into words specific individual taste sensations and how the meanings of expressions for taste are determined.

The key thesis is that taste is situated within a dynamic triangle delineated by culture, biology and language. Whether, and to what extent, we sense a food to be fresh, creamy or spicy depends on factors such as age, sex and social origin. Individual perceptions of taste, cultural imprints and linguistic patterns of expression mutually influence one another. Social and historically conditioned means of verbal expression tend to overarch individual taste perceptions. Even when we judge taste personally, we refer to collective knowledge and to cultural standards.

Symposium 10: Structural and Functional Properties of the Olfactory Bulb

Early Events in the Development of the Olfactory Pathway

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Differentiation and maturation of the primary olfactory pathway includes the initial appearance of olfactory sensory neurons and their subsequent innervation of the presumptive olfactory bulb.

Intermediate in this process, subpopulations of cells appear to migrate out of the developing olfactory epithelium and take up residence in the mesenchyme between the epithelium and the as yet undifferentiated rostral-ventral cerebral vesicle. The population of cells is diverse and includes GNRH expressing cells targeted for the hypothalamus as well as subpopulations of GNRH negative neurons, some of which express odor receptors. The apposition of the mesenchymal neurons and the axons that begin to extend from the olfactory sensory neurons that remain resident within the epithelium, suggest that the mesenchymal cells may contribute to a scaffold mediating the initial connections between the epithelium and bulb. We have been characterizing these events and will discuss emergence of the cellular and molecular features of cells within the migratory mass and the onset of odor receptor expression.

A G-Protein/Camp Signal Cascade is Required for Axonal Convergence into Olfactory Glomeruli

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Olfactory Sensory Neurons (OSNs) are generally believed to express only one of the large numbers of odor receptor genes – 1200 in the mouse. This receptor then confers upon the cell its ligand sensitivity. It also appears to be critical in determining where the axon will end up in the olfactory bulb. Thus the expressed receptor confers a unique identity on the OSN.

In classical brain development, activity plays a crucial role in the refinement of connections between sensory input cells and targets in the CNS. However the apparently dominant role of the odor receptor (OR) in olfactory neurons has led to the notion that activity is of much less importance in forming the connections between periphery and brain in this system. The data from knockouts of the various enzymes in the transduction pathway are ambiguous.

Using a combination of *in vivo* electroporation and viral transfer of genes into olfactory neurons we have produced mosaic epithelia with loss and gain of function for several of the enzymes in the signaling pathway. Alterations in either the G-protein or the Adenylate Cyclase – the enzymes immediately upstream of cAMP production - cause significant alterations in axonal convergence and targeting. This is in contrast to deletions of the CNG channel, downstream of cAMP, which has little effect on glomerular formation. These data suggest that while activity is important, it need not be electrical activity. Although neurobiologists are partial to changes in membrane voltage it appears that biochemical activity, in this case the production of cAMP, may be sufficient for developmental refinement of axon targeting. The adage that ''neurons that fire together wire together'' may have to be revised to include neurons that react together.

Olfaction Targeted

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The main olfactory system of the mouse is a mosaic of many populations of olfactory sensory neurons. Each population expresses one allele of one of the >1000 intact odorant receptor genes. The expressed odorant receptor determines both the odorant response profile of the olfactory sensory neuron and the projection of its axon. My laboratory focuses on genetic approaches to the axonal wiring problem.

Towards a Canonical Microcircuit of the Olfactory Bulb

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The surface of the Olfactory Bulb (OB) is composed by 1800 spherical structures known as glomeruli. Each glomerulus accomplishes two main structural roles: a) it is receiving converging axons from olfactory receptor neurons (ORN) expressing the same olfactory receptor (OR); b) is interfacing the ORN axons with the Mitral and Tufted cells (M/T cells) dendrites.

M/T cells projecting the apical dendrite to the same glomerulus display synchronous sub-threshold spontaneous activity defining a way to morphologically and physiologically isolate the cellular elements of a microcircuit.

However, the information regarding the number of principal neurons in the circuit and the relations between these elements is still lacking up to date.

Multiple patch clamp electrophysiological recording coupled with anatomical staining and numerical simulation allowed us to dissect the network connectivity and to investigate the functional role.

The estimated number of M/T cells in a microcircuit is 32 (+/- 11); the probability of synaptic connection is 0.68 while the probability of electric connection is 0.64. An extraordinary range of heterogeneity characterizes the synaptic transmission. Microcircuit simulation indicates that synaptic properties are responsible for the synergistic expression of simple network operations like signal detection and amplification, while preserving memory of the previous interaction and stability of circuit dynamic.

Symposium 11: Active Sensory Modification in Olfaction and Taste

Influence of Olfaction of Natural Raw Materials on Chemical and Physiological Parameter on Human-repercussion on Situationnal Stress

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Impact of odours on human behaviour is known since many years; indeed studies have demonstrated change in human behaviour after olfactive stimulations. Pheromones and then common odorous compounds influences have been revealed by these researches.

The goal of this study is to show the influence of natural raw materials used in perfumery such as essential oils on metabolism and on volunteer's perceptions. In this way, a multifactorial approach on cardiac rhythm, cortisol and alpha amylase salivary associated with psychometric tests reveals the influence of natural raw materials on situational stress.

The Use of Sensoriality in a Fragrances Company

Célia Albertini

Sensory Analysis & Market Research, Consumer goods fragrances EMEA

Research is carried out all over the world to try to understand and gain knowledge about how taste & olfaction mechanisms work. Although there is still a lot to learn about the biological & psychological workings involved, our perfumers' expertise enables them to formulate perfumes with specific olfactory characteristics.

We will illustrate how the internal creation process of a fragrances company is organised to orchestrate odorous molecules into fragrances which will scent finished goods such as personal perfumes of course but also cosmetics, laundry detergents, air fresheners or domestic cleaning products sold on the market and how the sensory tool is used to support & guide perfumers' creativity in order to satisfy consumers' expectations.

1. Development process in a fragrances company

   a. Fragrance description: the different olfactive families
   b. Who are the actors of the creation process?
   c. Sources of inspiration

2. Use of the sensory tool
   a. Sensory analysis & descriptive data
   b. Consumer/Market research & hedonic information
   c. Sensory perception in a multidimensional approach

Smells Modulate Mood and Physiology in Menopausal Women

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The present study investigated the effect of a pleasant smell on mood and physiology of menopausal women. Forty eight 55-65 years old women were involved (24 of them applied a skin care product containing a pleasant smell (test group) and 24 applied the same product but unscented (control group)). 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 one week familiarization with the product, participants 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 one week induced a) long lasting effects on mood (decrease negative mood and stress ($p<.05$) in the test group vs. control group) and b) short term effects (decrease in anxiety and fear, $p<.05$). These effects on mood were associated with an increase in facial zygomatic activity specifically in response to S1 in the test group ($p<.03$). Taken as a whole, these results suggest that the pleasant smell of a cosmetic product contributes to the well-being in menopausal women.

Keywords: smell, familiarization, menopause, emotion.

Fragrance and Mood

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It is clear that olfaction is uniquely designed to enable the communication of Mood and Emotion. One route to communicating at an emotional level is through odour character itself. This possibility is explored using a cross-modal matching technique based on emotive images. A clear underlying structure begins to emerge.

Mental Energy Enhancement by Frozen Snack Consumption

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The objective of this methodological study was to relate three complementary approaches, brain oscillation measurement by EEG, cognitive performance assessment and mood rating for highlighting a product benefit for consumers. We selected refreshing, a benefit often used by consumers to characterize certain types of snack and beverages. Refreshing is linked to the alleviation of unpleasant physiological symptoms such as mouth dryness and thirst. Although generally linked to the concepts of energy and arousal, very little is known about the role of refreshing on mental energy. The impact of a frozen snack optimized for refreshing perception was investigated on mood, cognitive performance and related functional brain state. In the first experiment, brain oscillations were measured in 6 participants using electroencephalography (EEG) during a rapid visual information processing task assessing specifically sustained attention and also requiring working memory for its successful execution. Mood was assessed with the Bond and Lader questionnaire. Measurements were acquired before and after treatment following a randomized, balanced, three-treatment, and three-session crossover design. Treatments were a frozen snack perceived as extremely refreshing by a group of 160 consumers, a standard frozen snack from the same product range and a glass of fresh water. Results revealed that brain oscillations during visual processing in the sustained attention task were enhanced by the refreshing frozen snack as compared to the other treatments. Oscillatory brain activity was significantly increased in the theta, alpha and beta bands, known to reflect activity of multifunctional brain networks involved in alertness, attention and memory processes. However, no significant effect of treatment was found on the cognitive performance or the mood ratings. In a second experiment, 18 participants completed the same experimental design, excluding the EEG recordings. Results revealed higher alertness ratings after consumption of both the refreshing and standard frozen snack, compared to the glass of water. However, significant improvements of task performance were found only for the refreshing frozen snack, as compared to the glass of water. This latter behavioral finding obtained in 18 participants support our EEG findings obtained in 6 participants, suggesting that the enhancement of brain oscillations induced by refreshing frozen snack is in favor of optimal task performance. From this, the monitoring of brain functions with EEG seems to be highly sensitive to evidence changes in mental energy induced by snack consumption in small groups of participants. In aggregate, our findings suggest that frozen snack can impact mental

energy. Whereas an improved subjective feeling of alertness was equally induced by both frozen snacks, only the snack with optimized refreshing properties displayed a beneficial impact on brain functions and related cognitive performances.

Effect of Aroma Release Profiles on Ad Libitum Food Intake

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As a response to obesity and over consumption this project focuses on increasing the (sensory) satiation power of foods by changing their physical and chemical characteristics. Important factors that play a role in the early development of meal termination and satiety are sensory processes [1]. The effect of one of these sensory processes 'smell' has been investigated recently in a study by Ruijschop et al. [2]. They found an increase of reported satiation after longer retronasal stimulation with a strawberry aroma (compared with control condition) during consumption of a sweetened milk drink.

The present study deals with the relationship between retronasal aroma stimulation and ad libidum food intake, using savoury stimuli. In a full factorial design, during 4 separate weeks at lunch time, 40 female participants (age 18–45) were asked to consume tomato soup until they were comfortably full. At each time when the participants swallowed 10 grams of a bland soup base, tomato soup aroma was administered in the nasal cavity with an Olfactometer, giving the impression of tomato soup. Ad libidum intake and appetite profile measurements were recorded. The retronasal aroma release profiles were varied in concentration (10x higher/lower) and duration (3s and 18s) resulting in 4 different profiles. Bite size and eating rate were fixed.

Preliminary results indicate an effect between the aroma concentration of tomato soup and the ad libidum intake, which is in line with the hypotheses. Moreover in this presentation experimental pitfalls and lessons will be discussed.

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Human Whole Saliva Proteome Response to Bitterness

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Human whole saliva (HWS) refers to a mixture of salivary glands-secreted saliva, gingival crevicular fluid, oral bacteria and food debris. It is mainly composed of proteins, ions and nitrogenous products. Saliva has several physiological functions, including taste perception. However, the interaction between HWS protein composition and taste perception remains elusive, although it has been shown that HWS proteome is modified by taste stimuli, in particular by bitterness. The present study has two major objectives: to compare the changes in HWS proteome after a bitter stimulus elicited by urea or quinine, and to characterize the response over time. HWS was collected from 12 healthy adult volunteers. Each subject tasted solutions of urea (0.36 M) and quinine (40 μM), i.e. at concentrations over the human recognition threshold values reported in the literature. Subjects donated saliva before, immediately and 15 min after taste stimulus. Protein separation was done using 2D electrophoresis and image analyses were made with Same spots v.3.0. The difference in composition of at-rest saliva vs saliva after taste stimulation was evaluated using paired t-tests. Results showed that urea and quinine produced different variations of proteome pattern. In the case of urea, 39 spots showed a significant abundance change after the taste stimulus, while quinine induced a less pronounced proteome alteration as 26 spots were affected. Interestingly, only 6 significant spots were common to both molecules. Two groups of spots emerged from the analysis. The first group (21 spots for urea and 11 spots for quinine) is made of spots which abundance changed immediately after the stimulus. The second group (18 spots for urea and 15 spots for quinine) comprises spots which responded more slowly to taste stimulation, i.e. for which the abundance change appeared only at 15 min. In other words, in our conditions, urea induced a greater and relatively more rapid disturbance of the salivary proteome pattern. Protein identification will be performed by mass spectrometry to allow a complete characterization of proteins that respond to bitterness. Further work will aim to study the correlation between individual sensory perceptions and HWS proteome variability.

Symposium 12: Olfaction and Taste in Medicine

Impact of the Early Olfactory Environment on the Well-Being and Health of Preterm Newborns

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Numerous studies indicate that prematurity is associated with substantial developmental impairment in the newborn. In an effort to decrease the immediate adversities and developmental deficits associated with prematurity, specific attention has been directed to the environmental stimulations to which the premature infant is exposed. Unadapted stimulation has been shown to be detrimental to the infant well-being and development although it is not clearly known whether these deleterious effects result mainly from sensory deprivation, unpleasant stimulations or over stimulation. Inversely, the provision of appropriate stimulations appears to produce beneficial effects on the premature infant by reducing stress and pain sensation, by promoting growth and by shortening their stay in

hospital. Until now, the soundness of this approach has been principally explored in the tactile/kinaesthetic, vestibular, auditory, and visual modalities, with little attention given to the olfactory modality. Indeed, very few studies have focused on how ambient olfactory stimulation affects the health of prematurely born infants. In the rat model, exposure of caesarean delivered preterm pups to novel or irritant odors immediately after birth resulted in reduced motor activity, higher stress and higher mortality, than did familiar or pleasant odors. In the human species, it has been demonstrated that the premature newborn is highly sensitive to olfactory stimulations, and is able to discriminate and categorize hedonically-contrasted stimuli. Furthermore, it has been observed that numerous odorous products used for daily medical care (disinfectant, hydro alcoholic solutions, detergent, solvent, lubricant, pomade) have detrimental effects by increasing respiratory instability and frequency of hypoxic episodes, by disturbing sleep organization and by increasing excitability and energy loss in these infants. On the other hand, introducing pleasant odors in the incubator has been shown to be of therapeutic value, particularly for premature infants suffering from respiratory instability and apneas. Altogether, these recent studies indicate that the early olfactory environment plays a critical and non negligible role in the well-being, health and development of immature infants.

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Influence of Tastants on Characteristics of Human Parotid Saliva

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During food consumption, saliva from parotid glands is known to play a major role in the initiation of digestion. It also plays an important role in taste perception (e.g. buffering action of bicarbonate ions on acid compounds, action of proline-rich proteins on tannins). Estimating the real contribution of saliva to taste perception is difficult since its composition changes continuously partly due to changes in flow rate. It is yet unclear whether the nature of the taste affects saliva composition.

Recently, we have developed a device able to measure flow rate, pH and protein concentration in parotid saliva at the exit of the human parotid duct. In this study, we present how parotid saliva characteristics are modified after stimulation by different concentrations of various tastants such as citric acid for sourness, sucrose for sweetness, NaCl for saltiness, $MgSO_4$ for bitterness and monosodium glutamate for umami.

The complete flow rate response after stimulation was usually obtained within one minute. It was found that flow rate increases with tastant concentration. Independent of the tastant nature, a significant increase of pH was observed with increase of flow rate. Therefore, it is possible to predict pH of parotid saliva after stimulation with tastants from flow rate measurements. Protein concentration significantly decreases with flow rate but the absolute amount of excreted proteins increases with flow rate. The potential effect of the nature of the tastant on protein secretion remains unclear.

In conclusion, our results show that pH and total protein concentration of parotid saliva can be predicted from flow rate measurements. However, tastant nature effects on the exact composition of proteins or ions need further investigation.

'Sniffin' Sticks': Olfactory Performance Assessed by a New Odour Recognition Test: Reliability and Normative Data

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Several tests are available for the assessment of olfactory functions. The most consistent among them allow examining diverse aspects of olfactory system, as the ability to identify and to detect odorants and the ability to discriminate among odorants. One of the most wide spread test kit in Europe, developed to measure such olfactory capabilities is the 'Sniffin Sticks' (Hummel et al., 1997; Kobal et al., 2000). The test, however, does not comprise an odour recognition task, namely an actual measure of memory function.

The aim of the present study was therefore to develop a reliable olfactory test to evaluate olfactory memory in relation to different age groups. Accordingly, we created a 16 items multiple choice odour recognition test based on pen-like odour dispensing device. The test was applied on two test occasions to a group of 108 healthy volunteers (51 male, 57 female, range 18-95 years) divided in three main age groups (young, adults and elderly). The results indicated that the performance decreased with increasing age of the participants ($p < .0001$), especially after the age of 65 years. The test-retest correlation coefficient (Pearson r) between sessions was also highly reliable (.90). No significant differences were found for the factor 'Gender'. These data suggest that the currently presented odour memory test is suited for the assessment of olfactory memory performance.

Does Taste Stimulation Activate Cephalic Phase Insulin Release in Healthy Humans?

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In humans little is known as to whether taste solutions applied to the tongue elicit cephalic phase insulin release (CPIR). The aim of this study was to re-examine if any effect of different taste solutions on CPIR occurs. Under fasting conditions healthy human subjects sipped, and washed out their mouths with eight taste solutions (sucrose, saccharin, acetic acid, sodium chloride, quinine hydrochloride, distilled water, starch, and sodium glutamate) for 45 s and spat them out again. The taste stimuli were not swallowed; they were applied in a randomized order, each on a separate day. Blood collection for determination of plasma glucose and plasma insulin concentrations was performed 3 min before and 3, 5, 7 and 10 min

after taste stimulation. Ratings of quality, intensity and hedonic characteristics were also obtained. A significant increase of plasma insulin concentration was apparent after stimulation with sucrose and saccharin. In conclusion, the current data suggest that the sweeteners sucrose and saccharin activate a CPIR even when applied to the oral cavity only.

Masking Undesirable Effects of Ethylhexanoate on Apple Flavour

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Esters naturally appear in apple juice as contributors to the aroma. During apple maturation, subsets of these esters are produced at increasing concentration. Hence, the amounts of these esters present correlates with the perceived sweetness of apples (Poll, 1985). In line with the thinking that previously experienced taste-smell combinations transfer taste properties to the aroma, we previously identified the ester ethylhexanoate as a key-component for the sweetness of apple juice aroma that can be used to enhance sweetness for this complex food system. However, changing a familiar food's aroma to enhance sweetness may also introduce less desired off-flavors. This study aims at suppressing off-flavors introduced by increasing ethylhexanoate concentrations by altering the relative concentrations of other naturally appearing aroma components, while maintaining sweetness enhancement.

This study shows that not only sweetness is enhanced by a potent odor such as ethylhexanoate, but also aroma attributes like "flowery" and "synthetic". A compensation for these undesired aroma effects is needed to achieve a balanced product, accepted by consumers. It is shown that, by changing relative' concentrations of ethylbutanoate as well as ethyl-2-methyl-butanoate, the undesired effects of ethylhexanoate can be masked in this specific system. In addition sweetness enhancement and scores for desired attributes like "apple-like" remained at the level produced by ethylhexanoate alone. Compared to the reference, other attributes such as "sour" or "fruity" where neither affected by ethylhexanoate nor by the combination of the three esters.

This study clearly shows that changing the aroma composition of a familiar, real food can enhance taste, but may also affect the overall product perception. When using aroma-induced taste enhancement in a commercial product, compensation for undesired changes in the product is needed, while maintaining the desired taste enhancement.

Structural and Ultrastructural Characterization of a Novel Class of Cells Expressing OBP-1F in the Rat Olfactory Epithelium

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Olfaction is based on the reception of odorant molecules reaching the olfactory receptors through a thin layer of mucus, whose composition is tightly regulated. Odorant binding proteins (OBP) are one of the major proteins of mucus which participate in peri-receptors events of the olfactory message. Among the three OBP described in rat, the OBP-1F is mainly synthesized and secreted by the lateral nasal glands (LNG) and Bowman's glands (Pevsner et al., 1988). Interestingly, the expression of OBP-1F was demonstrated by both qPCR and western blot in the rat olfactory epithelium (OE) itself, and was modulated by a 48h-fasting (Badonnel et al., 2007). In the course of in situ hybridization and immunohistochemistry investigations to clarify sites for OBP-1F production, we discovered a novel class of cells. These cells were identified in discrete zones of the OE, located in the posterior area of the nose. Dispersed along the thickness of the OE, these cells revealed a globular shape of about 20μm and histological features similar to mucopolysaccharides-secreting cells commonly described in both intestinal and respiratory mucosae as goblet cells. Ultrastructural analysis by both transmission and scanning electron microscopy completed the cytoarchitectural characterization of these cells, by showing numerous droplets with a homogenous matrix structure together with an eccentric nucleus.

Our observations demonstrate the presence of a novel class of cells expressing OBP-1F in the rat OE.

Discriminating Between Organic and Psychological Determinants of Multiple Chemical Sensitivity: A Case Study

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Multiple chemical sensitivity (MCS) is a controversial disorder characterized by a diverse set of debilitating symptoms purportedly induced by environmental chemicals. Many cases of putative MCS are believed to have a strong psychogenic component, making it difficult to differentiate between organic and psychogenic causes. In this case report, we describe a procedure that can aid in this differentiation. A patient who met a strict set of criteria for MCS was tested on two test occasions. On the first, the patient was found to have no olfactory dysfunction, as determined from standardized olfactory tests. On the second, odorants, as well as a blank stimulus, were presented to the patient with instructions as to whether they were harmful or harmless. The patient's task was to estimate the intensity of each odorant and report any induced MCS-related symptoms. Thus, potentially harmful odorants presented as harmless were judged significantly less intense, and triggered less symptoms, than harmless odorants presented as harmful. When the odourless stimulus was presented as harmful, the patient provided higher intensity evaluations and exhibited more symptoms than when it was presented as harmless. This straight-forward procedure allowed us to determine that the MCS symptoms of this patient

were largely psychological and may be of general value for identifying psychogenic cases of MCS.

Evaluation of Threshold and Suprathreshold Methodologies for the Classification of Individuals as they Correlate to the Genetic Taster Status for 6-N-Propylthiouracil (Prop)

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Throughout the 20th century different theories had been developed that could explain human responses to sensory stimuli. Threshold determination and psychophysical curves were established in order to determine levels of sensitivity to different sensory stimuli. However, advancement in the field is limited by the lack of simple and reliable methods for phenotyping people based on their sensitivity to specific tastes that can be used in large-scale consumer and population studies. The present study aimed to compare the accuracy and reliability of 4 standard methods used for classification of people as taster or non-tasters based on their sensitivity to PROP (6-n-propylthiouracil). A panel consisting on equal number of tasters and non-tasters (pre-classified by means of a PTC strip) were recruited and tested for threshold and suprathreshold sensitivity of Sodium Chloride and PROP and genotyped for TAS2R38. Two threshold methods, staircase and Harris-Kalmus, were used to obtain detection and recognition thresholds. Similarly Just Noticeable Differences (JND) and Weber's fractions were determined and results were compared to intensity ratings obtained with the general Labeled Magnitude Scale (gLMS). All methods were assessed for repeatability and accuracy in clustering people as tasters and non-tasters based on subjects' sensitivity to PROP. Results show both threshold methods been able to correctly separate people into two groups of tasters and non-tasters, with the staircase method having a lower variability within subjects as compared to Harris-Kalmus. On the suprathreshold front, we found that differences in sensitivity are obtained between tasters and non-tasters using both the Weber's fractions and psychophysical curves, however our data suggest that clustering people without previous knowledge of their taster status is less accurate when using JND/Weber's fractions as several subjects were misclassified. Intensity ratings appear to be more reliable to classify people into tasters and non-tasters without previous knowledge on the subject's genetic status. Our results show that staircase and gLMS are more reliable methods than Harris-Kalmus and JND/Weber's fractions for phenotyping people based on subjects' taste sensitivity.

Characterization of the Human Lingual Epithelial Sodium Channel

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The quality of food is evaluated by our sense of taste. Of the five basic tastes, salty taste serves as a detector for sodium containing food. In rodents, salty taste involves at least two transduction mechanisms. One is sensitive to the drug amiloride and specific for Na^+ and likely involves a tetrameric epithelial sodium channel, ENaC. Another one is amiloride-insensitive and triggered by various cations. In humans, the molecular mechanism of salty taste perception is even less clear. Here, we examine a potential role of ENaC in human salt taste perception.

First, we determined that several but one compounds that enhance the saltiness of sodium chloride solutions in sensory studies of human subjects also enhance ENaC-mediated sodium currents in *Xenopus* oocytes expressing recombinant ENaC channels. A structurally similar compound that failed to enhance saltiness did also not enhance ENaC-mediated sodium currents.

Next, we analyzed the presence of ENaC in taste tissue by RT-PCR, *in situ* hybridization and immunohistochemistry. By RT-PCR we detected mRNAs for all ENaC subunits in circumvallate and fungiform papillae in the rank order $\alpha \sim \beta > \gamma \gg > \delta$. By *in situ* hybridization, with the exception of the alpha-subunit in circumvallate papillae, we did not detect ENaC mRNAs in taste cells. These were exclusively observed in non-taste epithelial cells. Using specific antisera we detected all ENaC subunits in subsets of taste cells. Beta-, gamma-, and delta-ENaC-like immunreactivity was found in circumvallate and fungiform papillae, whereas alpha-ENaC immunreactivity was restricted to circumvallate papillae. Delta-ENaC was exclusively seen in taste pores. The data show that ENaC mRNAs are short-lived and that the subunit composition of ENaC remains to be elucidated. Together our data suggest that ENaC may have a role in taste transduction.

Symposium 13: The Solitary Nucleus: Taste Implications

Features Reflecting Organization in the Solitary Nucleus: Implications for Taste

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The morphologic basis organizing the passage of taste information from primary afferent, gustatory ganglion cells to second-order neurons in the nucleus of the solitary tract (NTS), as well as ways in which cells in subnuclei of NTS may be organized for 'taste'-mediated behaviors, remain intriguingly moot issues. The aim of this symposium is to reflect on some of the following: (1) What are the potential 'circuit' connections from geniculate ganglion somata to subnuclei in NTS, and other non-NTS structures? (2) What role(s) do cells of an NTS subnucleus play with respect to their receptive field character, breadth of tuning, and connectivity with taste discriminative, nongustatory viscerosensory, and/or oromotor circuitry? (3) Does genetic manipulation of a putative taste receptor result in changes in the tuning of cells in subnuclei of NTS versus

that in a wild type strain? (4) Does breadth of tuning of an NTS subnucleus cell reflect its afferent feedback from, or efferent projection to intranuclear (i.e., within NTS), local medullary, or supramedullary structures? (5) To what degree are features of NTS cells changed by the time line chosen to define cell responsiveness?

Types of Taste Circuits Synaptically Linked to a Few Geniculate Ganglion Neurons

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The present study evaluates the central circuits that are synaptically engaged by very small subsets of the total population of geniculate ganglion cells to test the hypothesis that taste ganglion cells are heterogeneous in terms of their central connections. We used transsynaptic anterograde pseudorabies virus labeling of fungiform taste papillae to infect single or small numbers of geniculate ganglion cells, together with the central neurons they connect with, to define differential patterns of synaptically linked neurons in the taste pathway. Labeled brain cells were localized within known gustatory regions, including the rostral central subdivision (RC) of the nucleus of the solitary tract (NST), the primary site of geniculate input and of output cells projecting as the ascending taste pathway to the parabrachial nucleus (PBN) of the pons. Cells were also located in the rostral lateral NST subdivision (RL), a site of trigeminal and sparse geniculate input, and the ventral NST (V) and medullary reticular formation (RF), a caudal brainstem pathway leading to reflexive oromotor functions. Comparisons between cases, each with a random, very small subset of labeled geniculate neurons, revealed ''types'' of central neural circuits consistent with a differential engagement of either the ascending or the local, intramedullary pathway by different classes of ganglion cells. We conclude that taste ganglion cells are heterogeneous in terms of their central connectivity, some engaging, predominantly, the ascending ''lemniscal'', taste pathway, a circuit associated with higher-order discriminative and homeostatic functions, others engaging the ''local'', intramedullary ''reflex'' circuit that mediates ingestion and rejection oromotor behaviors.

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Receptive Fields and Brainstem Taste Processing

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Spatially segregated oral taste bud groups transmit signals to the nucleus of the solitary tract (NST) via the VIIth or IXth cranial nerves. The NST distributes information to local reflex pathways and, in rodents, to the parabrachial nucleus (PBN), where forebrain projections arise. Previous studies have demonstrated distinctive sensitivities and functions for the VIIth and IXth nerves but less is known about differences in central processing. We used neurophysiology to address this question in the rat brainstem. In NST, taste signals arising from separated taste bud groups was common, but restricted mainly to either the anterior *or* posterior mouth, i.e., from the nasoincisor ducts and anterior tongue or soft palate and foliate papillae, giving rise to a strong orotopic organization. Further, neurons with VIIth nerve, anterior mouth receptive fields were more likely to respond with short latency, high frequency, selective sodium responses; those with posterior mouth, IXth nerve receptive fields to exhibit long latency, low frequency, selective responses to bitter tastants. Posterior-responsive neurons often lacked PBN projections suggesting a major role in local reflex circuits, a hypothesis supported by IXth nerve lesion effects (J. Travers and Norgren, 1987). Neurophysiology and Fos immunostaining both support a medial chemotopy for NST bitter responses but Fos expression peaked medial to the locations of bitter-selective cells detected by recording. Interestingly, microstimulation demonstrated that the *non*-Fos expressing region was more effective for eliciting oromotor responses mimicking the bitter-induced rejection response, implying a distinctive function for the far medial ''bitter-Fos hotspot. Surprisingly, many PBN neurons responded to posterior tongue taste stimulation despite the higher proportion of NST pontine projection neurons with anterior receptive fields. Further, compared to NST, more PBN cells, including bitter-selective neurons, had mixed anterior + posterior receptive fields. The ample PBN representation of the posterior mouth suggests that the functions of IXth nerve-derived taste signals extend beyond reflexive behaviors. Previous experiments have shown that the bitter-induced suppression of voluntary consumption is more severely disrupted when both, rather than either, the VIIth and IXth nerve are sectioned (St. John et al., '94). It is tempting to speculate that these convergent, higher-order, bitter-selective neurons provide inputs to forebrain circuits controlling such behaviors. Supported by NIH DC00416.

Connecting the T1R3 Taste Receptor to NTS: The Neural Processing of Sweet Taste

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Molecular studies have identified a class of taste receptor, known as T1r that mediates attractive responses to appetitive taste stimuli. Understanding the mechanism(s) of how T1r receptors give rise to behavior will require knowing how T1r-mediated taste information is processed by circuitry in the CNS. Our lab has explored how input from T1r3-containing taste receptors is received by neurons in nucleus tractus solitarius (NTS), the first central synapse for taste. T1r3 is a critical subunit of T1r receptors involved in the detection of pleasurable sweet-tasting stimuli (Damak et al. 2003; Zhao et al. 2003). Genomic and behavioral data raise the possibility of multiple taste receptors for sweets (Damak et al. 2003; Delay et al. 2006; Inoue et al. 2007), obfuscating exactly how T1r3 sweet input might be registered centrally. We have made electrophysiological recordings from 82 single NTS neurons in anesthetized T1r3 knockout (KO) mice (Damak et al. 2003), which carry a non-functional T1r3 allele, and C57BL/6 wild-type (WT) mice. Taste responses to a wide range of sweet stimuli (sugars, artificial sweets, sweet amino acids) and tastants of other qualities

(salty, sour, bitter, and glutamate) were measured. Cells (41 WT, 41 KO) were compared between genotype groups to deduce how NTS neurons process T1r3-mediated information about sweet tastants. Responses to all sweets were reduced in KO cells ($P < .05$). WT and KO neurons were sorted using an algorithm that identified WT cells with sweet response characteristics that did not emerge among KOs. Such WT cells would logically depend on input from T1r3 taste receptors. A statistical signal processing analysis was applied to each T1r3-dependent neuron to quantify its accuracy for discriminating sweet from non-sweet tastants, as based on response variance to a sizable number of stimuli. This model bears on whether a T1r3-receiving neuron could serve as a reliable indicator of sweet taste. Many of these neurons showed good accuracy for signaling sweet stimuli, although a portion did not. Thus, input from T1r3 sweet taste receptors is received by NTS cells with variable selectivities towards sweets. These findings have implications for how appetitive gustatory information from T1r receptors could be represented at the first central processing center for taste.

The Synaptic Interface Between the Rostral Solitary Nucleus and the Subjacent Reticular Formation

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Anatomical and behavioral pharmacological studies suggest that projections from the rostral (gustatory) nucleus of the solitary tract (rNST) to the subjacent reticular formation (RF) form part of a neural pathway for organizing the behavioral switch between oromotor patterns of ingestion (licking) and rejection (gaping) based on taste. In order to analyze the synaptic interface between the rNST and the subjacent RF, we used whole-cell patch recording to describe responses of identified pre-hypoglossal interneurons to electrical stimulation of the rNST in a neonatal slice study. This interface consisted of a small monosynaptic glutamatergic excitatory pathway and a larger polysynaptic pathway that was both excitatory and inhibitory. Some RF interneurons received both excitatory and inhibitory input from the rNST and provided evidence for a disinhibitory pathway from the rNST to the RF. Further evidence for disinhibition within this network was provided by a parallel set of calcium imaging studies in which blocking $GABA_A$ receptors increased the calcium response from identified RF neurons in response to rNST stimulation. Based on behavioral pharmacology, disinhibition has been proposed as one mechanism by which bitter stimuli can produce gapes. We further demonstrated that the excitatory and inhibitory synaptic interface between the rNST and the RF could dynamically drive a 3 neuron Hodgkin-Huxley model network of tongue protrudor, tongue retractor, and jaw-opener interneurons to mimic the EMG patterns of ingestion and rejection.

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Histochemical Changes and Apoptosis in Taste Buds of Rat Circumvallate Papilla Following Axotomy of Glossopharyngeal Nerve

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It is known that maintenance of mammalian taste buds is highly dependent on gustatory innervation – denervation of gustatory nerve cause the degeneration of taste buds. Although many studies have focused on histochemical properties of taste buds under normal condition, it is little known on the histochemical changes of taste buds following denervation. The present study was designed to examine the changes in histochemical properties of taste buds following gustatory denervation. Bilateral axotomy of glossopharyngeal nerve (nIX) was applied to Sprague-Dawley rats. Animals were killed 1, 2, 3, 4, 6 and 8 days following nIX axotomy, and circumvallate papilla (CVP) was labeled by specific antibody against phospholipase C β2 (PLCβ2; a marker for type II cells), neural cell adhesion molecule (NCAM; a marker for type III cells) or Jacalin (a marker for type IV cells). Apoptosis was also detected by immunohistochemistry of single stranded DNA (ssDNA). Under normal condition, approximately 15%, 8% and 7% of taste bud cells were labeled with PLCb2 (type II), NCAM (type III) and Jacalin (type IV), respectively. Apoptosis (ssDNA) was detected 1.6% of taste bud cells – approximately 20% of ssDNA cells showed immunoreactivity for PLCβ2, and rest of them were not labeled with NCAM or Jacalin. Following nIX axotomy, number of taste buds in CVP was not changed by 3 days post-injury (PO3), but number of taste bud cells per taste bud significantly decreased from PO1. Percentage of apoptotic cells (ssDNA cells) increased 2 folds from PO1, and all types of taste bud cells showed ssDNA immunoreactivity. Jacalin-labeled cells, i.e. type IV cells, disappeared by PO3. The present results indicate that gustatory nerve denervation induces the apoptosis in all types of taste bud cells, resulting rapid decrease of taste buds cells per taste bud.

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Brainstem Reflex Systems from Vagal Taste Nuclei

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The sense of taste is used in two ways: first, to discriminate the tasty from the toxic, and second, to trigger corresponding oropharyngeal reflexes, swallowing and gagging. These oropharyngeal reflexes are driven by brainstem neuronal networks that activate cranial motor nuclei, especially the nuc. ambiguus which controls pharyngeal musculature. The close functional linkage between vagal gustatory input systems and oropharyngeal motor networks is a unique feature of gustatory inputs arising from posterior taste fields of the oropharynx.

Gustatory control of pharyngeal musculature is especially prominent in the goldfish, *Carassius auratus*. The oropharynx in this species is specialized to permit intraoral sorting of palatable from inedible substances. The afferent limb begins with the vagus nerve which innervates tens of thousands of taste buds spread across the roof and floor of the pharynx. The neuronal machinery for the reflex system is incorporated into the vagal lobe which is an enlargement

of the dorsal medulla containing elements of both the nuc. of the solitary tract and the nuc. ambiguus.

The vagal lobe is a laminated structure with gustatory inputs ending superficially and motor output arising from deeply situated motoneurons. Reflex interneurons bridge the layer of white matter which separates the sensory and motor layers. The anatomy of the vagal lobe is conducive to investigation of the circuitry and neurotransmitters in slice preparations. Our electrophysiological studies have established that the primary gustatory afferents utilize glutamate as a neurotransmitter acting on both NMDA and AMPA/kainate type postsynaptic ionotropic receptors. In addition, presynaptic Group III (mGluR 4 & 8) metabotropic glutamate receptors serve to modulate transmission at the primary afferent synapse.

Functional imaging studies show that the reflex interneurons also utilize glutamate as neurotransmitter and synapse onto GABAergic interneurons of the motor layer as well as onto the ambigual motoneurons themselves. Thus glutamatergic neurotransmission – both at the primary afferent terminal and for the reflex interneuron system – is crucial for gustatory-triggered deglutition.

Symposium 14: Intracellular Stimulation by Membrane-Permeating Tastants that May Affect Signal Transduction

Intracellular pH Signalling in Sour Taste

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A decrease in intracellular pH (pH_i) of taste receptor cells (TRCs) is the proximate signal for sour taste transduction. The supporting evidence for this hypothesis will be presented using chorda tympani (CT) taste nerve recordings in rodents and measurement of pH_i changes in TRCs in polarized fungiform taste buds with fluorescence imaging techniques. For both fully dissociated strong acids and weak organic acids the CT response shows a good correlation with a decrease in TRC pH_i for HCl, citric acid, acetic acid and CO_2. Inhibiting CO_2-induced decrease in pH_i using membrane permeable blockers of carbonic anhydrases inhibited CT response to CO_2. Altering the activity of basolateral Na^+/H^+ exchanger, a major pH regulatory mechanism in TRCs, modulates the tonic part of the CT response to acids. Inhibiting the pH_i-induced decrease in TRC volume changes by hypertonic mannitol or altering the F/G actin ratio in TRC cytoskeleton modifies the phasic CT response to acids. Introducing an artificial H^+ entry pathway by incorporating nigericin, a K^+-H^+ exchanger, in the apical membrane of TRCs shifts the pH threshold at which a CT response is observed by approximately 2 pH units. These studies provide strong support for the hypothesis that a decrease in pH_i is necessary for eliciting a CT response to sour stimuli.

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Water Movement Through Aquaporins in Taste Cells – A Role in Osmotic Sensing

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The peripheral gustatory system plays a crucial role in identifying nutrients. As a consequence, this system shows plasticity and appears to be modulated by the underlying nutritional state of the organism in order to help maintain or restore important homeostatic processes such as salt and water balance. Modulation of taste cell responses is evident from studies which have shown that natriferic hormones including aldosterone, vasopressin and insulin alter responses to NaCl. In other transporting epithelia, in particular the kidney, these same hormones are also involved in altering water movement through aquaporin channels. Because of the many parallels we have observed between kidney and taste cells for NaCl transport, we are currently investigating how taste cells respond to water and then how the endocrine system may serve to regulate osmotic sensing in the peripheral taste system. Data will be presented showing how rodent taste cells respond to hypoosmotic solutions using functional assays as well as behavioral studies demonstrating the animal's ability to detect and respond to changing solution osmolarity. The most likely candidate for the initial transduction of hypoosmotic stimuli is rapid water entry into taste cells through aquaporin channels and subsequent activation of a stretch-activated Cl^- channel. Molecular and immunological evidence for the expression of aquaporin channels in taste cells will be discussed including functional data showing the importance of water movement through these channels for the taste cell response to non-isoosmotic stimuli. Supported by NIH DC007239 (KJW) and DC002507 (TAG).

Some Membrane-Permeant Sweet and Bitter Tastants Delay the Desensitization of G-Protein Coupled Receptors

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Sweet and bitter tastants stimulate the G protein-coupled taste receptors (GPCRs) *T1R*/*T2R*s, e.g., (1). In addition, some sweet and bitter tastants rapidly permeate into isolated taste-bud cells *in situ* (2), into taste-bud cells *in vivo* (4) and into various epithelial cells *ex vivo* (3). Interestingly, in the tube the same tastants inhibited the phosphorylation of rhodopsin induced by GPRC kinases (GRK) and protein kinase A (PKA) *in vitro* (4). These results propose that membrane-permeat tastants can potentially interfere with the desensitization of GPCRs by interacting with these signal termination-related kinases. Furthermore, they may have implications to the lingering aftertaste produced in humans after tasting such tastants. To further investigate this hypothesis directly, we recently used intact HeLa cells over expressed with the β2-adrenergic GPCR (β_2AR) as a prototype for the mechanisms of GPCRs desensitization (Shaul ME, Peri I, Malach E, Huang L, Spielman AI, Seger R, Naim M, unpublished data). We preincubated β_2AR-transfected HeLa cells with membrane-permeant tastants (saccharin, NHD,

D-tryptophan, naringin, quinine, caffeine) and found that: (i) isoproterenol (ISO)-stimulated cAMP formation is enhanced; (ii) this enhancement depends on time of preincubation and tastant concentration; (iii) this enhancement is independent of phosphodiesterase (PDE) and PKA activities; (iv) ISO-induced phosphorylation of β_2ARs as well as β_2AR internalization are delayed. Thus, membrane-permeant tastants inhibit signal-termination-related kinases intracellularly to delay β_2AR desensitization *ex vivo*, and this is likely to be relevant to the desensitization of other GPCRs whose desensitization involve GRKs. Supported by the US-Israel Binational Science Foundation, BSF-2003015.

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Artificial Sweeteners and Metallic Taste: Role of TRP Channel

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Many compounds have taste perceptions that change with concentration. Among them are the artificial sweeteners, divalent salts, such as FeSO4 and ZnSO4, and nicotine. All these molecules share the property that they can diffuse into cells and activate or inhibit ion channels. We will present studies regarding how one or more of these compounds affect TRPV1, TRPA1 and voltage gated sodium channels.

Symposium 15: New Approaches to Human Chemosensory Variation

Odorant Receptor Polymorphisms and Olfactory Perception

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Deciphering peripheral olfactory encoding requires a description of the ligands that activate each odorant receptor (OR). In mammalian systems, however, ligands are known for only a handful of over 1000 ORs, greatly limiting our understanding of olfactory coding. We performed high-throughput screening of ligands against a large repertoire of mouse and human ORs expressed in heterologous cells. Using interaction profiles of excitatory ligands for 52 mouse and 10 human ORs, we developed a predictive model relating physicochemical odorant properties, OR sequences, and their interactions. Our model can predict a novel OR's response to a tested odorant and a tested OR's response to a novel odorant.

Does a mutation of an OR change the perception to the odorants that activate the OR? Sensory variation in bitter taste and color perception has been related to mutations in sensory receptors. However, given the proposed combinatorial coding mechanism of ORs and the large number of ORs in the genome, it was not clear *a priori* that mutations in a single OR would be detectable as perceptual phenotypes. We have demonstrated the link between specific polymorphisms in a single OR gene, human *OR7D4* and altered perception of its cognate ligands, androstenone and androstadienone that activate this receptor. Our data support a model in which *OR7D4* activation partially contributes to the offensive odor quality of androstenone, such that subjects lacking the functional variant of this receptor are less likely to find this odor unpleasant. OR7D4 may function in parallel with one or more other ORs to produce the "complete" sensation of androstenone and androstadienone.

Phenotype Genotype Relationship in Human Taste

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Human subjects exhibit high inter-individual differences of sensitivity at threshold and supra-threshold levels. Statistical evaluation of individual sensitivities to tastants shows that no tastant is identical to any other one and that each subject presents his/her own specific taste quantitative spectrum. Among a series of tastants, some present a peculiar interest, e.g.: monosodium glutamate (MSG or NaGlu) shows a 500 fold variation for threshold concentrations across Subjects and for concentrations individually perceived isointense to NaCl 29 mM (Lugaz et al., 2002). 3000 French subjects were *screened* for their sensitivity to monosodium glutamate, among them, 302 (163 genetically independent) were selected to increase the relative proportion of non-tasters and participated in this phenotype–genotype study. *Phenotyping* included a series of psychophysical tests, namely: threshold determination; measurement of concentrations iso-intense to NaCl 29 mM, Time-Intensity evaluation of 29 mM NaCl and NaGlu; 30 repeated triangular tests for discrimination, a ranking test and a questionnaire on the difference of perceived quality, intensity, persistence, preference for NaCl and NaGlu, as well a tentative semantic description. Subjects could be classified as non-tasters, hypo-tasters and tasters.

For the genetic study we used 80 fungiform papillae collected from 20 subjects to investigate the candidate receptors Tas1R1, Tas1R3, mGluR4 and mGluR1 genes by RT-PCR and immunohistochemistry. Additionally, bucal swabs were collected from 258 subjects. Three non-synonymous single nucleotide polymorphisms (nsSNPs) were found in the coding region of Tas1R1 and 3 in Tas1R3 as well as 3 in mGluR1. The prevalence of these nsSNPs was low within this French population and similar to results of Kim et al. (2006). The prevalence of 3 of these 9 nsSNPs was significantly different between tasters and non-/ hypo-tasters, confirming the involvement of these receptors in signalling MSG. However, these SNPs are neither necessary nor sufficient to explain the non-taster/taster trait at individual level and do not explain the total variance of the data. It seems thus necessary to consider that other protein receptors or

mechanisms contribute and cooperate to the elaboration of the specific taste of glutamate.

The Unusual Diversity of the Human Olfactory Subgenome

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We have reported a widespread existence of segregating pseudogenes in the human olfactory receptor (OR) subgenome. These are loci which have two alleles, functionally-intact and disrupted by stop-codon-generating single nucleotide polymorphism. Such loci generate an ''olfactory barcode'' that leads to a different functional nose for every human individual. We have recently uncovered a significant association between one segregating pseudogene (OR11H7P) and sensitivity to the odorant isovaleric acid (PLoS Biol. 2007). Copy-number variants (CNVs) are another form of genetic variation, constituting deletions and duplications of DNA segments. CNVs are responsible for a large fraction of the genome variation in mammals. We hypothesized that individual differences in human olfaction may be further contributed to by CNVs. To help elucidate the impact of CNVs on the evolution and function of human ORs, we have generated a high-resolution CNV map of OR genes, employing high-resolution oligonucleotide tiling microarrays for 25 individuals from three ethnic populations. We identified 93 OR gene loci affected by CNVs, generating an unexpectedly complex mosaic of OR dosages across persons. We find that ''young'' ORs, evolutionary generated since the human-chimpanzee split, have more CNVs, showing that CNVs are an important part of the gene-birth process that led to a huge olfactory repertoire. Importantly, we find by quantitative PCR corroboration, that numerous OR loci are completely deleted, and that 13 ORs show loss of both gene copies (homozygous null) in some persons. This implies individual functional ''holes'' in the human chemosensory repertoire. All these OR deletions do not appear in chimpanzee, further supporting the notion of a fast-shrinking olfactory repertoire in humans. Some of the deletions represent pairs of OR genes that have undergone recombination. This results in fused genes that may harbor novel odorant-binding properties in some individuals. Future studies will address the specific chemosensory impact of such genomic variation map.

Molecular Genetics of Human Bitter Taste

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Humans, like other mammals, sample information about the chemical properties of their food through the sense of taste. Each of the five basic taste qualities sweet, umami, bitter, sour, and salty, fulfils a separate physiological function. Bitter taste is considered to be part of a warning system preventing intoxication by poisonous bitter compounds. Humans possess 25 *TAS2R* genes that encode G protein-coupled receptors for the detection of bitter chemicals. TAS2Rs appear to be glycosylated at a conserved site in extracellular loop II. The carbohydrate moiety mediates interaction with chaperones required for receptor functions. TASRs are exported to the cell surface by interacting with specific auxiliary proteins. They display broad ligand spectra, each receptor detecting numerous bitter compounds explaining humans' ability to perceive Thousands of bitter chemicals with only 25 receptors. TAS2R oligomerization does not seem to contribute to this ability. Bitter molecules interact, on the tongue and other parts of the oral cavity, with specialized receptor cells that are assembled into morphologically distinct structures called taste buds. The receptor cells tuned to detect bitter stimuli form a heterogeneous population of cells characterized, on average, by a set of seven out of 25 bitter taste receptors that they express. They use definite molecules for signal transduction, processing and output. In this scenario, bitter taste receptor proteins represent the devices that convert chemical structures into sensations and, through their biochemical properties, determine sensory sensitivities of subjects. They occur as genetically determined variants creating perceptual diversity in the human population. Excitation of bitter taste cells elicits nerve impulses that are conveyed to the cerebral cortex, where the activities of nerve cells represent information about the chemical content of the oral cavity. This information is integrated with information about other taste qualities as well as visceral and other information about food in the context of nutrition. Metabolic consequences of ingested food form taste memory traces that determine future handling of known and novel foods. The molecular evolution of TAS2Rs suggests that bitter taste receptors shaped nutrition and impacted on human health.

RNA Profiling Analysis of Rat Nasal Epithelium and Olfactory Bulbs

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Gene cloning and *in silico* mining of a number of mammalian genome sequences have identified between some 600 OR genes in the human genome to up 1500 OR genes in the rat genome. As such they constitute by far the largest gene family embedded in a mammalian genome. Of these a substantial percentage that varies between 60% in human down to around 20% for rodents and dog were identified as pseudogenes, i.e. genes in which a number of mutations closing their reading frames happened, leaving the remaining part as possibly active genes.

In order to approach their function and involvement in olfaction we have undertaken a series of RNA profiling analysis of the nasal epithelium and olfactory bulb of Brown Norway rats. Animals, three week old, were purchased from different companies and maintained from different period of time (up to 22 months) in the animal house. During this period they were subjected or not to different odorants under various conditioning conditions before to be sacrificed. Nasal epithelium and olfactory bulbs were quickly removed and RNA extracted. RNA profiling analyses were done by hybridization onto whole genome Agilent rat 44K micro-arrays and /or semi quantitative PCR.

The main results that will further be discussed were as follows.

–Rats of the same age and those obtained from two different sources and thus potentially exposed to slightly different environment conditions gave a nearly but not quite identical RNA profiling results.

–Two-third of the transcripts analyzed with the 44K micro-arrays and 50% of the 1300 OR genes for which there are probes onto the arrays, gave a signal above background.

–The most highly expressed OR mRNA is 250 times more abundant than the least expressed OR mRNA and represents 2% of Gαolf mRNA.

–A good correlation existed in the RNA expression profile of the two tissues

–Depending upon experimental conditions, exposure to odorant may induce either an increase or a decrease of the transcription of the corresponding OR genes in the nasal epithelium but not in the olfactory bulb in which the transcription rate stayed unchanged.

Symposium 16: From Taste Stimulus to Nerve Signals

In Search of Natural Taste Stimuli

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Discoveries of taste receptors are milestones for understanding the natural history of taste perception. The receptors, located in taste buds of the oral cavity, detect a variety of chemicals ranging from salts, sugars, alkaloids, amino acids and a diverse range of aversive substances. Many chemicals with strong tastes do not occur naturally or occur only in restricted environments and it is increasingly apparent that there are large species differences in taste perception. Also, there are clear regional differences in oral sensitivity to taste stimuli, in part related to presence of reflex as well as sensory afferents in taste nerves. This diversity makes it difficult to formulate inferences about molecular mechanisms. Carbohydrates, which naturally occur mostly as polysaccharides, are important constituents of foods with little taste that must be broken down to simple sugars, unless sugars themselves are present. Likewise, proteins are not genuine taste stimuli but must be broken down to amino acids, which may not occur in detectable amounts. T1R receptors detect sugars and amino acids, but there are large species differences. Of concern regarding food is how a naturally avoided component is rendered palatable in mixtures with attractive components or vice versa. Evidence is presented on the effect of brief selective adaptation to some components on identification of stimuli in humans; and on suppression of hamster neural responses to sugars by bitter stimuli and bitter stimuli by sodium chloride. T2R receptors detect bitter stimuli. Denatonium is sensed at 10^{-8} M by humans. However, denatonium in not a natural stimulus, which raises questions about the receptor activated and its natural stimulus; the receptor is not likely present in rodents and many other mammals that are orders of magnitude less sensitive to denatonium. In contrast, cycloheximide is aversive to rodents at 10^{-5} M but tasteless to humans at much higher levels. If cycloheximide, a potent naturally occurring antibiotic, were not experimentally exploited to inhibit protein synthesis, its aversive quality and ecological significance may not yet have been discovered. Yet salicin, an alcoholic glycoside found in willow bark, is bitter to diverse species. Salicin, as a bitter stimulus in hamsters, is compared with other stimuli, including denatonium and cycloheximide. To conclude, concordance/discordance between taste stimulus chemistries for candidate taste receptors and classes of rodent chorda tympani neurons is presented.

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P2 and mACh Receptors on Taste Bud Cells

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About 50 taste bud cells and many taste nerve endings form single taste buds. Taste bud cells are classified into four groups, type I to type IV, and taste nerve endings contain afferent and efferent nerves. Type II cells are equipped with taste receptors for sweet, umami, and bitter substances, but have no synaptic connections with taste nerves. Synaptic contacts between taste bud cells and taste nerve endings occur only on type III cells. Type II cells are shown to release ATP paracrinically in response to taste substances, and released ATP stimulates P2 receptors on taste nerves or type III cells. Also, acetylcholine may be released from taste bud cells or taste nerve endings to modify the function of neighbouring taste bud cells. It is thus likely that taste bud cells and taste nerve endings form cell-networks in order to process taste information by expressing a variety of neurotransmitter receptors. The identification of the subtype of neurotransmitter receptors functionally expressed is essential to understand the role of the cell network. We investigated the expression of P2 and ACh receptor subtypes on mouse fungiform taste buds in a variety of methods. Electrophysiological studies showed that 100 μM ATP applied to their basolateral membranes either increased or decreased the membrane conductance in a few cells per taste bud. Ca^{2+}-imaging showed that similarly applied 1 μM ATP, 30 μM BzATP (a P2X7 agonist), or 1 μM 2MeSATP (a P2Y1 and P2Y11 agonist) increased intracellular Ca^{2+} concentration, but 100 μM UTP (a P2Y2 and P2Y4 agonist) and α, β-meATP (a P2X agonist except for P2X2, P2X4, and P2X7) did not. RT-PCR suggested the expression of P2X2, P2X4, P2X7, P2Y1, P2Y13, and P2Y14. Immunohistostaining confirmed the expression of P2X2 on type III cells. These results showed for the first time the functional expression of P2X2 on TBCs. Similar electrophysiological, Ca^{2+}-imaging, and RT-PCR studies revealed that fungiform taste bud cells expressed M3, one of mACh receptor subtypes. The roles of P2 and M3 receptor subtypes in the taste transduction are discussed.

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What Gene Expression Patterns and Functional Responses Tell Us about Coding in Mammalian Taste

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Mammalian taste buds are able to detect a large number of taste compounds and categorize them into at least five classes that we

call the basic taste sub-modalities: salty, sour, sweet, bitter and umami. Taste-selective G protein coupled receptors (GPCRs) specialized to detect sweet, bitter and umami compounds have been described by molecular cloning and expression in heterologous cells in culture. Taste GPCRs for each quality are expressed in a separate subset of cells within taste buds. Hence, it was inferred that signals arising from each class of receptors are independently transmitted to separate afferent fibers in a so-called ''labeled line'' code. In contrast, physiological analyses have consistently shown that the majority of cells within taste buds and single fibers within afferent nerves carry responses to multiple taste qualities. Using gene expression profiling of individual cells, transgenic strains of reporter mice and Ca^{2+} imaging in a semi-intact preparation, we have developed a new model of the roles of individual cells within taste buds that offers a resolution of this discrepancy. Only 25-35% of all the cells in taste buds express taste GPCRs, and have been labeled Type II or Receptor cells. A separate class of cells, termed Type III or Presynaptic cells display ultrastructural synaptic specializations, express voltage-gated Ca channels, and have the enzymes for synthesizing, packaging and releasing neurotransmitters. We have established two strains of transgenic mice, PLCβ2-GFP and GAD-GFP that label Receptor (Type II) or Presynaptic (Type III) cells, respectively. We used lingual slices containing taste papillae from these mice to examine taste-evoked Ca^{2+} responses of each cell type. We found that Ca^{2+} responses of Receptor cells are narrowly tuned, i.e., each cell responds only to tastants of one sub-modality (e.g. bitter). In contrast, Presynaptic cells are broadly tuned, displaying Ca^{2+} responses to as many as all five sub-modalities. Receptor (Type II) cells secrete ATP in a taste-evoked manner, and this secondarily activates Presynaptic (Type III) cells. This provides a mechanism by which Presynaptic cells integrate signals from multiple Receptor cells. Thus, primary responses to sweet, bitter and umami stimuli arise in separate classes of cells, and are followed by secondary responses in Presynaptic cells. The coding significance of the integrated signal remains to be determined.

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Taste Signal Transnission from Fungiform Taste Bud Cells to Chorda Tympani Nerve Fibers in Mice

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It is known that a subset of taste cells generate action potentials in responses to taste stimuli. Action potentials, or their underlying voltage-gated Ca^{2+} and Na^{+} currents, of taste cells are thought to be important for transmitter release at the synapse or particular transmission sites to the nerve fibers. However, responsiveness of these cells to particular tastants and mechanisms for their signal transmission to the nerve fibers remain unknown. To address these issues, we first examined response profiles of mouse fungiform taste cells generating action potentials by using loose-patch configuration with isolated taste buds. Then we compared them with those of the chorda tympani (CT) nerve fibers. We found that taste cells and CT fibers share similar characteristics in response selectively (the breadth of responsiveness) to the four basic taste stimuli, the grouping based on hierarchical cluster analysis, and the occurrence of each class of taste cells with different taste responsiveness. The results suggest that information derived from receptor cells generating action potentials may be transmitted to taste nerve fibers without major modifications. Recent reports have highlighted the role of ATP as a key neurotransmitter from taste cells to gustatory nerve fibers. So, we next examined tastant-evoked ATP release from taste cells with action potentials. Just after recording spikes in response to a particular compound, the electrode solution was collected and applied for luciferase assay. Molecular expression of taste cells were also examined by using a single cell RT-PCR method. ATP was detected in taste cells expressing gustducin in response to saccharin, quinine or glutamate. In these cells, the amount of ATP increased in a firing rate-dependent manner. Taste cells, expressing glutamic acid dehydrogenase 67, responded to acids and/or many eletrolytes. But in these cells, ATP was below the detection limit of our luciferase assay. The results suggest that the amount of ATP released from single taste cells differ with the response properties, or that acid- and electrolyte-sensitive cells release another neurotransmitter. Supported by JSPS Grants-in-Aid 18077004, 18109013 (YN) and 19791367 (RY).

Lactisole Diminishes only Preference for and S Fiber Response to Sweet in Monkey

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The division between a sweet and bitter taste quality is a consistent feature in most species and evident already in the newborn human. While the discovery of a unique set of taste receptors for the sweet and bitter taste quality provided an answer to how sweet and bitter taste is created on the mammalian tongue, the question how this information is coded in taste nerves remains an enigma. We have addressed this previously by recording from taste fibers of rhesus, marmoset and chimpanzee and found that the responses to taste stimuli in single taste fibers group the fibers according to human taste qualities. To further our understanding of sweet taste, we studied here how lactisole, which in humans suppresses sweet taste, affected the preference for sweet and changed responses in chorda tympani single taste fibers of the crab-eating monkey, *Macaca fascicularis*. We found that the monkeys' intake of sweeteners diminished significantly when 1.25 mM lactisole had been added to all sweeteners tested. The fact that the animals did not discriminate between lactisole alone and water suggests that lactisole per se did not influence their preference. In 40 single taste fiber recordings we found that lactisole suppressed the response to sweeteners in fibers responding best to sweet, the S-cluster, but had no effect on the responses in fibers that responded to sour, bitter and salt, the H, Q- and N-clusters. Since lactisole blocks the T1R3 monomer of the sweet taste receptor T1R2/R3, these results suggest, not only that the perception of the sweet taste quality is linked to activity in fibers of the S-cluster, but also that S fibers convey taste from T1R2/R3 receptors, while the source of the impulse activity in non-S fibers are other kinds of receptors.

Channels into Perception: The Perceptual Properties of Thermal Taste and its Molecular Basis

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Individual variation in human oral sensation has been of great interest to researchers for many decades. Recently, 'thermal taste' was described, the phenomenon whereby some individuals perceive 'phantom' taste sensations as a result of thermally stimulating small areas of the tongue (Cruz & Green, 2000). Most commonly, thermal tasters (TTs), report sweetness when the tongue tip is rapidly re-warmed, and salty and sour upon cooling the tip and lateral edges, respectively (Cruz & Green, 2000). TTs perceive prototypical tastants with greater intensity than their thermal non-taster (TnTs) counterparts (Cruz & Green, 2000; Green et al., 2005), as well as orthonasally and retronasally delivered odorants (Green & George, 2004). We review the literature regarding the perceptual effects of thermal taste and the evidence that it functions via the TRPM5 channel (Talevera et al., 2005) and possibly other channels (e.g., ENaC, TRPV1t; Talevera et al., 2007). Additionally, we present recent research from our lab, which shows that thermal taste functions independently of 6-n-propylthiouracil (PROP) responsiveness (another index of individual variation in perception of oral sensations) and fungiform papillae density. We also show that TTs perceive astringency - a tactile sensation - and lingual temperature with greater intensity than TnTs and confirm the heightened responsiveness of TTs to prototypical tastants and retronasal stimuli. Taken together, these data suggest a global sensory advantage for TTS, and present the basis for the use of thermal taste as an index of general responsiveness to gustatory, and possibly, olfactory stimuli.

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Olfactory Superspecialism of *Drosophila sechellia*

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The *melanogaster* subgroup comprises several closely related species, most of which appear generalist in their food choice. Within the *simulans*-clade, however, *D. sechellia* is strictly specialized on the toxic fruit of *Morinda citrifolia*. Previously we showed an extreme olfactory tuning of *D. sechellia* to methyl hexanoate (MeHex), a volatile characteristic for the fly's sole host (Dekker et al., 2006). Here, we identified through GC-SS another volatile of *Morinda* fruit, 2-heptanone, which triggers a response on a neuron coinhating the MeHex neuron. Both neurons target two strongly enlarged glomeruli in the antennal lobes, which reminds of a macro glomerular complex tuned to host volatiles. Behavioural tests showed that 2-heptanone elicited a similarly strong attraction in *D. sechellia* as MeHex. We also tested the attraction of D. *s* and D. *m* to a mixture of some of the main volatiles of Morinda fruit: 2-heptanone, MeHex, hexanoic acid in their naturally occurring proportions. *D. sechellia* was more attracted to the mixture than to single compounds, whereas the generalist D. *melanogaster* was more attracted to the single compounds.

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 between 45 and 65 years of age. In the present study, we familiarized menopausal women who had difficulties in experiencing pleasure with a pleasant smell, and we assessed whether that familiarization improved their mood and emotions using behavioral and physiological test methods.

Forty eight female volunteers (55-65 years old), having difficulties in experiencing pleasure (anhedonia scores between 9 to 30) 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).

Both groups applied the appropriate skin care product at home daily for 5 days and filled in mood and emotions questionnaires before and after use. After one 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 one week induced long lasting effects on mood as reflected by significant decrease in annoyance ($p<.04$), disgust ($p<.05$), interest ($p<.01$) and stress ($p<.03$) 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 ($p<.02$) and fear ($p<.03$)). These effects on mood were associated with an increase in facial zygomatic activity specifically in response to S1 in the test group ($p<.03$).

Taken together, these results suggest that the pleasant smell of a cosmetic product can contribute to the well-being in a population of menopausal women who are less prone to experience pleasure.

Keywords: smell, familiarization, menopause, anhedonia, emotion

Induced Sensitivity to Androstenone: The Role for Main Olfactory System and Vomeronasal Organ

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In humans, there is a well-documented specific anosmia to sex boar pheromone androstenone (AND). It affects about 50% of adult humans (Amoore, 1977; Labows & Wysocki, 1984). Although there is a genetic basis for the perception of AND, this phenotype is dynamic. Individual experiences with odors often interact with genetic propensities to yield a changing phenotype. An animal model for this phenomenon has been developed using

inbred strains of mice CBA/J (CBA) and NZB/B1NJ (NZB) (Wang et al., 1993; Voznessenskaya, Wysocki, 1994). Using Y-maze paradigm we estimated sensitivity of NZB and CBA mice to AND. CBA mice could detect AND at a concentration 2000-fold more diluted then NZB mice (Voznessenskaya et al., 1995). After 2 weeks of exposure (16 h/day) all mice increased sensitivity to AND regardless of the initial level of sensitivity. We found significant and systematic increases in sensitivity to AND in animals exposed to the compound for 1, 2, or 3 weeks: mice with longer exposures became more sensitive. In more recent study we investigated the role of main olfactory system (MOS) and vomeronasal organ (VNO) in detection of and sensitization to the compound. Three basic experimental approaches were used: behavioral, vomeronasal removal (VNX) followed by histochemical verification and immunohistochemical. VNX caused a 4-16-fold decrease ($p < 0.01$) in sensitivity to AND in highly sensitive CBA mice (n=10), but did not affect AND thresholds in NZB mice (n=10). The data obtained indicate the involvement of VNO and MOS in detection of androstenone. We observed a specific pattern of Fos-positive cells in main olfactory bulb of CBA mice (n=6) but not in NZB (n=6) mice in response to AND stimulation. AND stimulation caused activation in accessory olfactory bulb in both strains of mice indicating the involvement of VNO in AND detection. Patterns of Fos-positive cells were recorded in response to androstenone stimulation (0.1% w/v) in VNO 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. Different distribution of activated receptor cells in CBA and NZB mice explains in part differences in sensitivity to the odorant. Induced AND sensitivity was correlated with elevated Fos-immunoreactivity in MOB and AOB in highly sensitive CBA mice. In almost insensitive NZB mice induced sensitivity correlated with elevated Fos-immunoreactivity in AOB only. Our data indicate that both systems: MOS and VNO are involved in sensitization to AND.

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Major Urinary Proteins (MUPs) as a Key Component of the Olfactory Coding In *Mus musculus* L : Combinatorial Co-expression of *Mup* Genes in Mice of Common Laboratory Strains

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The major urinary proteins (MUPs) are widely assumed to be a key component in the forming of the olfactory signature in *Mus musculus L.* (Hurst et al., 2001; Armstrong et al., 2005; Cheetham et al., 2007; Mudge et al., 2008). MUPs can bind volatile pheromone ligands and/or by themselves convey essential olfactory information about genotype, sex, social rank, reproductive state, age, and individuality of donors. The molecular mechanisms of the MUPs' participation in the forming of olfactory fingerprints remain to be elucidated. Recently we put forward the idea that the proportion of different MUPs in a given combination, rather than their concentrations, is essential for odor coding in *Mus musculus L.* (Novikov, 2007). This assumption was based on detailed studies of MUPs' SDS-PAGE profile from male and female mice of CBA/LacY and C57BL/6JY laboratory strains under different physiological conditions. We also investigated the ontogenetic profile of particular MUP bands in these strains and found significant positive correlation of MUPs' rank order between juvenile and adult animals of both sexes. Our data indicate that the specific ''adult proportion'' profile of different MUP fractions emerges very soon after weaning and resembles a ''bar code''. These results reflect the functional significance of co-expression of the *Mup* multigene family and give evidence for the important role of MUPs combinatorial pattern in the formation of genotype- and gender- specific pheromone signature in this species. In the light of recent findings on direct activation of the vomeronasal neurons by MUPs (Chamero et al., 2007) and combinatorial expression of pheromone receptors, V2Rs (Silvotti et al., 2007; He et al., 2008) the presented data provide valuable insights into fine molecular mechanisms of olfactory coding and social recognition in *Mus musculus L.* The perspectives of this model in dissection of social behaviors using *Mup* knockout mice and creation of sensitive biosensors based on recombinant MUPs matrix are also discussed.

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Olfactory Receptor Neurons of Mutant- and Normal-Strain *Trichoplusia ni* Male Moths Exhibit Differing Response Ratios Correlated with 'Evolved' Mutant Flight Behavior

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The changes in olfactory pathways that might allow shifts in sex pheromone blend composition and discrimination to occur have been speculated upon frequently. An example of such a blend ratio shift exists in the well-known case of the cabbage looper moth, *Trichoplusia ni* (1, 2), in which a mutation in the gene controlling production of a chain-shortening enzyme has occurred in a laboratory population. Females expressing the mutant enzyme were found to emit a ratio of pheromone components that was strikingly different from the ratio emitted by normal females (2). The blend-ratio shift was so great, and differed so much from the normal wild-type blend, that initially mutant as well as wild-type males were poorly attracted to the mutant female blend. After ca. 40 generations of laboratory selection, however, mutant males had 'evolved' to now broaden their spectrum of response so that they were now attracted equally well to both the new mutant female blend-ratio and the wild-type blend (2).

Results from our current study undertaken in 2008, indicate that there has been a significant change in the ORN response profile of mutant males that is correlated with their broadened behavioral

response. The sensitivity of the ORN type in mutant males that is tuned to the *T. ni* minor component (*Z*)-9-tetradecenyl acetate is significantly reduced compared to this type of ORN in wild-type males. The reduction in sensitivity of this ORN type, coupled with a lesser reduction of response in the ORNs tuned to the major component (*Z*)-7-dodecenyl acetate, results in a similar firing ratio in response to both the mutant and the wild-type female blends, thus permitting behavioral upwind flight responses to both blends. However, firing ratios in wild-type males in response to the mutant and wild-type blends is significantly different and explains the lack of upwind flight of wild-type males to the mutant blend.

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Olfactory Performance in Relation to Sex and Hormonal Profile in Humans

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The influence of several factors such as age or sex on human olfactory performance has been long since described. In the present research study we analysed changes of olfactory threshold and discrimination in relation to sex and sexual hormonal profiles in humans.

The recruited subjects were non-smokers and displayed homogeneous anthropometric and lipemic profiles. None were suffering from allergy or colds, nor were under any drug therapy. Average ages were 25.6 years and 25.4 years in men and women, respectively. The latter group was composed by non-pregnant subjects, and none was taking birth control pills. Measurements of the olfactory threshold for n-butanol were performed by means of the ''Sniffing Sticks'' technique (Hummel et al., 1997). According to the ''triple forced choice'' procedure (Doty, 1991), n-butanol and acetic acid were used for evaluating olfactory discrimination properties. In each subject, both olfactory tests were performed two hours after drawing blood samples for determining lipemic and sexual-hormone profiles. In the case of women, olfactory as well as blood tests were performed at the third, the fourteenth and the twentieth-third day of their menstrual cycle.

Lower threshold and higher discrimination values were measured in women with respect to those detected in men. In women, changes of the olfactory threshold were detected in relation to the menstrual cycle, with a peak decrease at the fourteenth day. On the other hand, high and constant olfactory discrimination values were measured across their menstrual cycle.

The results of the present study are consistent with those of previous reports. They confirm a higher olfactory sensitivity and discrimination capability in women with respect to men. They also show fluctuations of olfactory sensitivity in women across the menstrual cycle, and an enhanced sensitivity during the ovulatory phase.

Reception of Sex Pheromones in House Mouse is Affected by Exposure to Stress

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We studied the influence of exposure to stress on reception of sex pheromones in male mice. Three basic experimental approaches were used: behavioral, immunohistochemical and hormone assay. Test subjects were adult male mice of different social status. Patterns of Fos-positive cells were recorded in vomeronasal organ (VNO) receptor tissue in response to stimulation with bedding from receptive females. Plasma testosterone and corticosterone was detected using ELISA technique. Expression of GC receptors in VNO was investigated immunohistochemically. Patterns of sexual behavior were recorded for experimental and control animals. Pattern of activation in receptor epithelium of male VNO was recorded in response to exposure of receptive female bedding. We observed activated cells in basal and apical zone of VNO receptor tissue of intact males. Exposure of male mice to low temperatures (4°C, 2 hours) reduced number of Fos-positive cells in receptor epithelium (n=10). Exposure of male mice to cat odor for 10 days also suppressed the response of VNO receptor epithelium (n=8) and was coupled with significant increase of plasma corticosterone. Exposure to cat odor significantly ($p < 0.05$, n=10) decreased number of mountings with intromissions, number of attempted mountings and number of nasal contacts. Using antibodies against GC receptors (M-200, Santa Cruz) we showed that GPCRs are expressed in receptor VNO tissue. Taking into consideration that androgen receptors are not expressed (Chichet et al., 2007) in VNO we may explain the observed effects by the presence of GPCRs in VNO receptor tissue. The data obtained indicate that glucocorticoids may play an important role in pheromone reception in VNO. Supported by RFBR 07-04-01538.

Glutathione Conjugation of the Rabbit Mammary Pheromone 2-Methylbut-2-Enal

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In the process of smell, the olfactory signal is initiated by the binding of odorous molecules to olfactory receptors. In the receptor environment, associated events are supposed to modulate this signal. Thus, the xenobiotic metabolizing enzymes, potentially involved in the clearance of the odorous molecules, could modulate the availability of these molecules for the olfactory receptors, and consequently could participate indirectly in the olfactory signal termination. A mammary pheromone, which is an odorous aldehyde (2-methylbut-2-enal or 2MB2) has been recently characterized in the rabbit by our group. The aim of this work was to elucidate the metabolism of 2MB2 in the rabbit olfactory mucosa (OM). Results

of *in vitro* studies demonstrate that 2MB2 is conjugated to glutathione in OM, and that this metabolism is much stronger than in the liver. Besides, 2MB2 is less conjugated to glutathione in newborn rabbit OM than in weanling or adult animal mucosa. This last result is correlated with age-related variations in the expression of different olfactory glutathione transferase isoforms. In addition to *in vitro* studies, we developed an *ex-vivo* method in order to evaluate the metabolism of 2MB2 in a whole-tissue sample. This approach allows the nasal cavity to keep its complex structure, which is necessary for the olfactory physiological process. The disappearance of the volatile aldehyde is measured in the headspace around the isolated nasal cavity put in a closed vial, enabling a global measurement of the pheromone metabolism. These results demonstrate that 2MB2 is metabolized by the rabbit olfactory mucosa especially as glutathione conjugates, and suggest the use of this pheromone as an interesting tool for further investigations on the possible involvement of the xenobiotic metabolizing enzymes in the process of smell.

The Synaptic Pattern of the Olfactory Input of Intermale Mice Aggressive Behavior

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The mammalian olfactory system recognizes chemical signals which are connected with different aspects of animal behavior. The relation between olfaction and inter male aggression is well known. Olfactory signals provided by the opponent (intruder-mouse) are detected by olfactory epithelium of the attacking male. Then odor information is conveyed through the main and the accessory olfactory bulbs to the higher regions, including limbic system and cortical structures. Neuronal systems have a number of different neurotransmitters that are involved in realization of this behavioral reaction. It seems important to estimate their contribution in these mechanisms that may be partly understood by means of intranasal penetration of some chemicals into the CNS. We previously demonstrated the possibilities and the advantages of intranasal administration for GABA and glycine, involved in inhibitory circuits within the olfactory bulb. The long suppression of male mice aggressive behavior was observed. Present work continues our investigations in this field and is devoted to the study of intranasal delivery of glutamate. Behavioral observations of inter male mice aggression were performed before and after intranasal and intraperitoneal administration. Even given intranasally at low doses (1 mg/kg) L-glutamate evoked increasing in quantity and quality of attacks, at that increase in number of attacks was 36-88% as compared with control for different doses (1mg/kg-10mg/kg). Taking into consideration the fact that glutamate was found as the neurotransmitter at the olfactory nerve to mitral/tufted cell synapse and our data of GABA and glycine we can propose the existence of some synaptic patterns for aggressive behavior. Physiological basis and mechanisms of intranasal administration are discussed as well.

Sensitivity to Androstenone and Aggressive Behavior in Inbred Strains of Mice

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In mice, reproductive and aggressive behavior is guided by odors and investigatory behavior provides the behavioral mechanism for evaluating sex, physiological status and social rank of another individual. Failure to detect certain biological odors may seriously disrupt behavioral reactions. Mice engage in anogenital and/or naso-oral investigation prior to either initiating sexual advances in the presence of a female or aggression with an unfamiliar male. We studied a possible relationship between sensitivity to androstenone and aggression in NZB/B1NJ (NZB) and CBA/J (CBA) mice. Investigatory behavior did not precede the first attack when an intruder was introduced into the cage of the NZB male. Atypical for mice in general, NZB males often attacked females. This may imply that chemosensory cues and social behavior are de-linked in NZB males. Alternatively, as we hypothesize, NZB mice may have deficits in olfaction that lead to failure to discriminate sex and social rank of conspecifics. Failure to process biologically important odors may lead to elevated aggression. The level of aggression was quantified using a standard test with castrated male intruder: 88% of NZB males (n=25) exhibited high levels of aggression; however, only 8% of CBA mice (n= 25) engaged in any level of attack. We also investigated sensitivity to androstenone and aggression in the F_1 hybrids and in F_2 generation. Only 23% of F_1 males from the CBA (female) x NZB (male) cross were aggressive; however, 92% of the males from the reciprocal, NZB (female) x CBA (male), cross revealed high levels of aggression. In the F_2 generation, the level of sensitivity to androstenone is correlated with the level of male aggression (n=88, $p< 0.05$). Correlation between these two phenotypes in the segregating F_2 generation suggests either linkage of genes controlling these behavioral traits or pleiotropy. However, our preliminary DNA screen using micro satellite markers does not point to a shared locus. The absence of shared linkage between aggression and sensitivity to androstenone may result from the small number of animals (n = 88) used in the aggression test. In standard odor preference test CBA males showed strong preference for receptive female odor relative to male odor (n=8). However NZB males did not show preference for the odor from receptive female versus odor from male. In another set of behavioral tests CBA males showed significantly ($p<0.01$, n=8) higher investigatory activity towards biologically relevant odors then NZB males. In CBA males time of investigation of oestrus female urine during 10 minutes test was 193,0 ± 25,3 sec, in NZB males 73,4 ± 19,7. Male's urine in the same test was investigated for 81,7±10,6 sec. in CBA males, 62,9 ± 16,7 sec. in NZB males. The data obtained indicate the relation between specific anosmia to androstenone and aggressive behavior in NZB males.

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The Role of Predator Odors in Regulation of Oestrous Cycles in House Mouse

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Reproductive traits in mice are affected by a number of environmental, social and chemosensory factors. We investigated the influence of chemical signals derived from different sources – urine of feral cats (*Felis catus*) and urine from overcrowded mice (*Mus musculus*) on regulation of oestrous cycles in *Mus musculus musculus* under laboratory conditions. Cat urine applied every day to the bedding of female mice for a period from 14 to 21 days caused a significant ($p<0.001$) increase in the numbers of animals with extended oestrous cycles relative to control animals. Urine from overcrowded conspecifics applied to the bedding of female mice on a daily basis had even greater effect. For both treatments, the duration of the delay to the next oestrus ranged from 2 to 14 days. We observed regular cycles for all animals in control group. The average number of estruses was significantly reduced ($p< 0.001$) in animals exposed to cat urine and in the group treated with urine from overcrowded conspecifics ($p<0.001$). For control group for the period of 21 days it was 4.3 ± 0.2, for cat urine treatment group – 3.0 ± 0.2, overcrowded mouse urine treatment group – 3.0 ± 0.3. Using ELISA technique we monitored plasma corticosterone level in mice exposed to predator odor ($n=8$) and in the non-exposed mice ($n=8$). Under predator odor exposures plasma corticosterone was significantly elevated for extended periods. It may explain the observed effects. The results of the present study suggest that predator (feral cat) urine and urine from overcrowded mice may share similar information about unfavourable conditions for reproduction. The fact that mice respond to certain chemical signals in predator urine in a similar fashion may be fortuitous, and may have more to do with the coincidence that the urine contains similar cues resulting from protein digestion in carnivores and protein catabolism in nutritionally deprived rodents, rather than specific predator–prey adaptations.

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Human Body Odour Asymmetry and Mate Quality

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Research studies on body odours using axillary samples often do not distinguish between odour samples from the right and left armpits. In the present study, we tested the hypothesis of an asymmetry in the perceived quality of the odour from both armpits and of a link with handedness. A second aim was to determine how odour quality was linked to several physical characteristics known to be indicative of mate quality and to be involved in mate selection. Thirty-eight males aged 19 to 35, non-smokers, provided odour samples from their right and left armpits, among them 15 were left-handed. They were instructed to avoid strong food, limit alcohol consumption, and shower with a non-perfumed soap. Body and face asymmetry were assessed through repeated measurements of body bilateral traits and facial photographs. Chest-to-waist ratio, shoulder width, 2D:4D ratio and voice pitch were also collected. Forty-nine females aged 19 to 34 (half of them being under hormonal contraception) rated fresh odour samples from the left and right armpits of 8 to 11 men. Nine-point scales of attractiveness, intensity and masculinity were used. Ratings of the odours from the left and right armpits did not differ, except when handedness was taken into account. As expected, the smell of the dominant side was more intense and more masculine than the non-dominant side, but this effect was restricted to left-handed males when rated by non-pill users (especially those being in the fertile phase of their menstrual cycle). The link between axillary odour quality and physical markers of good genes are discussed within the framework of mate choice mechanisms.

Modelling the Moth Pheromone Transduction Cascade from Molecular Level to Sensillar Level

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Olfactory receptor neurons (ORNs) housed in antennal sensilla trichodea of male moths can detect female-released sexual pheromone with exquisite sensitivity. Moreover, the dynamic range of these ORNs is extremely broad, exceeding one million-fold of pheromone concentrations from threshold to saturation. In this work, we propose a quantitative model of the moth pheromone transduction cascade in its realistic multicellular sensillar environment, based on basic principles of chemical interactions and of electrical circuits. The model uses the known facts from anatomical, biochemical, pharmacological and electrophysiological experiments on ORNs and sensilla, and proposes reasonable hypotheses for the unknown or uncertain mechanisms.

The model is made of 3 related networks from the molecular to the sensillar level. 1) The biochemical network involves the perireception reactions (pheromone uptake, transport and deactivation) and the membrane reactions (involving receptor, G-protein and phospolipase C) which contribute to the production of second-messengers, IP_3 and DAG. 2) The chemo-electrical network includes the depolarizing channels (IP_3-dependent calcium channels, DAG-dependent cationic channels, calcium-dependent chloride channels), as well as the various inactivating and regulating feedback mechanisms that are needed to return the whole system to its resting state. 3) The electrical network takes into account the electrical properties of the ORN dendrite and soma, auxiliary cells, sensillar lymph and hemolymph.

By performing computer simulations of our model we successfully fitted the predicted kinetics of the sensillar potential to the corresponding experimentally measured kinetics at various doses, which allowed us to determine the values of the unknown parameters. This model suggests a specific role for the various channels at different stimulus intensities. It gives insight on calcium regulation for example and on the significance of the recently found direct interaction of the receptor with the cationic channel. Thus it helps to conceive new experimental tests and opens a virtuous circle of combined experimental and modelling investigations.

Molecular Elements of Pheromone Reception in Moths

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Many insects use multicomponent pheromone blends for mate attraction. The remarkable ability of male moths to accurately detect lowest concentrations of female-released sex-pheromones is mediated by specific sensory neurons on the antenna, which respond to distinct pheromonal compounds. This specific responsiveness implies that the sensory neurons are equipped with distinct receptors. We have identified candidate pheromone receptors of moths, which form a relatively conserved group of olfactory receptors and are expressed in sensory neurons housed in pheromone-responsive sensilla types. By immunohistochemical approaches the receptor protein could be allocated to dendritic processes of sensory neurons. Receptor expressing cells were found to be surrounded by cells expressing pheromone binding proteins (PBPs), which are supposed to mediate the transfer of hydrophobic pheromones through the aqueous sensillum lymph towards the receptors in the dendrite membrane. In functional studies using cell lines which heterologously expressed definite receptor types it was demonstrated that the candidate receptors indeed recognized pheromonal compounds. Studies employing PBPs revealed an increased sensitivity and specificity of the system, suggesting that both, a distinct PBP and receptor, contribute to the specific recognition of a pheromone component by the mothś pheromone detection system. In addition to receptors and PBPs, a possible role of ''sensory neuron membrane protein'' (SNMP) in pheromone reception has been suggested. From several moth species we have identified two SNMP-subtypes, which are expressed in cells of pheromone-sensitive sensilla. Whereas SNMP-1s were expressed in only one of the generally two neurons in a single sensillum hair, SNMP-2s were expressed in cells co-expressing PBP, apparently the supporting cells. Our results indicate that SNMP-1s and SNMP-2s are differentially expressed in cells of pheromone-sensitive sensilla and suggest distinct functions for the two SNMP-subtypes in the olfactory system.

Decreases in Pheromonal Responses in the Mouse Accessory Olfactory Bulb after Maturation

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Chemical signals excreted from animals affect the sexual behavior of conspecific male and female animals. Sexually experienced male rats prefer oestrous to di estrous urine odor (Pfaff & Pfaffmann, 1969; Lydell & Doty, 1972). Sexually inexperienced males do not exhibit these preferences, indicating that male rats develop the ability to process odor information regarding a female's state of sexual receptivity after sexual experience. We have shown that sexual experience in male rats enhances the transmission of reproductively salient information concerning potential oestrous status to a specific periglomerular cell region of the accessory olfactory bulb (AOB) (Eur. J. Neurosci., 2008), suggesting that maturation augments excitability of the vomeronasal system. In the present study, we explored Fos-immunoreactive (Fos-ir) structures in the AOB of immature and mature mice after the vomeronasal organ was exposed to conspecific urine. Exposure of the vomeronasal organ of immature female mice to male urine led to the appearance of many Fos-ir cells in the AOB. However, the density of Fos-ir cells in the AOB after exposure of mature female to male urine was lower than that after exposure of immature females. These results suggest that the sensitivity of the vomeronasal system of immature females is higher than that of mature females.

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Smells Modulate Mood and Physiology in Menopausal Women

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The present study investigated the effect of a pleasant smell on mood and physiology of menopausal women. Forty eight 55-65 years old women were involved (24 of them applied a skin care product containing a pleasant smell (test group) and 24 applied the same product but unscented (control group)). 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 one week familiarization with the product, participants 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 one week induced a) long lasting effects on mood (decrease negative mood and stress ($p<.05$) in the test group vs. control group) and b) short term effects (decrease in anxiety and fear, $p<.05$). These effects on mood were associated with an increase in facial zygomatic activity specifically in response to S1 in the test group ($p<.03$). Taken as a whole, these results suggest that the pleasant smell of a cosmetic product contributes to the well-being in menopausal women.

Keywords: smell, familiarization, menopause, emotion.

Odorant Binding Proteins and Mouse Urinary Proteins: Potential Biomimetic Sensing Systems

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The lipocalins are a family of functionally diverse, small proteins that comprise 160–180 amino acid residues. They have important

biological functions from bacteria to humans. The β-barrel structural element of the lipocalins represents a rigid folding unit. The backbone conformation of the β-barrel is highly conserved throughout the lipocalins. This β-barrel structure can support loops with highly variable lengths, sequences and conformations at its open end. This is analogous to the mode in which antibodies present their six hypervariable loops (complementarities-determining regions) on top of a structurally conserved framework. However, compared with antibodies, lipocalins exhibit several biotechnological advantages because they are smaller in size, are composed of a single polypeptide chain and they exhibit a simpler set of four hypervariable loops that can be more easily manipulated at the genetic level. This architecture appears to be well-suited to the implementation of novel binding activities via combinatorial protein design. Lipocalins have potential to be used both as ''sensor-proteins'' by themselves in cell-free systems.

This presentation reports an initial investigation into using this family of proteins for sensing volatiles in an artificial sensing platform. As part of the EU Project GOSPEL IST-507610 initial studies were carried out to immobilise mouse urinary protein and a range of odorant binding proteins from different species on quartz crystal transducer platforms to determine whether they would function as specific sensors for volatile organic compounds. Reversible and repeatable responses were obtained, and with a range of pyrazine derivatives tested, the system demonstrated limits of detection in the range of 50-100 ppb.

Woundmonitor

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Smell used to be a common diagnostic tool in medicine, and physicians were trained to use their sense of smell. Array based gas sensor technology now offers the potential of a robust analytical approach to odour measurement for medical use. 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. We are developing a fast reliable method for detection of microbial infection by monitoring the headspace from the infected wounds funded via an IST-027859 EU project WOUNDMONITOR. We present results obtained by analysing the headspace volatiles emitted from Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa in order to identify volatile markers of infection. The results obtained from this GC-MS study are enabling us to build a mobile system for non invasive wound monitoring using an array of gas and odour sensors, to be used for point of care monitoring of patients. Sensors based on metal oxide and conductive polymer films were produced and modified and refined aiming to detect the most probable key markers for the bacteria types that is described as most frequently found in clinical conditions. For sampling from swabs or dressings from patients a solid phase micro extraction approach was used for pre-concentration of the low concentrations of volatile compounds emitted. An instrument was constructed that incorporated an automated solid phase micro extraction desorption system, a hybrid sensor array, electronics, and data processing to enable the system to be used for clinical validation.

The instrument is being validated over the next year in two hospitals where patients with serious burns are treated.

Topic 19: Perfumes

Effect of Flavour Complexity and Flavour Familiarity on the Acceptance of Yoghurt after Repeated Exposure

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In current market research product testing usually focuses on first impression only and not on liking after repeated exposure. Liking can change in time; it is dynamic rather than static. The main objective of the present study was to investigate the impact of flavour familiarity and flavour complexity on the development of liking of yoghurts after repeated exposure. The experimental design aimed to deliver results for 3 base flavours varying in flavour familiarity (flavour A, B, and C). Of each flavour, 2 variants were developed, a simple and a complex one, which resulted in 6 types of yoghurt. The design was a balanced cross-over design. Subjects (N=51) consumed each of the yoghurts daily for 5 days (Monday to Friday), as a dessert, after dinner at home. Differences in acceptability between familiar and novel, simple and complex flavours with regard to variety-seeking tendency were also investigated. Results showed that novelty and complexity were strongly interrelated. A rank-order test of these two attributes by a screened sensory panel revealed that both variants of flavour A were simple and familiar, one variant of flavour B was simple and novel, the other was complex but less novel. The two variants of flavour C were complex and novel. There were differences in the development of liking between the simple/familiar vs. complex/novel yoghurt variants. Initially highly liked, simple/familiar variants, tended to declined or remain stable. In contrast, complex/novel variants, tended to show an increase in pleasantness ratings over repeated exposure, although they were initially less liked. Low variety-seekers rated familiar flavours significantly higher on pleasantness than both novel flavours, whereas among high variety-seekers pleasantness ratings were comparable among all products. It is advisable to introduce familiar flavours into market and later increase the complexity (novelty) gradually in order to guarantee sustained liking.

A New Testing Method for Sensory Evaluation of Olfactory Masking Agents

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Introduction: Despite the long-time use of fragrance as a malodor-masking agent, little is known about the cellular events by which odors suppress malodors. Recently we found that some fragrance materials attenuate malodor-induced transduction current in the olfactory receptor cells (ORCs) (1) and block the CNG channel which has an important role of odor-induced transduction current in the ORCs (2). We wanted to clarify the relationship between malodor-masking at the sensory level and cellular responses. A sample preparation method and a questionnaire for sensory evaluation of olfactory masking agents are presented.

Method: Iso-valeric acid that produces rancid body odor was chosen as a malodor substance, while twenty-two popular fragrances including iso-amyl acetate, which was reported to block the CNG channel, were chosen as a masking agents. A saturated vapor was produced by enclosing each of above substances in a sealed 50-ml injection syringe for twenty-four hours. Test sample was prepared by putting a 10-ml saturated vapor of iso-valeric acid into a 500-ml plastic container and, 50-ml of saturated vapor of each masking agent was added into it. Immediately after the preparation, twenty sensory panelists with more than ten years experience in fragrance evaluation, smelled each container and filled a questionnaire about levels of malodor (e.g., 0= no odor, 5= very strong odor) and the qualitative masking ability of each sample (e.g., 0 = no effect, 5 = excellent). The score of malodor levels was made in accordance with the Offensive Odor Control Law in Japan (3).

Results: Dihydromyrcenol and iso-amyl acetate showed higher ability to suppress the malodor than fruitate. The agents with higher masking ability decreased the levels of malodor. The results indicate that the sample preparation method and questionnaire are appropriate for testing olfactory masking agents.

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Food Aroma Presented at Sub- or Peri Threshold Concentrations Decreases Bite Size

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Previous results indicated that bite size varies with a food's familiarity, and with its hedonic and textural properties. More over, smaller bite sizes prove to be more satiating than larger ones and bite sizes typically become smaller when the consumer becomes more satiated. These results indicate that bite size control is sensitive to general food properties as well as to the internal state of the consumer. To further explore the role of food sensations in bite size regulation, effects on bite sizes were investigated for food aromas presented at concentrations near detection threshold. For this purpose, semi-solid vanilla custard dessert was pumped into the mouth of subjects while a cream aroma was presented retro-nasally in the nose in one of two concentrations using an olfactometer. Termination of the pump, which determined bite size, was controlled by the subjects via a pushbutton. Higher aroma intensities resulted in smaller bite sizes, which demonstrate that bite size control is sensitive to food sensations that vary from bite to bite, even at aroma concentrations below or near perception threshold. This result suggests a rapid feedback mechanism in which the aroma is perceived during the filling of the mouth, and where the outcome of this evaluation is used to terminate the bite. The results contribute to our understanding of bite-size and food intake regulations, and may be of relevance for weight-management.

Effects of Personality on Preference and Perceived Intensity for Perfume

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Effects of personality on preference and perceived intensity for odor stimuli were investigated using 14 kinds of popular perfumes. Thirty female college students who usually wear perfumes took part in the experiment. The big five personality test was used to evaluate the participants' personality. Evaluations of odor quality on each perfume have been carried out through a free description method where the participants made free descriptions, together with an accordance rating method where each perfume was rated its congruity with 18 adjective descriptors. Each stimulus was presented manually by participants for 5 sec by squeezing a plastic bottle (4.5 × 10 cm) with a thin exhaling tube. A square piece of gauze (1 × 1 cm) infiltrated with approximately 0.30 ml of each perfume was placed in each bottle.

Results showed that personality factors, ''extraversion'', ''openness'', and ''neuroticism'', had significant effects on perfume-preference. Those who showed higher scores in ''extraversion'' and ''openness'', and lower scores in ''neuroticism'' than other participants preferred perfumes described as more ''refreshing'', ''fresh'', and ''fine'' than other perfumes. On the other hand, ''extraversion'', ''conscientiousness'', and ''agreeableness'' had significant effects on relative perceived intensity of certain perfumes. To be more precise, higher ''extraversion''-, ''agreeableness''-, and lower ''conscientiousness''-individuals showed higher perceived intensity for relatively more ''fresh'', ''refreshing'', ''graceful'', and ''gentle'' perfumes than other perfumes.

The current study demonstrates that personality factors are likely to influence our preference and perceived intensity for perfumes. The causality of the significant effects of personality, however, remains to be addressed.

Topic 20: Taste

Saltiness Perception as Affected by Salt Delivery in the Mouth

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The reduction of sodium content in food, as advocated by the World Health Organization, is needed to improve population health. Several strategies are actually under investigation to enable a reduction in salt concentration without affecting consumer acceptability of manufactured food products. In this context, the present study aims at investigating if changes in salt profile delivered to the human mouth increase saltiness perception. Salt solutions were delivered into the oral cavity of eleven panelists during a 30-s period using a custom made set of liquid pumps controlled by computer. Various delivery protocols were designed to produce small changes in salt concentration over time: constant, 20% variation every 5s and 38% variation using 2s pulses. For the last two series, the design began either with low or with high concentration. All five protocols delivered the same total salt concentration ($6.3g.L^{-1}$) at a constant flow rate ($40mL.min^{-1}$). For each protocol, trained panelists rated the saltiness intensity over the period of liquid delivery plus 10 seconds (aftertaste), using the Time Intensity method. The area under the Time Intensity curve was then extracted to compare the different salt delivery profiles. This study showed that the saltiness perception was affected by the salt delivery protocol used. Short pulses high in salt were able to increase saltiness perception compared to the constant stimulation. Moreover, saltines perception was affected by the timing at which the pulses were presented.

Do Tu Memory of Saltiness

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Background Recent work on memory in the chemical senses has suggested that these work fundamentally differently than memory in vision and audition. In incidental learning, which is how most ecologically interesting learning in the chemical senses takes place, memory seems to be based on 'detecting what has not previously been encountered', i.e. it is the correct rejection of new previously unencountered stimuli (distractors) which allows subjects to separate a memory target from distractors. Furthermore, pleasantness (hedonicity) of stimuli has often been found to influence these memories. In the experiment we report here, we wanted to test if ''novelty detection'' and pleasantness also play a role in memories of saltiness of incidentally learned natural stimuli. Memory plays an obvious role in food choices and development of food preferences and for this reason we used a real food, potato soup, as medium in the experiment.

Method Twenty six Ss participated in a sensory specific satiety experiment providing scores of various appetite and sensory parameters. Ss returned to the lab the day after the SSS experiment to participate in a memory experiment. No mention was made of the memory task before they arrived at the lab for the second session. Ss were presented with a random sequence of 30 stimuli, 14 of which were target samples (T) identical to the salty soup (6.82 g salt/L) they had eaten in the first (incidental learning) session. The other 16 stimuli consisted of 4 presentations each of 4 different distractors, identical to the target, except that two them (D- and D--) contained less salt than the target (5.0 g salt/L and 2.5 g salt/L respectively) and the other two (D+ and D++) contained more salt (8.62 g/L and 9.82 g/L). Ss evaluated if a stimulus was the same they had eaten the day before and provided a hedonic score for each stimulus. Ss had a short break after completing the memory task and then participated in a discrimination experiment with the same stimuli.

Results All distractor stimuli produce memory- and discrimination d-primes significantly different from zero. ANOVAs show that the observed differences in memory of T paired with the different distractors cannot be explained by differences in discriminability. D- and D-- both are remembered better than D++, despite no significant differences in discriminability between these 3 distractors and the target. The hedonic data reveal a significant difference between T and D- and D--, but not between T and D+ and D++. Thus, hedonic differences, but not perceptual discriminability, correlate with memory ability. Hit and correct rejection rates show that memory behaviour is guided by detection of novel stimuli rather than genuine recognition of the target encountered previously. It is interesting to note that distractors which do not differ in discriminability from the target nevertheless are clearly different in the affective hedonic test providing further support for the ''authenticity test'' proposed by Ep Köster.

Conclusion We find support for the suggestion that ''novelty detection'' and affective properties of stimuli are crucial for the workings of memory in the chemical senses.

Development of Four Channel Taste Stimulator System

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It is strongly required taste stimulator which can present various tastants without any tactile stimulus in one experiment, for interaction between tastants or cross modal experiment, especially olfaction. Such kind of multichannel gustatory stimulator was, however, difficult to constructed, as more parts, such as pressure meters, electric valves and flow regulators would be necessary increasing the variation of testate stimuli. A stability of making gustatory stimulus is moreover crucial. Since the stability of the gustatory stimulus worsens according to becoming if a necessary number of parts increase, we develop a new suction type gustatory stimulator system instead of previous adding air pressure for driving water and tastants. If someone wants to make four channels' gustatory stimulator by adding air pressure type system, twelve pressure regulators, four water flow controllers and eight electro-magnetic valves were required. Our newly developed system used just one pressure regulator, two water flow controllers and four electro-magnetic valves, which realized simple construction. In addition, the gustatory

stimulus timing control precision was improved. For example, our system can create 212 +- 27msec (mean +- S.D., N=20) gustatory stimulation if we set the gustatory width set to 200 msec. This controllability of the gustatory stimulation (timing and multi-channel control) will be useful for precise analysis of the signal processing mechanism of taste response in the field of (neuro-) physiology or human study. We developed this stimulator for human study, although this mechanism can also apply for the study field of biology or medical examination.

Conditioned Taste Aversion is Severly Impaired in Adolescent Rats Artificially Reared on a N-3 Fatty Acid Deficient Diet

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It is well known that n-3 deficiency in rodents is associated with impairments in a variety of learning and memory tasks. Artificially rearing rat pups on a n-3 deficient diet has been shown to induce poorer performance in hippocampal-dependent learning and memory tasks. We have investigated the effect of artificial rearing on a deficient n-3 diet in taste aversion learning acquisition during late adolescence (PN45).

Forty male Wistar rats were assigned to three groups: a control maternally reared group (Ctrl; n=12) and two artificially reared groups either on a standard adequate diet (Stand; n=14) or on a deficient diet (Def; n=14). Animals were trained in a variety of taste learning tasks, then a standard taste aversion conditioning procedure with i. p. lithium chloride (0.15M; 2% b. w.) injections following consumption of a cider vinegar solution (3%), was applied.

An impairment of taste aversion acquisition in both artificially reared groups was demonstrated in a one-bottle test. Also, the behavior of the group reared on the deficient diet evidenced a selective deficit that persisted in a later vinegar-water choice test.

The results suggest that early postnatal dietary treatments may modify acquisition of learning abilities, previously considered to be fully developed earlier in life. Taste learning and memory tasks may be a tool for investigating the effects of n-3 deficiencies on brain development.

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Topic 21: Olfactory Epithelium

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 co-culture 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.

Key words: main olfactory system, in vitro cultures, olfactory axons.

The Possible Functions and Properties of the Ecto-Atpase on Surface of Vertebrates Olfactory Epithelium

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The ecto-ATPase activity was determined on non destructed olfactory epithelium surface of different vertebrate animals using biochemical and electronic cytochemical methods. The properties of this enzyme were different from those of other ATPases, which take part in energy transduction and ions transport processes. It was defined that this ATPase had wide substrate specificity and its activity depended on Mg^{2+} and Ca^{2+} ions. The EDTA and EGTA made a strong inhibitory effect and high concentration of ATP too. The mitochondrial inhibitor oligomycin and Na, K-ATPase inhibitor – ouabain did not influence the activity of this enzyme. However this activity was suppressed by some SH-reagents, NaF and La^{3+} ions. The molecular forms of ecto-ATPase from vertebrates' 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 glycoprotein subunits. The effects of calcium and magnesium concentrations on frog olfactory epithelium ecto-ATPase activity were studied. It was shown that one form of ecto-ATPase was more sensitive to Ca^{2+} concentration than to Mg^{2+} and another form quite the opposite was more sensitive to Mg^{2+}. The investigation of the ultrastructural localization in the frogs olfactory epithelium demonstrated that the ecto-ATPase was located in the external part of the olfactory cilia membranes. These results are discussed with regard to possible involvement of this ecto-ATPase in Ca^{2+} exchange by excitation mechanism of the olfactory cells. Another molecular form of enzyme was located in vesicles in olfactory mucus near the olfactory knobs. This molecular form of enzyme may be related

to the protection of olfactory cells from ATP action, which is contained in the olfactory mucus from support cells secretion and receptor cells destruction. Among surface phosphohydrolases in cow and mouse olfactory epithelium also isoforms of alkaline phosphatases were found, but in frog olfactory epithelium this activity was related only to ecto-ATPase.

Topic 22: Theoretic

Trial Measurement of Synchronicity Judgment Gustation and Olfaction

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Synchronicity of gustation and olfaction in food intake seriously will affect recognition of food perception. The integration process, currently, was well investigated in the point view of retronasal or orthonasal olfactory presentation. The synchronicity, in temporal point of view, is rarely investigated for integration process of gestation and olfaction. In this study, therefore, we focused on the relation taste perception and time shift between taste and olfactory stimuli. We used taste stimulator which was able to present pure gustation without tactile stimuli, and the timing of taste stimuli to tip of participants' tongues was measured in real time by three optical sensors. We also used olfactometer originally developed by Dr. Kobal, with real time stimulus monitoring using ultrasonic sensor. The shift of presentation time of taste and odor stimuli was set from -1500 ms to 1500ms. ''0 ms'' means exact simultaneous taste and odor stimuli, ''-1500 ms'' means odor stimulus comes into participant's nostril 1500 ms faster than taste stimuli to tip of tongue, and . Participants were instructed to judge which stimuli perceived faster.

As a result, participants could not decide order of taste and odor stimuli. Another cross modality perception, such as vision vs. audition, vision vs. touch, and touch vs. audition, shift of 300 ms between cross modal stimuli is said to be enough for judgment of stimulus order. These results indicate the combination between gustation and olfaction has stronger than other cross modal sensation.

Mechanistic and Energetic Predictions in Odorant-OBP and Odorant-or Systems – Molecular Modeling Approach

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Smell is one of the most complex senses. During the early steps, the chemoreception involves at least 2 kinds of proteins, the Odorant Binding Proteins (OBP) and more importantly the Olfactory Receptors (OR). The capacity to unravel the role played by each of these systems is of primary importance to understand the molecular mechanism underlying the perception of odors.

With the recent advances in computational power and parameters developments, molecular modeling has definitely become a force to reckon with. Here, for rat, porcine and human OBPs, the experimental affinity for different odorants (linear, cyclic, and more or less polar) was shown to be recovered with high accuracy (within $\sim$ 1 kcal/mol). Concerning the OR-odorant interactions, no quantitative experimental value can be obtained and a theoretical approach thus takes all its importance to describe the odorant binding site as well as the potential selectivity of the receptor. A comparison is performed between experimental (calcium imaging) and theoretical approaches (affinity calculation) on human OR17-209 with camphor, isobutyl-acetate, methyl-acetate and tridecanal. This study has allowed identifying several important residues, notably those belonging to the binding cavity.

The capacity to nail down residues originating the interactions with odorants is of crucial importance for further mutagenesis experiments dedicated for example to produce engineered protein dedicated to targeted activities.

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Representation, Digital Coding and Clustering of Tastes for Gestation Information Transmission

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In this paper, we propose techniques converting taste information into digital data to provide representation, digital coding, and clustering of various tastes for gestation information transmission. Tastes have complex chemical compositions, it is difficult to precisely represent and identify chemical composition. Furthermore, the human being associates a taste in combination with a feeling. It may be more effective to present a taste using a feeling and emotion of human being rather than chemical analysis. Assuming that there are about 40-50 adjective factors describing tastes perceivable to human being a number of tastes can be expressed in combination of the adjective factors. More than twenty emotional adjectives describing various kinds of tastes are selected as expressional receptors. Each of taste is expressed by a definite adjective. The tastes expressed using emotional receptors are coded using a predetermined method for transmission. After being transmitted, the taste data is decoded using fuzzy c-mans (FCM) clustering algorithm. Since the taste data are expressed over 20 dimensions, a longer computing times and a larger size of memory are required for FCM algorithm. To solve these problems, a principal component analysis (PCA) algorithm is used to map data coded in a higher number of dimensions to 2 or 3 dimensions and then a FCM algorithm is used for clustering. As a result, a small amount of memory for storing only representative tastes can be used and 95% or more of original data can be expressed in 3 dimensions without data information loss. The proposed techniques are confirmed by experimental trails. The coded taste data are constructed as meta-data images and can be effectively reproduced in synchronization with an image. This primary result can be contributed taste information transmission for more natural and realistic

communication applicable to remote postal service, medical, education and entertainment industry.

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Topic 18: Crossmodal Perception

Do Odour-Colour Associations Bias the Orienting of Visual Attention

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We investigated whether the presentation of an odour would affect participants' performance in a visual temporal order judgment (TOJ) task involving colour stimuli. We were interested in verifying whether the odour-colour associations that have been documented previously would influence visual attention. In particular, we investigated whether the presentation of a task-irrelevant odour would modulate people's perception of simultaneity of pairs of colour patches by orienting participants' attention as a function of the compatibility between the odour and the colour. Three sticks (spearmint, cinnamon, and blank) from the Sniffin' Sticks Test were used as the olfactory stimuli. The targets consisted of 2 coloured squares that were compatibly-coloured with either the cinnamon or the spearmint odour. In a preliminary pilot study, each participant indicated the subjective best-matching colour-odour pairs. During the experiment itself, the 2 squares were presented on a monitor on the left and on the right of a fixation cross at varying interstimulus intervals (ISIs). A task-irrelevant odour was presented during the presentation of the visual targets and the participants were instructed to decide which colour appeared first. The results revealed no effect of odour condition on the point of subjective simultaneity (PSS). There was, however, a significant effect of odour condition on the just noticeable difference (JND), with performance tending to be less sensitive in the spearmint odour condition than in the other conditions. This result suggests that (in this case) performance wasn't modulated by the odour-colour associations, as no advantage in the speed of detection of any of the colours as a function of the odour condition was found (PSS). Instead, odours appeared to exert a more non-specific effect, as indicated by the modulation of the sensitivity of participants' responses.

Toward a Domain-specific Scale to Verbally Measure Odour-elicited Emotions

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For most of research on odors and emotions, the measurement of subjective experience elicited by olfactory stimuli has been limited to self-report questionnaires derived from two types of approaches: i) the discrete emotion theory, postulating the existence of a small number of so-called basic emotions based on phylogenetically stable neuromotor programs or ii) the dimensional theory that reduces the subjective experience of emotions to positions in a two or three dimensions space that most economically accounts for some similarities and differences in affective experience. Recently, we have developed a domain specific 6-scale model (Geneva Emotion and Odour Scale, GEOS), based on empirical data, to specifically account for the highly differentiated responses and feeling states produced by odours. The goal of the present study was to specifically evaluate whether this new scale is more appropriate for the olfactory domain than the current emotional prominent scales. To evaluate the comparative validity of the three models, we examined to what degree respondents reported their experience of each of the emotions derived from the discrete emotion model, the dimensional model or the GEOS, to each of the presented olfactory stimuli. The set of stimuli included both everyday odours and fine fragrances in order to cover a large range of odour types. The comparison, based on 3 main criteria: (i) the relevance of the scales to describe the emotional effects elicited by the different olfactory stimuli, (ii) the inter-rater agreement in using the different emotion scales and (iii) the power of the scales to discriminate among various odorous substances, provided strong evidence for a better account of the domain-specific model (GEOS) of odour-elicited emotional experiences compared to the two classical models. Our findings lend support to the view that an accurate description of odor-elicited affective feelings seems to require a specific affect vocabulary and taxonomy, which differ from those provided by classical emotion theories.

Sequential Unfolding of Novelty and Pleasantness Appraisals of Odors

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We investigated the appraisal processes of odors and consequent emotional responses. The main goal of the study was to test whether an odor is detected as novel or familiar before it is evaluated as pleasant or unpleasant, as predicted by many appraisal theories of emotion. Participants performed a delayed matching to sample recognition task in which they were presented with pairs of unpleasant or pleasant odors (sample and target odors). Within a pair, the sample and target were either identical or different to assess participants' novelty detection; unpleasant and pleasant target odors were contrasted to examine participants' appraisal of pleasantness. We measured facial expressions (electromyography) and physiological reactions (electrocardiogram and electrodermal activity). The earliest effects on facial muscles (frontalis) and heart rate occurred in response to novelty detection. Later effects on facial muscles (corrugator and frontalis) and heart rate were related to

pleasantness evaluation. This study is the first that demonstrates the existence of a sequence of appraisals in the emotional reaction elicited by odors.

Information-Theoretic Study of Quality Coding in a Realistic ORN Population Model

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The strategy followed by nature to perceive the majority of volatile compounds is to have a set of non-specific Olfactory Receptor Neurons (ORNs) that encode the identity of odorants in a combinatorial fashion. In this work we study the advantages of this coding scheme in ORNs using measures of information theory. To do so, we built computational populations of ORNs and analyzed the amount of information (mutual information) transmitted by the population. The individual ORN model used is that proposed by Rospars et al. in 2003. This mathematical model captures the interaction between the odorant molecule and the ORN binding protein allowing for the description of different ORN types. Building ORN populations with different ORN types, we evaluate the coding efficiency (information transmission) of the population in terms of parameters as the spread of the ORN receptive field and others.

Cross-Modal Interactions: Way to Counterbalance Salt Reduction in Solid Foods?

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Odor-taste interactions have been reported several times in liquid and semi solid food models. Most of these studies focused on sweetness enhancement by odor and highlighted the importance of congruency between odor and taste for the enhancement to occur. Concerning odor-induced saltiness enhancement (OISE), very few data are available in water solutions and no study focused on OISE in solid food model systems. The aim of the present study was to investigate OISE in a solid food model (cheese) in order to evaluate the influence of cross-modal interactions (aroma-texture-taste) on saltiness perception.

Four model cheeses varying in texture (2 Dry Matter (DM) levels; 2 Fat/DM levels and 1 salt level) were designed and flavored with 3 commercial tasteless aromas (''Comté Cheese'', Sardine and Carrot) differently associated with salty and cheesy food products. Thirty untrained subjects were instructed to evaluate taste intensity (sourness, bitterness, saltiness and sweetness), aroma intensity, texture attributes (firmness, moistness and graininess), aroma congruency with the product and their hedonic appreciation of the 12 flavored and 4 unflavored model cheese samples. A comparison of the saltiness perception of the flavored and unflavored model cheeses revealed cross-modal interactions between odor and taste and no influence of texture on taste perception. The results showed a significant saltiness enhancement induced by ''Comté Cheese'' aroma which was associated with saltiness and judged as congruent with the model cheese. Sardine aroma (associated with saltiness) also induced a significant saltiness enhancement even if it was judged as less congruent with the food product. However, the Carrot aroma (not associated with saltiness and judged as not congruent with the food product) did not enhance saltiness. These findings revealed that well selected aromas could induce saltiness enhancement in solid food products of various textures. We thus propose that cross-modal odor-taste interactions could be a way to counterbalance the decrease in saltiness and acceptability of food with reduced salt level in line with recommendations of public health authorities.

Topic 24: Olfaction in Medicine

Activity and Expression of Drug Metabolizing Enzymes in Olfactory Mucosa of Rats Treated by Hepatic Inducers

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Several drug-metabolizing enzymes (DME), such as cytochrome P450- dependent monooxygenases (CYP) and transferases have been characterized in the olfactory epithelium. Some of them are preferentially expressed in this tissue, while others are similar to those present in the liver. The role of these enzymes remains unclear. Since the olfactory mucosa is in direct contact with the external environment, these enzymes can contribute to the detoxification of chemical compounds. In addition, these enzymes could be involved in the olfaction process, especially in the biotransformation of odorants. Indeed, the rapid inactivation and clearance of odorants is a prerequisite for the capability of the olfactory system to receive iterative incoming signals. If such a hypothesis is plausible, it can be assumed that induction or inhibition of DME would have an impact on clearance of odorants and therefore on the olfactory signal. The aim of the present study was to identify treatments which modulate olfactory DME in the rat. The effects of known inducers of hepatic DME (arochlor, dexamethasone, phenobarbital, methylcholanthrene and ethoxyquin) on activity and mRNA expression of a panel of DME have been evaluated. All these treatments caused changes in activity and expression of several DME in the olfactory mucosa. The effects were found to be lower than those observed in the liver. Administration of dexamethasone resulted in significant increases in CYP-dependent activities and in CYP2G1 and CYP3A9 expression in the olfactory mucosa. Significant induction of UDP-glucuronosyltransferase (UGT) activities and UGT2A1 expression was also observed after dexamethasone treatment. Ethoxyquin administration induced a significant enhancement of quinone reductase and a decrease of CYP-dependent activities. The other treatments (arochlor, phenobarbital, methylcholanthrene) slightly increased the activities of UGT and quinone reductase. This study demonstrated that it is possible to modulate drug-metabolizing enzymes in the rat olfactory mucosa by chemical treatments. The impact of dexamethasone and ethoxyquin on olfactory signal will be further investigated.

Evaluation of the Olfactory Function in Patients with Multiple Sclerosis

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A controversial issue within the neurological science is whether Multiple Sclerosis (MS) influence the ability to smell. Here we investigate this issue by administering the Sniffin' Sticks Extended Test to 100 MS patients subdivided in terms of age (range 19-55 yo), sex (75 females and 25 males) and disease type (Relapsing-remitting, RRMS or Secondary Progressive, SPMS). We also acquired high-resolution MR images for 20 RRMS patients to quantify the number and the volume of the demyelinating plaques in the central olfactory structures. Results show that 38.7% of our MS group demonstrated hyposmia, but none of them could be considered totally anosmic. Moreover, we have been unable to find any significant correlation between the olfactory scores and the number (ZTDI: ρ = - 0.11, p = 0.66) and the volume (Z TDI: ρ = - 0.35, p = 0.14) of the IFTLs' plaques. Altogether these findings suggest that about 2/5 of the RRMS population suffer from olfactory loss which, however, is not significantly correlated to either the number of plaques in IFTLs or their volume. Nevertheless the results show some evidence suggesting that the volume of plaques might be more informative than plaques number. These results are discussed in terms of current theories put forward to explain the presence or absence of olfactory deficits in MS patients.

Action of Anorectic Peptides on Olfactory Function

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We have previously shown that olfactory acuity is modulated by nutritional status: hungry rats display better olfactory sensitivity than satiated rats. This suggests that olfactory function is under the control of neuroendocrine signals that modulate feeding behavior. Insulin and leptin are likely to be such signals since the olfactory bulb (OB) is rich in both insulin receptors (IRs) and the long isoform of the leptin receptors (LRs).

In this study, we have performed histological, electrophysiological and behavioral experiments in order to determine whether insulin and leptin are involved in the modulation of olfactory function. First, we demonstrate that IRs and LRs are strongly expressed in the OB. The IRs and LRs are localized (i) on the soma of the mitral cells, (ii) in external plexiform and (iii) granular layers. IRs are also found in the glomeruli neuropiles. Furthermore, patch-clamp experiments show that both insulin and leptin modulate the spontaneous activity of the mitral cells in a dual way: either through an increase or a decrease in their mean firing frequency. Finally, we show that both peptides modulate the olfactory perception. Indeed, by intracerebroventricular (icv) injections, insulin has a dose dependent effect, *i.e.* a low dose increases olfactory sensitivity, while a higher dose decreases it. Leptin icv injections decrease olfactory sensitivity in a dose-dependent manner. Moreover, Obese Zucker fa/fa rats, carrying a non functional LR, display higher olfactory sensitivity than their lean controls.

Taken together these data suggest that anorectic peptides modulate olfactory sensitivity partly by altering the transmission of the sensory stimulus in the OB. These results support the notion that the sense of smell participates in the regulation of ingestive behavior by responding to hormonal cues of nutritional status.

3d Ligandbased Virtual Screening for Deorphanisation of a Human Olfactory Receptor

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All living organisms are able to detect and discriminate myriads of structurally diverse odorants through their interaction with olfactory receptors (ORs) (1), and the perception of odors results from a combinatorial coding (2).

In a recent study, we used previously obtained functional data (3) to perform a molecular modelling study of ligands of the human OR1G1 using Catalyst/HypoGen software (Catalyst version 4.11, Accelrys Inc., San Diego, 2004, running on Unix O2 workstation) (4). HypoGen takes into account molecular flexibility, considering each compound as a collection of conformers. The software generates models which describe ligands as sets of chemical functions. These hypotheses should be able to predict the activities of different compounds having the same receptor binding mechanism. Using OR1G1 biologically identified ligands as training set, we obtained two models (A and Z), which satisfactorily explained the experimental activities, and permitted to predict novel agonists for OR1G1 (5). Both models are constituted by two hydrophobic features and one Hydrogen Bond Acceptor, but shorter distances between features characterize model-Z. In the present work, we used models A and Z to perform a virtual screening by Catalyst Database Searching on a transposition in Catalyst environment of the commercial FlavorBase 2004 (http://www.leffingwell.com). OR1G1 appears to be broadly tuned because 80% of the FlavorBase compounds are mapped by model A and/or Z. All molecules described with "waxy", "fatty" and "rose" odors are predicted as OR1G1 agonists by model A and/or Z. Our results can open the way to decipher the odotopes of OR1G1 agonists and more broadly provide cues to the understanding the olfactory coding.

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Estimating Odorant Trigeminal Threshold with the Lateralization Method Associated to a Constant Stimuli Procedure

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Most odorants activate both olfactory and trigeminal systems. However, different concentrations are required to activate the olfactory or the trigeminal system. Indeed, human olfactory thresholds were reported to be lower than trigeminal ones. As a consequence, there is a methodological difficulty to evaluate an odorant trigeminal threshold due to actual odor perception within the range of concentrations to be tested. It has been assumed that trigeminal thresholds can be inferred from lateralization thresholds. The lateralization procedure relies on the possibility for human subjects (Ss) to localize the nostril in which a trigeminal volatile compound is delivered. Such localization is not possible for pure olfactory compounds or when the odorant concentration is too low to activate the trigeminal system. The objective of the work presented in this paper is to propose a methodology to evaluate odorant trigeminal threshold using the lateralization method associated to a constant stimuli procedure which reduces adaptation, habituation and expectation errors. Thirty two healthy Ss (18W, 40±12 and 15M, 40±10) participated in a three sessions experiment designed to evaluate their lateralization threshold for 3 odorants: 1-butanol, hypochlorous acid and 2-phenylethanol (a control odorant which does not activate the trigeminal system). Eight replicates of 4 different concentrations of 1-butanol were delivered to each Ss nostril in a complete random order. Ss were asked to indicate which nostril had been stimulated. The same procedure was applied for hypochlorous acid but for the control odorant only 8 replicates of a single high concentration were delivered to each Ss nostril. An air-dilution olfactometer (OM4b, Burghart, Germany), configured in a birhinal mode, allowed precise stimulus control. Stimulus concentrations in vapor phase were measured. Individual psychometric functions were obtained from the constant stimuli procedure. Individual and panel lateralization thresholds were estimated using a mixed generalized linear modeling for binomial data taking into account individual variations. This statistical analysis enabled threshold estimation for each subject even if 100% of the trials at the highest concentration level were not correctly lateralized. Lateralization threshold were discussed in terms of odorant and Ss profile.

Olfactory Mucosa as a Target for Insulin

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The nutritional status of individuals influences odour detection. In primates and humans, fasting results in an increased perception of some food-related odours, whereas satiety regarding one type of food is correlated with a reduction in olfactory detection specific to this food. To date, the neural bases for these olfactory performance modulations by the nutritional status have been only identified in the olfactory bulb. Among nutritional signals, insulin has been shown to act on mitral cells. However, olfactory bulb consists only in the second level of odours detection. Data obtained in our laboratory suggest that metabolic status could also control the first level of odours detection. The aim of this study was to investigate a potential role of insulin in the olfactory mucosa (OM). We first established the expression of insulin receptor (IR) in rat olfactory mucosa. IR protein was located in olfactory receptor neurones, sustentacular and basal cells and in endothelium of the lamina propria vessels. Insulin binding capacity of OM was found equivalent to that of olfactory bulb or liver. We demonstrated an increase of IR expression and insulin binding capacities in different metabolic and nutritional states using qPCR and binding experiments. We also showed that olfactory signal could be decreased following insulin application on OM. These data provide the first evidence that insulin modulates the most peripheral step of odour detection at the olfactory mucosa level. But the wide distribution of IR in different type of cells in the OM suggests other potential role for insulin, mainly in the maintenance of cells dynamics.

Olfactory Alliesthesia in Anorexic Women

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Alliesthesia, the non selective hedonic (positive & negative) change in sensing stimuli related to internal state, is one of the sensory mechanisms presumed to regulate food intake. One way to ascertain its effective regulatory impact on intake is to investigate it comparatively in healthy and food-disordered patients. Thus, this study assessed olfactory alliesthesia in healthy control (CO; n=29, aged 22.6±2.2, BMI: 20.4±1.9) and anorexic females (AN; n=19, aged 26.5±7.1, BMI: 15.0±1.9). They were presented (automated procedure) a range of odorants representing selected categories of foods/non-foods. The odour evaluations were run on alternate days, while the subjects were hungry or sated (after a standardised meal). They had then to report their liking, wanting to eat, and arousing value of the olfactory-represented food and non-food objects.

It came out that : 1/ non-food odorants received similar liking scores in CO and AN groups, without significant contrast between the metabolic states; 2/ liking for food odorants decreased in the CO group (HSD Tukey test, $p<0.0002$), but not in the AN group. Detailed analyses indicate that this apparent absence of liking change in AN women may be due to their initially low hedonic

ratings while hungry ($p<0.04$); when sated, the CO and AN groups evinced a similar low scores in liking; 3/ wanting scores followed the same trend than liking scores; 4/ the reports on the arousing effect of the odorants were not discriminative of both groups, or of the group by metabolic state interaction.

In sum, AN subjects show dampened hedonic fluctuations of food-odors regardless of their internal state. They appear to be anallies-thesic. More generally, metabolic state-related changes in the hedonic value of food odors may bear regulatory impact on food intake at the next meal. Specifically, the low hedonic value of food odours reported by hungry AN subjects may be correlative or causal in their demotivation/rejection towards food intake. Thus, the assessment of liking/wanting to given food odorants in non-satiated AN patients may be a way to evaluate the gravity of their anorexic status.

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Peripheral Olfactory Function in Rats after Chemotherapeutic Treatment with the Anticancer Drug Docetaxel

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Clinical studies have documented that chemotherapeutic cancer treatment in humans is often associated with weight loss and decreased enjoyment of food. Beside taste, olfaction plays a role in the food intake regulation. We assessed whether chemotherapeutic cancer treatment compromises olfactory function in rats treated with docetaxel (Taxotere®, TAX), an antineoplastic drug which disrupts the structures necessary for cell survival and division. Electro-olfactogram responses (EOG) can indicate morpho-pathological changes in olfactory epithelium. Male rats received either a single, two or three intravenous injections (one per week) of an estimated 10% lethal dose (LD10) of TAX. After a recovery period of 7-9 days the peripheral olfactory function was measured with EOGs from the epithelium overlaying the septum and the turbinates. We tested three different odorants, isoamyl acetate, benzaldehyde and cineole at three different matched recording sites delivered in vapor phase. To assess changes in sensitivity we recorded concentration response curves during days 3-6 after a single drug administration using submerged EOG recording technique allowing a precise control of stimulus concentration. Single, double or triple doses of TAX caused significant attenuation of weight gain over the duration of the experiment. Whatever the duration of the treatment all recording sites were responsive to the three odorants and all stimuli evoked typical EOGs with rapid rising phase and a slower decline. Odorant stimulation as well forskolin and IBXM in the range of 10-6 to 5x10-4 M elicited typical EOG responses in a concentration–dependent manner. Response thresholds and curve shapes were not to be affected by the single treatment. Vapor-phase supraliminal stimulations revealed effects related to the strength of the treatment. Surprisingly, the maximal amplitude of the EOGs for a given odorant and location was greater in rats after two taxotere injections than in untreated animals. The study failed to demonstrate a detrimental effect of taxotere on the peripheral olfactory function in rat.

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Chronic Corticosteroid Anti-Asthma Aerosol Treatment Affects Olfaction in Mice

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Asthma is a common disease affecting a great number of persons that need continuous treatment often for years. In humans, some drugs are administered via aerosol to reach the lungs where they are active. Some persons sporadically report modification in their olfactory sensitivity after chronic aerosol treatment, therefore we explored the possibility that one of the most commonly used anti-asthma agent, the corticosteroid fluticasone propionate, could influence the olfactory function by travelling through the nose. A group of six mice were exposed to a clean air stream while a group of six mice were exposed to fluticasone propionate aerosol, 20 minutes twice a day for 42 consecutive days, before being tested for olfactory performance and eventually sacrificed. Histology and immunohistochemistry for Olfactory Marker Protein (OMP) were performed on the olfactory mucosa and olfactory bulb.

On the Cookie finding test, the mice exposed to fluticasone propionate showed an impairment in retrieving a piece of food when it had to be discovered using olfaction, while they were as fast as controls when the food was visible.

On the other hand, the presence of a clearly detectable odor (thymol) in the environment similarly modified exploration activity in both treated and control mice, suggesting that olfaction was not affected to a high degree, and olfactory function was still available, provided that the stimulus was above threshold. Histology indicated that the height of the mucosa in fluticasone-treated mice was larger than in controls, a result confirmed by OMP immunolabelling, while no difference was apparent in OMP immunoreactivity of the olfactory bulbs.

The present results suggest that a mild impairment of olfactory function is present in mice chronically treated with fluticasone propionate aerosol, apparently accompanied by modifications in the olfactory mucosa.

Limited Involvement of the Olfactory System in a Murine Parkinson Model

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First signs of Parkinson's disease (PD) in humans often include olfactory deficits and histological modifications of the olfactory system. Several animal models for PD are now available, but most of them involve the acute administration of a toxic agent, resulting in a quite fast onset of the disease, while in humans PD is a neurodegenerative disorder that requires years for becoming clinically apparent. We investigated whether in one model of PD, obtained in mice via the injection of the toxic agent 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP), the motor deficits and the impairments in dopaminergic neurons are accompanied also by olfactory deficits, both clinically and histologically apparent.

A group of five mice served as controls, while a group of six mice were injected with MTPT. Mice were then tested for olfactory retrieving of hidden food with the Cookie finding test: no significant

difference was apparent between controls and treated mice when food was hidden, as well as when it was visible. On the other hand, MPTP-treated mice showed all the motor signs that were expected, by being very slow in moving in an open field arena, and by being slower than controls in coming down from a vertical pole. Immunohistochemistry revealed that in the olfactory bulb there was no difference in tyrosine hydroxylase labeling, while a strong decrease was present the substantia nigra and tegmentum, suggesting a differential involvement of olfactory dopaminergic neurons after MPTP treatment. In the olfactory bulb, a decrease in Olfactory Marker Protein immunolabelling was present in MPTP-treated mice compared to controls. In addition, in MPTP-treated mice OMP-immunolabelling was fainter in lateral glomeruli compared to medial glomeruli, while no difference was apparent in control mice between lateral and medial glomeruli.

The present data suggest that the MPTP model for PD is not accompanied by clearly detectable olfactory deficits, while variations in OMP labeling point to an involvement of olfactory structures and deserves further studies.

Topic 25: Taste in Medicine

Causative Factors and Treatment of Taste Disorder

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Introduction: Taste disorders occur most often in aged people, their number increases with age. We examined causative factors in 408 cases of taste disorder and studied therapeutic effects of zinc at different ages of patients. Subjects: All the patients complaining of taste reduction originated from the Department of Nihon University Itabashi Hospital. Their ages ranged from 21 to 84. The therapeutic effects of treatment of taste disorder with zinc were studied in 252 patients.

Methods: The causative factors for taste disorder were classified as: drug-induced, zinc deficiency, systemic diseases, inflammation of the upper respiratory tract, head injuries, glossitis, loss of flavor and idiopathic factors. Patients with taste disorders due to zinc deficiency had lower than normal serum zinc concentrations (<69 μg/dl), the triggering causes of the deficiency were not known. The taste functions were evaluated with the filter-paper disk method and by application of solutions of sucrose, sodium chloride and tartaric acid. The zinc agent, polaprezinc, was given at daily doses of 150 mg/day (75 mg, b. i. d.). Polaprezinc is a zinc-carnosine complex formulated as white odorless granules whose primary use is peptic ulcer treatment. 75 mg of polaprezinc contains ~17 mg of zinc.

Results: Drug-induced taste disorders were the most frequent (32% of cases), followed by taste disorders due to idiopathic factors, systemic disorders, and zinc deficiency. Among the 408 patients, 116 cases (28%) showed low serum zinc concentration regardless of the causative factors of the disorder. In the aged group taste disorders had significantly higher incidence, they were provoked by drugs and systemic disorders. In the aged group significantly lower causative factors were either idiopathic or inflammation of the upper respiratory tract. Zinc administration was an effective treatment in 70% of all the patients studied, 74% of the aged patients. No significant differences ware observed in the curative effectiveness rates between different age groups.

Quantitative Taste Function Studies in Adults Undergoing Treatments of the Ear

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Aim: This study aims to establish what effect different forms of middle ear and skull base surgery have on taste sensitivity, compared with controls. The secondary aim is to establish whether the different disease processes being treated have an effect on taste.

Design: A prospective, controlled clinical trial.

Materials and Methods: The sample comprised 25 patients undergoing surgery of the middle ear/mastoid and 7 patients with inner ear (acoustic) surgery; the control were 25 patients with similar middle ear conditions and 20 patients with acoustics all with conservative management. Both groups were reviewed at baseline and at 4-6 months. The sample group was also seen 2-4 weeks postoperatively. In addition, 33 patients having undergone previous ear surgery were also reviewed, and 31 patients with facial palsy were reviewed as well. Exclusion criteria included those under 16 years old, those unable to sign consent, and those experiencing a stormy perioperative course. Patients' taste was measured quantitatively using a TR-06 Rion electrogustometer, and correlation was made with subjective questioning about taste and clinical examination.

Results: Mean differences on the affected side of the tongue from baseline to final follow-up at 4-6 months were significant for the middle ear/mastoid ear surgery sample group ($p=0.024$) and for the recovery of the facial palsy group ($p=0.010$). The remaining groups were not significant: acoustic surgery $p=0.21$; middle ear controls $p=0.64$; acoustic controls $p=0.10$; previous ear surgery $p=0.83$.

This research establishes the implications of middle ear and mastoid surgery, and the impact of facial palsy and acoustic neuromas, on taste thresholds.

T2R Gene Family Expression in the Tongue of Patients with Taste Disorder

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Purpose: The receptors for sweetness, umami and bitterness are believed to be G protein-coupled receptors (GPCR). G protein forms a gene family of taste receptor genes. Genes associated with bitterness are called the T2R gene family. This gene family has not yet been sufficiently elucidated in humans. We evaluated gene expression of the T2R family in the human tongue in normal healthy subjects and in patients with taste disorders. Fifty four

healthy subjects were selected with no taste disorders (12 males, 38 females) were selected. Their age was 20 to 73 years the average age was 34 years. There were 51 patients (20 males, 31 females) who visited our clinic due to taste disorders and were confirmed by a taste test using the filter paper disk method. The age of the patients was from 25 to 88 years and the average age was 61 years.

Methods: Samples were collected by scraping the foliate papillae of the tongue and total RNA was extracted using TRIzol (Invitrogen). A reverse transcription reaction was performed for total RNA using Super Script III, and PCR was performed using Ex Taq (Takara). Electrophoresis was performed using a 2100 Bioanalyzer (Agilent). Gene expression was evaluated in 10 genes: T2R3, 8, 9, 10, 13, and 16 and THTR4, 5, 9, and 11.

Results: When the frequency of gene expression was compared between healthy subjects and the patients with taste disorders, T2R3, 8, 9, and 10 and THTR4 and 5 showed significantly decreased frequencies of expression in the patients with taste disorders. When evaluated for the taste disorders, the expression of T2R3, 8, 9, and 10 and THTR5 were significantly decreased in patients with decreased serum zinc levels. Moreover, the patient group with taste disorders related to bitterness tended to show lower taste gene expression compared to healthy subjects. Especially T2R3, 8, and 9 and THTR4 and 5 showed statistically significant decrease in gene expression.

Conclusions: Compared with healthy subjects, patients with taste disorders showed a decreased expression of taste genes in the tongue. In particular, in patients diagnosed with zinc deficiency, expression of taste genes was decreased. It was suggested that a decreased expression of taste associated genes could be involved in the mechanism of taste disorders in humans.

The Study of the Taste Receptor Gene Expression on the Tongue of the Patients with Dysgeusia

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In the past two decades, tremendous progress has been achieved with the discovery of the taste receptor genes. G-protein coupled receptors (GPCRs) are thought to be the taste receptors for sweet, bitter, and umami. T2R are a family of GPCRs that are implicated in bitter taste sensing. THTR family is known to have high homology with T2R family. Since, in our study, patients with dysgeusia frequently complain of spontaneous bitter taste. We have investigated the taste receptor gene expression on the tongue of the patients with dysgeusia, concerning T2R family and THTR family. The genes we analyzed were T2R 3, 8, 9, 10, 13 and16, and THTR 4, 5, 9 and 11.

The subjects comprised 41 patients who presented to the ENT department of Nihon University Itabashi Hospital complaining of dysgeusia. We took specimens by scraping their foliate papillae with the lid of the Eppendorf tube. From these specimens, taste receptors gene expression was detected by RT-PCR method and electrophoresis.

For the dysgeusia patients, the frequencies of detecting gene expression of T2R 3 and 9, and THTR 4, and 9 were significantly greater compared with normal control subjects.

Revisiting Reliability of the Rion TR-06 Electrogustometer

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Aim: To establish our own test-retest reliability results for the currently most accepted electrogustometer (Rion TR-06) in order to interpret confidently clinical results and to establish a standard by which newer electrogustometers can be rated.

Materials and Methods: Electrogustometry was performed with the TR-06, using stainless steel single 12.5mm^{2}probes and 0.5sec anodal pulse duration on 25 healthy young students with no perceived gustatory abnormalities, nor conditions known to cause these disorders (12 male, 13 female; 21-29 years, M=22.9). Tests of right and left anterior tongue were performed at baseline and repeated 4 weeks later (24-30 days, M=27.6). The testing employed a two-alternative forced-choice (stimulus/blank), ascending threshold procedure with a criterion of five consecutive correct responses initially followed by two consecutive correct responses subsequently, commencing at 8dB, in 4dB steps. Our TR-06 used the -6dB to 34dB (4-400 μA) range without attenuators. Results were processed in microamperes (μA) to avoid logarithmic conversions.

Results: Mean thresholds (±SD) for the participants were 6.09 (±1.6) μA (6.20 right; 5.98 left). These differences were insignificant ($p<0.316$). Test-retest reliability coefficients were 0.816 (right side; $p<0.001$) and 0.856 (left side; $p<0.001$).

Conclusion: The Rion TR-06 electrogustometer demonstrated consistent reliability over time in this cohort of young participants. We discuss factors that can influence reliability and validity, concluding more tests on the TR-06 are required before exacting comparisons can be made with newer automated electrogustometers. Standardization of the various psychophysical methods of electrogustometry should be agreed.

Topic 26: Taste Basic

Taste Responses to Various Umami Substances in Mice

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Various taste substances were included in food, and they contribute to taste of the food. Among them, umami substances play important role to decide the taste. However, there are few studies about their taste characteristic except for representative umami substances such as glutamate. Using mice behavior studies (48h two bottle preference test and conditioned taste aversion test) and gustatory nerve

response, we investigated taste characteristic of unique umami substances including L-theanine, sodium succinate, and betaine. Furthermore, we examined synergy of umami with IMP. In mice, sodium succinate had umami, and it showed the synergy with IMP. L-theanine showed the synergy with IMP, but did not present umami with the theanine alone. On the other hand, the betaine hardly presented umami and did not show the synergy between IMP.

The Palatability of Fatty Acids to Mice as Measured by Short-Term Two-Bottle Choice and Licking Tests

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Free fatty acids (FFAs) were reported to be recognized in the oral cavity and possibly involved in fatty foods recognition. However, dietary oil generally consists mainly of triacylglycerol and a low concentration (less than 1%) of FFAs. To understand the importance of oil recognition in the oral cavity, we investigated the effect of various concentrations of a fatty acid or corn oil on fluid intake as well as mice's preferences in a two-bottle choice test and a licking test in mice. Linoleic acid (LA), which is a main component of corn oil, was used as a representative FFA. In the two-bottle choice test between a pair of different concentrations of corn oil, the mice consistently adopted the higher concentration of corn oil. In the licking test for corn oil, the licking rates for the serial concentration of corn oils (0, 1, 5, 10 and 100%) were increased in a concentration-dependent manner. On the other hand, in the two-bottle test for a pair of different concentrations of LA (0, 0.125, 0.25 and 1%), 0.25% and 1% LA were preferred to mineral oil, but 0.25% and 1% LA were preferred equally in mice. In the licking test for LA, the mice showed the largest number of initial lickings for the 1% LA, while the licking rates for the high concentration of LA decreased. These results suggest that mice could discriminate the concentration of corn oil and LA in the oral cavity. We also suggest that the small amount of lipase-digested FFA in the oral cavity has at least some role in the recognition that occurs in the oral cavity in mice. In the case of human, which has less lingual lipase, may detect free FFA spontaneously generated in cooking and process of maturation of foods. This study was supported by the Program for the Promotion of Basic Research Activities for Innovative Bioscience.

Biosynthesis and Trafficking of Human Bitter Taste Receptors

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A complex set of G protein-coupled receptors is devoted to the detection of noxious substances, most of which taste bitter to humans, across a wide range of animal phyla implicating important roles during evolution. The 25 human TAS2R receptors are all expressed in gustatory papillae. As natural and synthetic bitter substances are plentiful and usually cause aversive reactions, TAS2Rs profoundly influence our daily live. Because of their fundamental role in human nutrition, considerable effort was made to identify agonist-receptor pairs utilizing heterologous expression in combination with functional assays. Unfortunately, functional expression of native TAS2Rs is difficult because of limited cell surface localization. From this, we conclude that TAS2Rs require a specific cellular environment for proper biosynthesis and routing.

Here, we report on our recent advances in determining critical steps involved in hTAS2R maturation. By functional heterologous expression of hTAS2Rs with their native N-termini, thus excluding the commonly added export-epitopes, we observed a high degree of individuality among the receptors ranging from receptors, which do not require those tags, to receptors that are non-functional without them. Yet another group of hTAS2Rs exhibited a variable functional impairment. For some of these latter receptors, we demonstrated an improvement of function by co-expression of receptor transporting proteins 3 and 4. Cell surface biotinylation experiments revealed that these auxiliary proteins improve plasma membrane targeting of hTAS2R16, thus explaining the observed functional rescue. These findings did, however, not explain the differences in export-tag dependence, a feature that must ultimately reside in the amino acid sequence of the individual receptors. We therefore compared the amino acid sequences of all hTAS2Rs and recognized a highly conserved consensus site for N-glycosylation within the 2nd extracellular loops. Again, using functional expression and biochemical analyses in mammalian cell lines, we subsequently showed that glycan structures added to the conserved site is a major determinant for hTAS2R function.

Understanding the Interactions Between Saliva and Taste Perception

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Saliva is known to play an important role in food perception. During eating the food stimulates salivation. Saliva dissolves the taste molecules and transports them to the taste receptor. Consequently, both the volume and composition of the saliva influence the taste perception. Therefore, understanding the mutual interactions between orally processed food and salivation is crucial in order to understand perception of foods. Part of these complex interactions is the effect of taste stimulation on saliva. A method was developed to measure salivary responses simultaneously, by continuous measurements, allowing monitoring compositional changes in time. Manipulation with the four basic tastes, sweet, salt, sour and bitter, also gave insights into the relationship between stimulus composition and salivary response. In each session different concentrations of one of the four taste stimuli and water were presented to the assessors. The concentrations were all based on the taste threshold for the specific tastes, obtained from literature. The tastant concentrations used were multiples of the taste threshold concentration. The stimulus was given at equal time intervals using an automated pump system (a gustometer), which also provided the assessors with

instructions via a computer screen, for example to score the intensity of the stimulus. During each session parotid saliva was collected continuously using a Lashley cup. Saliva fractions, taken every minute, were weighed to establish saliva flow rates per minute. The saliva fractions were analysed for pH, amylase activity and protein concentration. These measures were compared to the different stimuli, and to each other. Comparison between stimuli could be done in two different ways: relative to concentration levels or to perceived intensity levels. It was found that different tastants affect saliva response in different ways, both in threshold and growth rate. This means that depending on the concentrations of the compared stimuli, different results will be obtained with regard to the differences between stimuli. By measuring these responses simultaneously and continuously, salivary response mechanisms can be better understood. This will help completing the picture of how interactions between saliva and the food matrix affect food perception.

Effect of Tobacco on Gustatory Sensitivity, Evaluation of the Deficit and Recovery Time-Course During Smoking Cessation: A New Tool to Help Smokers to Quit

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Background: Literature results have shown that chronic exposure to cigarette smoking affected taste function in human. However, neither the quantitative impact on taste sensitivity, nor the time-course of taste recovery at weaning, or the affected regions of the tongue were precisely examined.

Methods: Electrogustometric (EGM) thresholds (iontophoretic application of saliva cations) were measured repeatedly (double blind tests) from t-30 days to t+292 days (n= 23) after cigarette quitting, every week then every month after t+30d, at nine loci on the tongue surface in healthy smokers, without dental deafferentation or medication. EGM was measured in 35 healthy controls who never smoked. Results were examined individually.

Results: Smokers (n = 54) exhibited significantly higher EGM thresholds than non-smokers ($p<0{,}0001$; $p<0.001$ depending on locus; Mann–Whitney U-test). The higher the Nicotine Dependence (Fagerström scores), the higher the thresholds. During smoking cessation, EGM thresholds decreased progressively, for example, at side tip: from 35 to 4.6 μA and eventually reached the taste sensitivity range of non-smoking controls depending on locus and time: tip of the tongue, total recovery < 30 days, edges and posterior tongue, total recovery > 30 days, dorsal part of the tongue, total recovery in some cases: > 6 months except for heavy smokers who had not yet fully recovered on dorsal loci after 10 months.

Conclusion: Taste sensitivity recovered sooner at the tip of the tongue where the density of papillae is higher. EGM provides a reliable quantification of taste sensitivity disturbance and recovery in smokers with immediate reading. This evaluation of taste impairment provides (i) a good indicator of the harmful consequence of smoking, thus constituting a possible alternative to biological primary biomarkers (CO, nicotine and related metabolites); (ii) an efficient motivational help for tobacco weaning through the observation by the patient of his/her own but objective evaluation of taste sensitivity recovery.

Genetics of Taste in Genetically Isolated Populations: From Phenotypes to Genotypes

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Variation in the bitter-taste receptor gene, TAS2R38 confers the ability to taste 6-n-propylthiouracil (PROP). In this study, we determined the PROP phenotype distribution and the correlation between taster status and TAS2R38 gene in a genetically isolated populations form Italy. Moreover, we tried to relate TAS2R38 haplotypes and PROP-tasting phenotypes to adiposity in the same populations: we hypothesized that the non-taster phenotype would be associated with higher BMI and waist circumference (WC) in females. Participants were 540 healthy inhabitants of the genetically isolated village of Carlantino in Southern Italy. Some SNPs were selected to analyze TAS2R38, TAS2R4 and BDNF genes. PROP tasting was assessed using a filter paper method. Height, weight and WC were measured. The same study has been replicated in Stoccareddo village (198 collected inhabitants) situated in Northern Italy, and a bigger sampling is in progress in Friuli-Venezia Giulia region. Here, 2500 inhabitants of 6 villages are under study. As regards to bitter perception, the PROP phenotype distribution found in Carlantino was in agreement with data already reported for other Caucasian populations. A strong association between taster status and the TAS2R38 gene in Carlantino's population was showed. However, only a part of the variation in phenotype was explained by TAS2R38 genotype. In this light, additional genes have been analyzed, but no correlation was found. We plan to investigate other TAS2Rs receptor genes to verify their involvement in bitter perception. Non-taster females had higher BMI and WC than female phenotypic tasters. Neither TAS2R38 haplotype nor PROP phenotype was strongly related to BMI or WC in males. In Stoccareddo village, while the PROP phenotype distribution was overlapping with those of Carlantino, the contribution of TAS2R38 gene was slightly higher. The study of additional villages can help to understand the role of TAS2R38 gene in bitter taste perception and in human health status.

Structural and Genetic Biology of Bitter Taste: A Combined Approach

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Bitter taste evolved to prevent animals from ingesting toxic substances, provoking an aversion response. Understanding the molecular basis of bitter taste perception may help improve the palatability of intake products. Bitter taste perception involves a family of G-Protein Coupled Receptors (GPCRs), encoded by 25 functional genes in humans. Little is known about structural, genetic and functional characteristics of these receptors. Here we propose a combined experimental and bioinformatics approach to deepen in the knowledge of bitter taste receptors' structure and ligand specificity. We aim at building a structural model for bitter taste receptors and at developing a platform to characterize ligand-receptor specificity. We initially considered the most characterized bitter taste receptor, T2R38 that binds phenylthiocarbamide and 6-n-propylthiouracil [1]. We determined the evolutionary relations with other GPCRs by applying Psi-Blast searches. Aligning the protein sequences and constructing homology modeling structures, we identified 15 putative residues involved in ligand binding and proposed mutants which could affect the ligand affinity. Molecular biology experiments are being performed to validate our hypothesis. To establish a stable Gα15-protein expressing cell line we transfected HEK-293 with a vector carrying GNAT15 cDNA. The correct integration was assured by using FRT sites and confirmed by hygromycin and zeocin negative and positive selection, respectively, and by control PCR. The proposed mutants will be then transfected in HEK-293 and the receptor activation or inactivation will be tested by a calcium imaging assay [2]. Based on the functional assays results we will improve the structural model and enhance our predicting power, similarly to what was done for odorant receptors [3]. Our approach will contribute to the understanding of the structural and molecular determinants of bitter molecules binding to these receptors.

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In situ Calcium Imaging of Epithelial Cells Surrounding Taste Buds in Mouse Fungiform Papillae

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Single taste bud in mouse fungiform papillae contains about 50 cells (TBCs) which classified to four cell types, type I to type IV. In the periphery of the taste bud, they border at the perigemmal cells and, together with them, form a shell of the taste bud. It had been reported that the application of ATP to basolateral membranes of TBCs increased the cytosolic calcium concentrations ($[Ca^{2+}]_{in}$) in some TBCs in single taste bud. However, responsiveness of perigemmal cells to neurotransmitters was unknown. In this study, we focused on perigemmal cells and investigated whether these cells were sensitive to neurotransmitter under an in-situ optical recording condition with peeled lingual epithelia of mouse. In brief, the lingual epithelia of a ddy-strain mouse were peeled with a subcutaneous injection of protease solution (1.5 mg/ml, 8 min.), then were loaded with acetoxymethyl (AM) ester derivative of calcium indicator, Fura-2. The epithelia mounted on a recording platform placed under an upright fluorescent microscope with a 60X-water- immersed objective. The applications of 5 μM serotonin and 100 μM acetylcholine increased $[Ca^{2+}]_{in}$ in perigemmal cells. The application of 1 μM ATP also increased $[Ca^{2+}]_{in}$ in most of perigemmal cells . It seemed that responses to ATP were generated initially in several perigemmal cells and were transmitted to neighbouring cells one after another. Here, we found that some perigemmal cells showed dye-couplings by the diffusion of the probe dye, Lucifer Yellow, from injected cells to their neighbours. Octanol, gap-junction inhibitor, also blocked the ATP-induced increase $[Ca^{2+}]_{in}$ in a part of perigemmal cells. These ATP-induced responses were inhibited by removal of extracellular Ca^{2+}.These results indicate that some perigemmal cells are coupled by gap junctions, and ATP receptors are expressed in a part of perigemmal cells. It is pointed out that electric and chemical synapses contribute to form cell-networks of TBCs. Here, we discuss the role of perigemmal cells on TBCs functions.

Topic 27: Molecular Biology – Olfaction

Odorant Receptors in Olfactory Cilia and Axons

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Odorant receptors (ORs) are supposed to act not only as molecular sensors for odorants but also as cell recognition molecules guiding the axons of olfactory sensory neurons to their appropriate glomerulus in the olfactory bulb. This concept implies that OR proteins are located in sensory cilia and in the axons. To approach this critical issue, antibodies were generated against two peptides, one derived from olfactory receptor mOR256-17, one derived from the 'mOR37' subfamily. By means of immunohistochemistry and double-labelling studies using transgenic mouse lines it was demonstrated that the newly generated antibodies specifically recognized the receptor proteins. Western Blot analyses of isolated cilia preparations revealed a single immunoreactive band for each OR. To scrutinize the hypothesis that OR proteins may also be present in the axonal processes and the nerve terminals, serial sections through the olfactory bulb were probed with the antibodies. Two glomeruli in each bulb were stained by anti-mOR256-17, one positioned in the medial, one in the lateral hemisphere. Fiber bundles approaching the glomeruli through the outer nerve layer also displayed intense immunofluorescence. In immunoblotting experiments using microdissected OB tissue anti-mOR256-17 recognized a band with a molecular mass different from that in cilia. Altogether, these data demonstrate that OR proteins are indeed present in the cilia and the axons of olfactory sensory neurons and indicate that OR versions with different molecular features may be present in these two compartments.

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 co-converged 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.

The OR37 Subfamily: Establishment of the Clustered Expression Pattern

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Odorant receptors (ORs) are encoded by the largest gene family in vertebrate genomes, comprising of more than 1000 members. Each olfactory sensory neuron (OSN) in the olfactory epithelium (OE) selectively expresses one particular receptor gene from this large repertoire; moreover, only one of the two alleles is chosen per cell. OSNs which express a distinct OR gene are usually broadly dispersed throughout the OE; in contrast, cells expressing a member of the OR37 subfamily show a unique pattern: they are located exclusively in a small central patch of the OE. The regulatory mechanisms which govern OR gene choice of individual OSNs in a topographically restricted manner are still largely elusive. To obtain more insight into the underlying principles we have employed a transgenic approach in mice that allowed to permanently label all cells that selected a defined OR gene for expression. For this purpose a knock-in mouse line was generated in which expression of one member of the OR37 subfamily (OR37C) leads to the co-expression of Cre-recombinase (OR37C-IRES-Cre). By crossing this line to Cre-reporter mouse lines, all cells could be visualized that transcribed OR37C at any time during development. As expected, labelled OSNs were found in the central patch which is typical for OR37 expression. Surprisingly, however, numerous additional OSNs were found which were broadly dispersed throughout the OE. Using *in situ* hybridization, mRNA for OR37C could only be detected in those cells located in the typical 'OR37 patch', suggesting that all ectopic OSNs had ceased OR37C expression. The question whether these cells may undergo premature apoptosis was addressed by immunohistochemical analysis for active caspase-3; none of them, however, expressed this pro-apoptotic marker. A close examination of the ectopically positioned OSNs revealed that they all extended an axon towards the olfactory bulb (OB), and indeed many glomeruli could be detected which contained a few labelled fibers. The location of these glomeruli in the medial and lateral domains of the bulb indicated that these represented glomeruli that receive input from OSN populations expressing other ORs than OR37C. Altogether, these data indicate that OSNs which initially express OR37C outside the typical patch do not continue, but switch to the expression of a different OR gene, suggesting the involvement of a feedback mechanism downstream of gene choice that restricts OR37C expression to the central patch.

G Alpha Protein Subtypes in the Teleost Olfactory System

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Heterotrimeric G proteins act as molecular switches which relay the signals from G-protein coupled receptors to intracellular signaling pathways which trigger further activities. In contrast to mammals, teleost species have only one olfactory epithelium, in which three different types of neurons (ciliated, microvillous, and crypt neurons) corresponding to main and accessory olfactory systems are located. All of four known olfactory receptor families (ORs, V1Rs/ORAs, V2Rs/OlfCs, and TAARs) are expressed within the teleost olfactory epithelium. However, little is known about subtypes of G alpha proteins expressed in teleost olfactory epithelium, their coupling to olfactory receptors, and their cellular localization. We have established the complete zebrafish G alpha protein family by data-mining in publicly available genomic databases. The number of G alpha proteins in zebrafish is almost 1.5 times as high as that in mammals. Many genes including those expressed in the mammalian olfactory system such as Golf, Go, and Gi2 have duplicated paralogs. RT-PCR analysis revealed that most genes of the G alpha family are expressed in the zebrafish olfactory epithelium. These data are consistent with the notion that each receptor family employs specific G alpha proteins for the signal transduction. To analyze this assumption, we are currently examining the cell types expressing each G alpha gene in the zebrafish olfactory epithelium by in situ hybridization and co-labeling with cell type marker genes. This study will serve to understand the molecular mechanisms underlying the signal transduction of each of the three types of olfactory neurons.

Differential Expression of Class I Odorant Receptor Genes in *Xenopus tropicalis*

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Olfaction is important for animal survival. A great variety of odorants are recognized by a huge number of odorant receptors (ORs) which are expressed on the surface of olfactory sensory neurons (OSNs). Each OSN expresses only one OR gene from a large family

of OR genes and an OR gene choice is stochastic. OR genes are classified two classes. Class I and class II are referred as the fish-type and tetrapod-specific, respectively. The class I ORs are thought to recognize water-soluble odorants and class II ORs are for air-borne odorants.

Amphibian frogs have two distinct life stages, aquatic larva and terrestrial adult. Frogs are a unique animal which has both water-filled (MC) and air-filled (PC) nasal cavities, which express fish-type and tetrapod-specific ORs, respectively. Earlier studies in *Xenopus* showed differential expression of several OR genes during development. However, global analysis of entire OR gene expression has not yet been carried out.

We have identified 60 functional class I OR genes in the *Xenopus (Silurana) tropicalis* genome and assessed the expression of all them. Class I OR genes consist of 4 subclasses (3 of them are shared with fish and one is common among tetrapods) and genes in each subclass are located within one or small number of gene clusters. All class I OR genes but one exception which is expressed both MC and PC are preferentially expressed MC in adult frog. These results suggest that one particular class I OR may function as a receptor for airborne odorants as well as water soluble odorants. Most of the genes are expressed in both larva and adult. Interestingly some OR genes are expressed at specifically high levels in larva whereas the expression of all OR genes is almost even in adult. These results indicate a possibility that several ORs use different mechanisms to express the genes between larva and adult.

Functional Expression of Olfactory Receptor Genes in a Heterologous Cell Line

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Smelling is a very complex event in which molecules from the environment are inhaled through the nose and dissolved in the mucus of the olfactory neuroepithelium where they subsequently bind to olfactory receptors (ORs). Once a receptor has been activated, a cascade of events is initiated that transforms the chemical-structural information contained in the odorous stimulus into a neural signal, i.e. a membrane potential. This signal is projected to the first relay place in the brain, the olfactory bulb, from where it is transported to higher regions of the brain.

Recombinant expression of G protein-coupled receptors (GPCRs) in heterologous systems is widely used for high throughput screening of ligands. In the past, ligand activation of mammalian ORs has been studied in a variety of host cells. But today, most of the approx. 380 ORs, the largest family of GPCRs, are still orphan receptors. This is due to the fact that in heterologous cell lines the transport of the recombinant OR proteins to the cell surface are insufficient. Consequently, the percentage of functional ORs that are accessible to the odorants is low. Co-expression of the OR with additional proteins (e.g. chaperones) did improve the transport to the cell surface for some of the OR proteins. But 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. Activation specificity of OR17-40 with known ligands was consistent with data from the literature and receptor activation occurred in a dose-dependent manner. A library of odorant molecules with a broad spectrum of molecular structures was used to study the activation profile of OR17-40. The identified 'hits' were confirmed by establishing concentration-dependent curves and determining EC_{50} values for the newly found agonists. For some of the identified agonists, a series of structurally related molecules was tested for its ability to activate OR17-40 and their EC_{50} values determined.

Strong Positive Selection and High Intron Dynamics in Teleost *TAAR* Genes

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Trace amine associated receptors (TAARs) have recently been identified as a fourth family of mammalian olfactory receptor genes. We have performed a thorough search for *taar* genes in genomes of five teleosts, two basal fish and seven higher vertebrates. *Taar* genes segregate into three classes, with class III being exclusively teleost. Teleost family size ranges from 18 to 112 genes (pufferfish and zebrafish, respectively), while mammalian families contain at most 19 genes (opossum).The TAAR family originated in the common ancestor of bony and cartilaginous fishes, after its divergence from jawless fish. Most extant teleost *taar* genes have emerged late in evolution, well after the split between basal teleosts (zebrafish) and *neoteleostei* (stickleback, medaka, pufferfish). This recent surge in evolutionary rate affects the genomic layout, as zebrafish *taar* genes are largely arranged according to phylogenetic proximity in two big clusters (both syntenic to the single sarcopterygian gene cluster). Moreover, global negative selection in teleost *taar* genes is less pronounced than in mammalian *taar* genes. The accelerated evolution of especially class III teleost *taar* genes is reflected in minimal global negative selection, an unprecedented degree of local positive selection, the complete loss of the aminergic binding motif, as well as unusually high intron dynamics, which led to four independent intron gain/loss events during evolution of neoteleosts. Consistent with a function as olfactory receptors we observe that from a representative subset of eight zebrafish *taar* genes all but TAAR1 were expressed in sparse subsets of olfactory receptor neurons.

Study of Chemotopic Representations of Glomeruli in the Rat Olfactory Bulb

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The olfactory bulb (OB) of vertebrates represents chemical information in distinctive zones. This property has been called "chemotopy". We wanted to gain insights into how molecular properties of odorants are organized in the OB, especially, where their representational sites are located. For this purpose, we tried two means of analysis on experimental image data of the rat OB: i) we obtained representational maps by statistically testing image pixels corresponding to glomeruli,

and ii) searching for representational modules by application of clustering techniques. Clustering involved first fuzzy c-means with $k=\{2,\cdots,30\}$ finding clusters which were coherent and distinct from each other with respect to the activations of points. We then performed the Wilcoxon-signed rank test in order to determine significant differences of within-cluster and outside-cluster activations given chemical odorant properties. Discarding clusters which were not distinct with respect to their chemical representation or which were superseded in their chemical representation by overlapping smaller clusters (sub clusters) we obtained a number of clusters which are distinct in their activations as well as in their chemical representation. Our conclusions are threefold: We find the centers of representations for several chemical dimensions. We confirm the finding that there are zones in the olfactory bulb of rats, which are different with respect to glomerular activation and chemical representation. We present representational maps that show where these modules are located and to which chemical properties they correspond.

Topic 29: Olfaction in Insects

Physiological and Morphological Characteristics of the Serotonin Immunoreactive Neuron in the Antennal Lobe of the Male Oriental Moth *Helicoverpa assulta*

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Olfactory receptor neurons send projections directly to the antennal lobe, the primary olfactory centre of the insect brain. Here, the terminals make synapses with second-order neurons in spherical structures termed glomeruli. Two main types of antennal-lobe neurons, projection and local interneurons, have been characterized in several insect species. In addition, a few unique centrifugal neurons give efferent input from other brain areas. One pair of serotonin-immunoreactive (SI) centrifugal neurons, each innervating one antennal lobe, is suggested to modulate the processing of olfactory information (Kent et al. 1987, J Neurobiol; Kloppenburg et al. 1999, J Neurosci). Whereas the morphology of the SI neuron is relatively thoroughly described in several insect species, only one study has so far reported about the physiological properties of the neuron (Hill et al. 2002, Chem Senses). Based on intracellular recordings and staining combined with immuno-cytochemistry, we present data dealing with morphological and physiological characteristics of the SI antennal-lobe neuron in the noctuid moth *Helicoverpa assulta*. The neuron has a large soma in the lateral cell cluster of the antennal lobe and projects via the protocerebrum to the contralateral antennal lobe where it innervates each glomerulus including the male specific units and the ordinary glomeruli. Fine processes arborize in bilateral areas of the protocerebrum. Thus, the morphology is similar to that of the SI antennal-lobe neuron initially described in the sphinx moth *Manduca sexta* (Kent et al. 1987). The neuron presented here fired long lasting action potentials. Two distinctly different amplitudes appeared, a small spiking type showing a high spontaneous activity and a large spiking type showing low activity. The small spiking amplitude responded with an increased frequency of action potentials to all stimuli applied to the antenna whereas the large spiking amplitude showed no responses. The presence of two spike categories in the same recording indicates that the neuron serves multiple functions.

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Perception of Plant Volatiles in the Hawkmoth Manduca Sexta (Sphingidae) and Their Contribution to Oviposition Behaviour

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Peripheral detection of plant odorants and central nervous processing of this information has been studied in *Manduca sexta* females. *M. sexta* oviposition preferences are, however, known to be strongly influenced by host plant species as well as geno- or ecotypes. Also manipulation of the plants gene expression as well as damage inflicted by feeding conspecifics or other herbivores is known to have a strong effect on oviposition behaviour. The olfactory basis of these choices is presently not established. We investigate the missing links between female sensory physiology, behaviour, and decision-making.

Electrophysiological experiments reveal that the olfactory ranges of *M. sexta* females and males are far broader than previously known. Comparison of female antennal responses to odorants from differentially preferred host plants point at compounds, which modulate oviposition behaviour. Their role in host location and host acceptance is assessed with bioassays using the scent emanating from host plants and synthetic blends of physiologically active host plant odorants.

Shape Variations of the Double-Impulses Generated by the Antennal PH-Sensitive Neuron in the Ground Beetle *Pterostichus aethiops*

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Antennal flagellum of the ground beetle *Pterostichus aethiops* houses approximately 70 large 100–200-μm long bristle-like contact chemoreceptor innervated by four chemosensory and one mechanosensory neuron. Previously, it was repeatedly demonstrated that one of the chemosensory neurons responds to the pH of the stimulating salt solution. This neuron was called the pH sensitive neuron. In our extracellular sensillum-tip recordings, in contrast to other chemosensitive neurons of the bristle, the pH-sensitive neuron generated very large action potentials, 2-4 mV peak-to-peak with initial positive polarity. The shape of these impulses is complex because they are composed of two separate electrical events in quick succession and most probably produced by two separate spike generation sites of the same neuron. Minor differences in the time interval between these two events occuring among antennal taste sensilla may cause considerable differences in the shape of the resulting double-impulse. Usually, the double-impulses of the pH-sensitive neuron described are two-tipped. In some sensilla, however, only a

small characteristic notch or discontinuity in the rising phase of these impulses indicates their complex nature. In rare cases, the second spike of these double-impulses may be negative. The polarity of spikes probably depends on the location of the spike generation site (axonal or dendritic).

The responses of the pH-sensitive neuron to phosphate buffers and alkalized 100 mM NaCl at various pH were tested. In experiments with alkalized NaCl at pH 6-10, no correlation between the responses and pH was observed.

Electrophysiological Responses from Neurons of Antennal Taste Sensilla in the Polyphagous Predatory Ground Beetle *Pterostichus oblongopunctatus* (Fabricius 1787) to Plant Sugars and Amino Acids

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The responses of antennal contact chemoreceptors, in the polyphagous predatory ground beetle *Pterostichus oblongopunctatus*, to twelve 1-1000 mmol l^{-1} plant sugars and seven 10-100 mmol l^{-1} amino acids were tested. The disaccharides with an α-1.4-glycoside linkage, sucrose and maltose, were the two most stimulatory sugars for the sugar-sensitive neuron innervating these contact chemosensilla. The firing rates they evoked were concentration dependent and reached up to 70 imp/s at 1000 mmol l^{-1}. The stimulatory effect of glucose on this neuron was approximately two times lower. This can be partly explained by the fact that glucose exists in at least two anomeric forms, α and β. These two forms interconvert over a timescale of hours in aqueous solution, to a final stable ratio of α:β 36:64, in a process called mutarotation. So the physiologically-active α-anomere forms only 36% of the glucose solution which was reflected in its relatively low dose/response curve. Due to the partial herbivory of *P. oblongopunctatus* these plant sugars are probably involved in its search for food, for example, for conifer seeds. Several carbohydrates, in addition to glucose, such as cellobiose, arabinose, xylose, mannose, rhamnose and galactose are known as components of cellulose and hemicelluloses. They are released by brown-rot fungi during enzymatic wood decay. None of them stimulated the antennal sugar-sensitive neuron. They are therefore not implicated in the search for hibernation sites, which include rotting wood, by this beetle. The weak stimulating effect (below 3 imp/s) of some 100 mmol l^{-1} amino acids (methionine, serine, alanine, glutamine) to the 4th chemosensory neuron of these sensilla was characterized as non-specific, or modulating the responses of non-target chemosensory neurons.

Computation of the Selectivity of *Bombyx mori* Pheromone Binding Protein for Bombykol Over Bombykal, a Molecular Dynamics Approach

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We report calculations dedicated to estimate the selectivity of the *Bombyx mori* Pheromone Binding Protein towards the two closely related pheromonal components bombykol and bombykal. The selectivity is quantified by the binding free energy difference, obtained either by the Thermodynamic Integration or by the MM-GBSA approach. In the latter, the selectivity is decomposed on a per-residue basis, allowing nailing down the residues considered as crucial for the selectivity of the protein for Bombykol over Bombykal. The selectivity for Bombykol is recovered by both approaches in accordance with experimental results.

Fruit Preference in the Fruit Fly *Drosophila melanogaster*

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Olfaction in the fruit fly has been extensively studied from a molecular and physiological point of view and provided us with a detailed outline of olfactory signals from detection to integration in higher brain centres. However, the fly's chemical ecology remains relatively understudied. With regards to food, *D. melanogaster* is widely thought to have a preference to banana (thus the common name Banana Fly). Here we studied the fruit preference of wild-type *D. melanogaster* in different types of bioassays types depending on the assay, several fruits were preferred over banana. However, the use of olfactory cues in the decision-making process may depend on the distance from the source.

Gene Expression in Olfactory Organs of *Drosophila melanogaster*

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To assess the signaling mechanisms utilized by primary olfactory neurons in *Drosophila* to transduce the presence of an odorant into action potentials we have approached a genome-wide screen of the olfactory-receptor neuron (ORN) gene expression from antennae and maxillary palps. The mRNA population of specific ORN neurons is tagged by the target expression of a FLAG-PolyA binding protein (PF) using the binary Gal4-UAS system in *Drosophila* transgenic flies. ORN-specific mRNA population is then purified by mRNA-PF co-immunoprecipitation. Further RT-PCR and direct sequencing analysis of the purified ORN-tagged transcriptome will pinpoint candidate genes to be involved in the olfactory-signal transduction cascade. Moreover, CO_2-regulation of gene expression (i.e. Gr21a and Gr63a) will be tested in control vs. CO_2 exposed flies using the mentioned mRNA-tagging approach.

Morphological and Physiological Characterization of Antennal Lobe Local Interneurons in *Drosophila*

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The *Drosophila* olfactory system has become the most extensively investigated model system for neural coding of olfactory information, due to the identification of olfactory receptors, the almost complete receptor-receptor neuron- glomerulus map and extensive investigations on odor specificity of receptors. The axons of olfactory receptor neurons that express the same olfactory receptor converge onto the same glomerulus. There they form synapses with projection neurons. These projection neurons receive input in a single glomerulus. Local interneurons branch exclusively within the antennal lobe and interconnect different glomeruli. Many of the local interneurons are GABAergic. It has thus been postulated that inhibitory interactions play an important role in odor coding. However, recent studies have demonstrated that lateral excitation mediated by cholinergic excitatory local interneurons spread the odor information over different glomerular channels. It is not well understood how these inhibitory and excitatory networks are balanced to optimize odor coding in the antennal lobe. The morphological and physiological characteristics of these excitatory and inhibitory local interneurons, especially at the single neuron level, have not been intensively examined. To reveal the neural basis of the antennal lobe neural circuit, we characterized antennal lobe local interneurons morphologically and physiologically using whole-cell patch clamp recordings. Taking advantage of local interneuron-specific GAL4 enhancer trap lines, we recorded intrinsic electrophysiological properties of single local interneurons and analyzed their detailed morphology using confocal microscopy. Morphological and physiological variations were found among and within the GAL4 lines. Correlations between morphological and physiological characteristics were established in some cases. Here, we discuss the functional operation of the antennal lobe neural circuit mediated by the variety of local interneurons.

Divergent Gustatory Sensitivity to Sugars Among *Drosophila simulans* Strains

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Polymorphism of Ala218Thr in Gr5a, a sweet-taste receptor gene in *D. melanogaster* wild populations has been known to be responsible for the behavioral preference for trehalose solutions. In contrast, the corresponding amino residue of *D. simulans*, a closely related species to *D. m.*, is not polymorphic and is always Thr, the low sensitivity allele of the gene. Nevertheless we found that wild *D. s.* strains are divergent for the trehalose/sucrose gustatory preference. We analyzed preference between two different concentrations of a sugar and found that the differential sensitivity for some sugars is significantly different among strains and that some strains are more sensitive to certain sugars but less sensitive to other sugars. Therefore the sugar sensitivity of the fly may not be controlled by a single mechanism. Genetic and molecular studies of the gustatory sugar sensitivity in *D. s.* are in progress.

Odor Processing in the Mushroom Bodies of Honeybees, a Calcium Imaging Study

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Mushroom bodies (MB) are higher order multimodal sensory integration centers in insect brains. They integrate and process olfactory information. In honeybees (*Apis mellifera*), each mushroom body consists of 170.000 Kenyon cells. The MB-calyces are considered to be the main input sites of Kenyon cells; the MB-lobes are their main output regions.

We used the calcium imaging technique combined with odor stimulation in order to analyze the neural activity of Kenyon cells in MB-calyces *in vivo*. Subgroups of Kenyon cells were retrogradely filled with a calcium sensitive dye (Fura 2 mixed with a fixable dye) by means of mass injection. This allows us to examine both, the neural activity during odor stimulation and subsequently the morphology of the imaged neurons with a confocal microscope.

In the honeybee, several studies have dealt with the characterization of olfactory input into the antennal lobe, the first olfactory neuropil, and the output from the antennal lobe to the MB via projection neurons. So far, little is known about odor processing in Kenyon cells. Imaging studies by Szyszka et al. (J. Neurophysiol. 2005) revealed a sparsening of Kenyon cell responses to odors both in the temporal and spatial domains. This sparsening effect results from neural processing in the lip region of the calyces and may be a result of general or specific inhibition. Inhibition in the insect brain is usually caused by the neurotransmitter GABA. Therefore we used different GABA-receptor blockers and investigated the effect on Kenyon cell responses. We are particularly interested in the neural coding of odor concentration. We could show that Kenyon cells are indeed responding in a concentration-dependent way. We also found that GABA inhibition acts mainly on higher odor concentrations and is therefore a form of gain control.

Olfactory Response in the Receptor Organs of *Drosophila melanogaster* is Modulated by the Environmental Temperature

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The sensorial systems should be able to adapt to environmental changes to produce meaningful information to the animal. In nature, changes in temperature modify the volatility of odorants and their concentration in the air. If the olfactory system does not adapt to these changes it could give wrong information about distance or direction to odour sources.

Behavioural studies in our lab have shown that the environmental temperature affects olfactory perception in *Drosophila melanogaster*. When flies were submitted to a period of higher temperature before the behavioural test they display less sensitivity to high odour concentrations than their controls. The effect is the opposite, they have more sensitivity, when submitted to a lower temperature treatment.

In this work we have used two electrophysiological measures: electroantenograms (EAG) and Single Sensilla Recordings (SSR) to check if temperature modifies the olfactory response already in the receptor organs.

We see that the EAG's amplitude is higher in flies that have been exposed to a higher temperature than their controls in response to high concentrations of all the tested odorants: Ethyl Acetate, Ethanol and Acetone. When the flies were exposed to a lower temperature the amplitude was reduced and the signal termination was slower. These results demonstrate that at least part of the olfactory perception-modulation by temperature is happening at receptor level.

We have also investigated the effect of temperature in individual olfactory receptor neurons using SSR. We have seen that in the ab2 sensilla of flies exposed to high temperature neuron ab2A (that express Or59A) showed a reduced response to Ethyl Acetate, but neuron ab2B (that express Or85a) is more reactive to Acetone than the corresponding control. These results indicate that the modulation produced by temperature is both neuron and odour dependent.

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Odor Processing in the Drosophila Lateral Horn

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In Drosophila, the antennal lobe, the first olfactory relay in the olfactory pathway, receives sensory input from olfactory receptor neurons in the antennae. From there, second order projection neuron axons form synapses in two higher olfactory centers: the mushroom body and the lateral horn. The representation of odors in the Kenyon cells of the mushroom body and third order neurons in the lateral horn is a result of the deconstructed map of activity in the antennal lobe.

We aim to understand how the integrated sensory pattern of activity at a higher level in the lateral horn can elicit behaviour, in particular innate behaviour.

We have been using in vivo recordings done ''blindly'' and on a GAL4 line with restricted expression in a small number of third order neurons, combined with high-resolution neuroanatomy to identify the underlying circuit. We have observed different strategies for odor representations at the higher level and we are continuing to investigate this further.

Topic 30: Physiology

Beta/Gamma Alternation in the Olfactory Bulb of the Anesthetized Rat: in the Pursuit of Understanding Mechanisms

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In the anesthetized rodent olfactory bulb (OB), the network temporal dynamics is characterized by local field potential (LFP) oscillations whose frequencies alternate between beta (15-30Hz) and gamma (40-90Hz) ranges, this alternation being highly dependent on the respiratory cycle. The presence, amplitude and frequency of these rhythms have been shown to depend on the animal behavioural state or learning experience. Understanding the origin of these rhythms and their alternation is of a great importance to understand olfactory coding at early cortical stages.

Here, we investigated the mechanisms underlying the generation of these oscillations using a current source-density (CSD) analysis of LFP bulbar signals. In parallels, we used a pharmacological approach to understand the origin of alternation between both regimes. Both studies were performed in the OB of freely breathing animals. *CSD:* The use of 16-channel silicon probes give us access to the LFP in all layers of the olfactory bulb and we took advantage of the laminar structure of the bulb to perform a linear CSD analysis. Efficient automatic detection of phase and amplitude of oscillatory episodes allowed us to average CSD maps across oscillation cycles of the same frequency range. Finally multiunit activity (MUA) was used to relate CSD maps to spiking activity in the different layers. *Pharmacology*: $GABA_A$ agonist (muscimol) and antagonist (bicuculine) were locally injected in the neighborhood of recording probes. Time-frequency maps of bulbar LFPs obtained under bicuculine or muscimol conditions were compared to control ones.

Our results show that beta and gamma rhythms display different CSD maps, which underlies different generation mechanisms. In particular, we observe that gamma rhythm seems related to a balanced interplay between excitatory mitral cells and inhibitory granule cells. On the contrary, beta oscillation appears dominated by mitral cells with only poor synchronization of granule cells. Preliminary results of pharmacological study revealed that gamma oscillations seem to emerge when the ratio activation/inhibition in the network is high while they tend to disappear when this ration is low. Overall these results give new insights on the network spatio-temporal activity underlying oscillatory dynamics in the mammalian OB.

Exploring the Number of Modular Dimensions in Olfactory Bulb Activations of the Rat

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Glomeruli are the initial sites of processing in the olfactory bulb (OB). Each glomerulus receives signals from olfactory receptor neurons of roughly one receptor type. As a consequence of this orderly projection and the topological distribution of glomeruli, similar chemical features are represented by nearby positions at the OB (''chemotopy''). Among others, Leon and Johnson et al. (2006, 2007, 2008) and Mori et al. (2006, 2007) have provided evidence that there exist molecular representational primitives and correspondingly some distinctive zones (''modules''). In order to investigate the issue of what could be a reasonable number of modules with respect to differences in activations, we applied fuzzy c-means

clustering techniques, together with bootstrap stability validation to imaging data of the rat OB. We compared the results with measures of information theory and other cluster-stability methods.

Uptake of Odorant Binding Proteins in the Olfactory Epithelium

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The detection of odorants is mediated by distinct chemosensory neurons in the main olfactory epithelium (MOE) of the nose. It is supposed that the hydrophobic odorous compounds are dissolved in the nasal mucus by means of specialized globular proteins, the odorant binding proteins (OBPs) in order to reach the chemosensory cells. To assure the responsiveness to odors of each inhalation, odorant molecules have to be removed rather quickly from the immediate vicinity of the sensory neurons. Therefore, it has been hypothesized that mechanisms exist which remove OBP/odorant complexes from the ciliary environment. To scrutinize this concept, recombinant mouse OBP1a was fluorescently labelled, loaded with odorous compounds and applied to the MOE. It was found that within less than a minute, labelled OBP disappeared from the surface and appeared in the sustentacular cells, glial-like cells which form the apical border of the MOE. This uptake occurred only when the OBP was loaded with appropriate compounds. A candidate system for mediating an uptake of OBP/odorant complexes into sustentacular cells represents the scavenger receptors which are involved in internalizing lipocalin/hydrophobic ligand complexes. RT-PCR and in situ hybridisation studies revealed that only the low density lipoprotein receptor Lrp2/megalin was specifically expressed in the sustentacular cells. Immunohistochemical analyses localized megalin to the microvilli of sustentacular cells. Immunoreactivity was visible throughout the olfactory epithelium; whereas the respiratory epithelium was devoid of megalin. To analyze whether megalin is capable of internalizing OBP/odorant complexes, *in vitro* uptake studies were performed using a megalin expressing cell line. It was found that internalization of OBP/odorant complexes occurred within short period of time and internalized OBP occurred within lysosomes of the cells. Also in the *in vitro* system, uptake of OBP1a/odorant complexes only took place when OBP1a was loaded with appropriate odorous compounds. A preincubation of the cells with the ''receptor associated protein'' (RAP), an inhibitor of Megalin-function, blocked the uptake process; RAP also blocked internalization of OBPs into sustentacular cells of the olfactory epithelium. These data support the notion, that the uptake of OBP/odorant complexes into sustentacular cells mediated by megalin represents an important mechanism for a local elimination of odorants.

Taste Signalling Elements in the Gastrointestinal Tract

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In the gastrointestinal (GI) tract, a variety of digestive processes are continually adapted to the changing composition of ingested foods, which requires a precise chemosensory monitoring of luminal contents. Gustducin-expressing brush cells scattered throughout the GI mucosa are considered candidate sensory cells for accomplishing this task. A large cluster of gustducin-positive cells is located exactly at the boundary between the fundic and the oxyntic mucosa of the mouse stomach, at the so-called ''limiting ridge''. In close association with the candidate chemosensory cluster, two populations of enteroendocrine cells were found: one population containing the satiety regulating hormone ghrelin, the other population comprising serotonin-secreting enterochromaffin cells. The particular arrangement of gustducin-expressing cells and enteroendocrine cells at the limiting ridge suggests a direct interplay between these cell types with immediate implications, not only for digestive processes in the stomach, but also for parameters controlling the satiety status.

Reelin May Act as a Guidance Cue for Outgrowing Olfactory Axons

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During early development of the olfactory system, axons from sensory neurons in the nasal neuroepithelium grow out and pass through the so-called cribriform mesenchyme to reach their targets in the olfactory bulb (OB). Therefore, it has been proposed that cells in the cribriform mesenchyme provide guidance cues for axonal outgrowth or fasciculation. To identify proteins at the surface of cells in the cribriform mesenchyme which might affect axonal guidance, microarray analyses were performed. Among the identified genes encoding surface proteins, Reelin was considered as an interesting candidate since Reelin is an extracellular matrix protein influencing neuronal migration as well as axonal outgrowth in the brain. In mouse embryonic stages, Reelin was found to be expressed in a subset of cells in the cribriform mesenchyme when the first olfactory axons navigate through this tissue. By contrast, no expression of Reelin was observed in the olfactory epithelium. The Reelin-positive cells in the cribriform mesenchyme are in close contact with olfactory axons. Reelin expression was also detectable in the OB, presumably in the periglomerular and mitral cells which are the targets of olfactory axonal projection. These observations suggest that Reelin localized to the periphery of cells in the cribriform mesenchyme and the OB as well as to the extracellular matrix may act as a guidance cue for olfactory axons. This concept is substantiated by the finding that a receptor protein for Reelin was found to be situated in segments of olfactory axons associated with Reelin-positive cells in the cribriform mesenchyme or the OB, respectively.

The Grueneberg Ganglion – A Chemosensory Organ?

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The detection of odors and pheromones in mammals is mediated by chemosensory neurons of the main olfactory epithelium (MOE) and the vomeronasal organ (VNO), which generally express the olfactory marker protein OMP. We have found that OMP is also

expressed in cells of the so-called Grueneberg ganglion (GG), a cluster of neuronal cells in the vestibule of the anterior nasal cavity. Chemosensory responsiveness of olfactory neurons is based on the expression of distinct receptors: odorant receptors in the MOE or pheromone receptors in the VNO, respectively. To scrutinize whether neurons in the GG may indeed be chemosensory cells, they were subjected to molecular phenotyping. It was found that a distinct vomeronasal receptor type (V2r83) was expressed in the majority of GG neurons which were concomitantly endowed with the G proteins G_o and G_i; both are also present in sensory neurons of the VNO. Expression of odorant receptors was only observed in very few cells during perinatal stages; a similar number of cells expressed adenylyl cyclase type III and $G_{olf/s}$. These findings demonstrate that the GG mainly comprises cells with a VNO-like phenotype. GG neurons lacking expression of V2r83 are endowed with trace amine associated receptors (TAARs) which have been identified recently as a novel class of olfactory receptors. Interestingly, expression of V2r83 as well as TAARs predominantly occurs in perinatal stages and is significantly reduced in adults, suggesting that the GG might be particularly important for neonates.

Similar to other nasal sensory cells, GG neurons extend axonal processes which fasciculate to form nerve bundles that project caudally along the roof of the nasal cavity and through the cribriform plate, finally terminating in the olfactory bulb of the brain. In summary, the expressions of olfactory signaling proteins as well as the axonal projection to the olfactory bulb strongly support the notion that the GG may indeed have a chemosensory function.

Anion Selectivity and Blockers of the Calcium-Activated Chloride Current in Mouse Olfactory Sensory Neurons

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The binding of odorant molecules to olfactory receptors in the cilia of olfactory sensory neurons initiates a transduction cascade that leads to an increase in the concentration of cAMP, followed by an increase in the intraciliary concentration of Ca, which enters into the cilia through cAMP-gated channels. In turn Ca activates Cl channels in the ciliary membrane and, because olfactory sensory neurons maintain an elevated intracellular Cl concentration, Cl ions will exit from the cilia, contributing to the amplification of the odorant response. The Ca-activated chloride current constitutes a large part of the transduction current in olfactory sensory neurons. This current has not been fully characterized and its molecular identity is still not well established. In the present study we aim to characterize the biophysical properties of such chloride conductance in isolated olfactory sensory neurons from mouse. We used the whole-cell patch-clamp technique in the voltage-clamp configuration to record currents elicited by Ca photorelease in the ciliary region of olfactory sensory neurons. The reversal potential of the current in symmetrical chloride concentration was about 0 mV. The permeability sequence, calculated from changes of reversal potentials measured in external anion substitution experiments, was $I>SCN>NO_3>Br>Cl>MeS$. Chloride current blockers, such as Niflumic acid, DIDS and NPPB, reduced the current amplitude.

The biophysical characterization of the native olfactory Ca-activated Cl current might be compared to data obtained from ion channels candidate to be the olfactory channel involved in transduction, or with data from genetically modified mice, to establish the channel molecular identity.

Voltage-Gated NA^+ Currents of each Cell Type in Mouse Taste Buds

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Action potentials play an important role in neurotransmitter release in taste bud cells (TBCs). For example, type II cells release ATP paracrinically via hemi channels opened on the firing of action potentials. Type III cells also need action potentials to activate voltage-gated Ca^{2+} channels for exocytosis. We investigated voltage-gated Na^+ currents, a primary component of action potentials, of mouse fungiform TBCs under in-situ whole-cell clamping and RT-PCR studies. Cell types of TBCs electrophysiologically examined were identified immunohistochemically. RT-PCR studies suggested that taste buds expressed two TTX-sensitive α subunits, Nav1.3 and Nav1.6, and a TTX-resistant one, Nav1.5. Many TBCs generated Na^+ currents, and several TBCs exhibited them in the presence of 1 μM TTX. The magnitude of TTX-resistant component was ~6% of total voltage-gated Na^+ current magnitude. The relative magnitude of the TTX-resistant component was larger in type III (SNAP-25-immunoreacitive) cells than in other cell types. The recovery of the total Na^+ current magnitude at -70 mV from inactivation was well fitted with double exponential. Time constants for the recovery were ~20 ms (tau1, ~60% of total currents) and ~800 ms (tau2, ~40%) in type II (IP_3R3-immunoreacitive) cells. These results suggest that type II cells hardly fire in higher frequencies. Type II cells may develop unidentified mechanisms in order to encode the concentration of taste substances as the frequency of action potentials.

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Increases in Intracellular Calcium Levels in the Mouse Olfactory Neurons Induced by IGF-I and 2-APB

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Olfactory sensory neurons (OSNs), which receive odorants, transmit odor information to the olfactory bulb (OB). Some volatile odorants having toxic effects physiologically damage on the OSNs. One of their particular features is that they are continuously renewed during adulthood to overcome these disadvantages. It has been suggested that insulin-like growth factor (IGF)-I plays an important role in bulbo-epithelial interactions in the olfactory system. In general, axon elongation and guidance depend on the localization, concentration, and temporal dynamics of cytoplasmic calcium signaling. Kanzaki et al. (1999) have reported that

translocation of TRPV2 to the plasma membrane is augmented by IGF-I. Here we show that TRPV2 mRNA is predominantly expressed in early mature olfactory sensory neurons. In olfactory mucosa, intensive TRPV2 immunostaining was observed at the olfactory axon bundles but not at the soma. Furthermore, we demonstrated that cell bodies of briefly cultured OSNs expressing TRPV2 mRNA are IGF-I Receptor-immunopositive. The increase in intracellular calcium levels in the OSNs isolated from adult olfactory mucosa was found to be induced by the application of 2-aminoethoxy- diphenyl borate (2-APB), which is an agonist for TRPV1, TRPV2 and TRPV3, and was not observed in the absence of outer Ca^{2+} or in the presence of SKF96365, a TRPV2 inhibitor. Application of IGF-I also induced an increase in $[Ca^{2+}]_i$ in normal Ringer's solution but not in Ca^{2+}-free Ringer's solution. In the presence of SKF96365, application of IGF-I did not induce an increase in $[Ca^{2+}]_i$. Observations in the present study suggest that TRPV2 localizes to growing olfactory axons and contributes to the elevation of intracellular calcium levels in the OSNs in response to IGF-I.

Evaluation of Quantitative Characterization of Odor Adaptation

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It was generally considered that the perceived odor intensity was decayed exponentially with time when people were exposed to odorants continuously. Recently, however, some reports have been published which do not support this theory. On the other hand, no method for evaluating adaptation had been established yet, new method for quantitative evaluation was therefore required.

In this research, two indexes were suggested; one was the ''Adaptation index'' which could decide whether adaptation occurred or not, the other was the ''Attenuation index'' which could indicate the time index required for adaptation about the data showing adaptation.

In the experiments, the evaluating system was used to record the time profiles of perceived odor intensities based on subjects' responses by moving hand lever with visual feedback, and odor intensities at first presentation were controlled between ''easy detectable'' and ''strong''.

The method was investigated that classified ''adaptation had been occurred during this experiment'' or not.

''Adaptation index'' was calculated as the ratio of temporal integration of intensity value for former and latter half of one experiment. This was matched to a result of ''adaptation or non-adaptation'' judged by experimenter's inspection that was previously reported. Using this simple mathematical algorithm, the time profile of odor intensity was able to distinguish ''adaptation occurred or not''.

Furthermore, ''Attenuation index'' was suggested to quantitatively evaluate the time required for adaptation, which was defined the time when the perceived odor intensity had been decaying to a sensible level.

These two indexes, ''Adaptation index'' and ''Attenuation index'', enabled to evaluate the quantitative characteristics of human odor adaptation.

Perceptual Odor Blending is Influenced by Chemical Complexity of Odorant Mixtures

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Perfumers and flavorists are familiar with odor blending phenomenon and often report that a minimum number of odorants has to be mixed for a good odor blend to arise. Previous studies in humans and animals suggested that a configural process could be involved in the perceptual analysis of mixtures of odorants. It has been proposed that the perceptual blending phenomenon corresponds to a configural perception of odorant mixtures. In the present study, we investigated the influence of chemical complexity on the configural perception of odorant mixtures. Six mixtures including 2 to 6 odorants as well as each unmixed odorant were assessed for their odor quality by a panel of 55 healthy untrained subjects (Ss). Two verbal description tasks were performed: Free Description and Choice between Attributes. For each mixture, a target odor name was expected; i.e. a name for odor quality emerging from each mixture through the perceptual blending phenomenon. A typicality rating task was also performed. In this task, Ss had to rate, on a linear scale, how typical was the stimulus in relation to the target odor name. Analysis of typicality ratings as well as comparison of the frequency of quotation of the target names, in both verbal tasks, between the mixtures and their components, revealed that perceptual blending did occur in 2 of the tested mixtures. Indeed the typicality was found to be significantly higher for mixtures as compared to their components and mixtures were more often described with the expected target name as compared to their components. Moreover it appeared that perceptual blending was more pronounced in the mixtures containing the higher number of odorants. Taken together, these findings confirm that mixtures of odorants can be perceived in a configural way by humans and that such a configural process may explain why odorant mixtures can elicit novel odor percepts. Additionally, we propose that a configural process would be more likely engaged when more than 4 odorants are mixed which is in line with the limit in the capacity of humans to identify odorants in a mixture (Laing & Francis, 1989).

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Olfactory Discrimination of Binary Mixtures of Amino Acids in Zebrafish (*Danio rerio*)

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Zebrafish (*Danio rerio*) are model organisms for studies of olfactory discrimination in vertebrates. In fishes, large odorant mixtures are always perceived as different from single components, whereas

binary and ternary mixtures of two unequally stimulatory amino acids are perceived initially as the more stimulatory component of the binary (ternary) mixture. We investigated olfactory discrimination of binary amino acid mixtures in zebrafish. We made one component of the binary mixture more stimulatory than the other component by increasing its concentration >30 times above its equal EOG magnitude concentration. We were evaluating the zebrafish behavioral responses to olfactory stimulation by counting the number of turns of the fish greater than 90° during 90s after the stimulus delivery. During 30 conditioning sessions the zebrafish started to respond evenly to the conditioned binary mixture of L-Ala and L-Arg. L-Ala was the more stimulatory component of the mixture. The zebrafish responded equally well to the conditioned mixture and to its more stimulatory component alone indicating that initially zebrafish can not discriminate the more stimulatory component from the binary mixture. The less stimulatory component of the mixture and all other tested amino acids were always discriminated from the conditioned mixture. Zebrafish also discriminated the binary mixture of L-Ala and L-Arg with L-Arg more stimulatory than L-Ala from the conditioned mixture. Initially binary mixtures of amino acids were, like in catfish, detected as their more stimulatory components, however during the repeated presentation of the more stimulatory component alone and its binary mixture they learned to discriminate between the two.

Olfactory Discrimination of Similar Amino Acids in different Groups of Geneticaly Altered Zebrafish (*Danio rerio*)

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Fish olfactory system enables amino acid discrimination. Odors are represented in the olfactory bulb by patterns of glomerular activation typical for each odor. We predicted that amino acid discrimination in zebrafish can be anticipated from their chemotopic projections on olfactory bulb glomeruli as shown by calcium labeling technique (Friedrich and Korsching, 1997). To study olfactory discrimination in zebrafish (*Danio rerio*) we conditioned different groups of wild type and transgenic zebrafish to specific amino acids and tested their discrimination from other amino acids. Conditioned zebrafish swam more during 90 seconds test periods after stimulation with the conditioned stimuli than after stimulation with other amino acids that they discriminated from the conditioned stimuli. With the exception of L-Ile/L-Val and L-Phe/L-Tyr the wild type and the transgenic zebrafish discriminated all the tested amino acids from the conditioned stimuli (Miklavc, 2005). In most wild type zebrafish L-Val and L-Ile triggered almost identical bulbar activation patterns (Friedrich and Laurent, 2001) the same was true for the pair of amino acids L-Phe and L-Tyr. Wild type and transgenic zebrafish were conditioned to either L-Phe or L-Tyr and to either L-Val or L-Ile. Most wild type zebrafish conditioned to L-Val did not discriminate L-Ile from L-Val, however, most zebrafish selected for L-Ile/L-Val discrimination in nearly all cases discriminated L-Ile from L-Val. When we conditioned a different wild type zebrafish to L-Ile most zebrafish discriminated L-Ile from L-Val. Transgenic zebrafish (donated by R. Friedrich) Huc-Yc exhibited more distinct OB spatio-temporal activity patterns for L-Phe and L-Tyr than wild type zebrafish. Wild type of zebrafish did not discriminate L-Phe from L-Tyr irrespective of which amino acid we conditioned whereas the transgenic zebrafish discriminated L-Phe from L-Tyr.

Newborn Rabbits Process Odour Mixtures in an Elemental and a Configural Way

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The processing of odour mixtures is elemental or configural in adult mammals, but questions remain for young organisms. Here, using the ability of newborn rabbits to rapidly learn a novel odour after coupling with the mammary pheromone (MP), we pursue our investigation of the pups' ability to discriminate a blend from its constituents. In a previous study (Coureaud et al., 2007, *Chem. Senses 32*, A19), the pup perception of a binary odour mixture (AB) for which perceptual blending occurs in humans (Le Berre et al., 2008, *Chem. Senses 33*, 193-199) seemed to be both elemental and configural. Indeed, after the MP-induced learning of the AB mixture, pups responded behaviourally to the mixture and its constituents, demonstrating elemental abilities. But after the learning of only one of the constituents, they did not respond to the mixture, suggesting also a perception partially configural. However, the non response to the mixture could also been explained by a novelty effect. According to this hypothesis, pups would not respond to the AB odour mixture after learning of the component A (or B) since the other odorant, unfamiliar, detected in the mixture would restrain their response. Here, we started to evaluate such possibility by the MP-conditioning of pups (age: 2 d, n=86, 15 litters) to the odorant A, the odorant B or to another odorant, C. The pups were later tested for their response to A, C and AC, or B, C and BC (in humans AC and BC do not constitute blending mixtures). The results show that pups responded then to the odorant they have learned, but also to the AC mixture (when they have learned A), and to the BC mixture (when they have learned B). Such responses might not result from a masking effect, since pups conditioned to AC (n=23, 5 litters) responded later both to A and C, and pups conditioned to BC (n=25, 5 litters) responded to B and C. To sum up, these results suggest an absence of novelty effect, and are coherent with the hypothesis of a partially configural perception of the AB odour mixture by the newborn rabbit.

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Purinergic Signaling in the Olfactory Epithelium

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Extracellular nucleotides are important signaling molecules that mediate various biological effects via cell-surface receptors termed purinergic receptors. It has previously been shown that ATP activates sustentacular supporting cells (SCs) of the olfactory epithelium (OE) of larval *Xenopus laevis*. Here we characterized the

ATP-induced responses of SCs using functional calcium imaging. We were able to show that ATP elicits intracellular calcium waves in SCs. The waves initiate in the apical part of the SCs and propagate towards their end feet in the basal part of the OE (wave velocity 17.10 ± 1.02 μm/s). Furthermore, we defined the purinergic receptor subtype(s) involved in the ATP-induced responses. ATP evoked increases in $[Ca^{2+}]_i$ in both the presence and absence of extracellular calcium. Depletion of the intracellular calcium stores abolished the $[Ca^{2+}]_i$ responses. The above results together with the determined order of potency of the used purinergic agonists and antagonists implicate an involvement of $P2Y_2/P2Y_4$-like receptors. Thus, our findings suggest that the release of nucleotides in the OE could stimulate a distinctive spatiotemporal pattern of $[Ca^{2+}]_i$ increases in SCs. This allows speculating about a novel form of intraepithelial communication. The physiological role of purinergic receptors in SCs of larval *Xenopus laevis* remains to be determined, but our findings suggest to view the OE as a tissue and to start to concentrate on possible functional interactions between the different cell types in the OE. [Supported by DFG Research Center Molecular Physiology of the Brain (CMPB, Project B1)].

Recording the Activation of the Human Olfactory System Using Odorant Evoked Contingent Negative Variation

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N1 and P2/P3 olfactory event related potentials (OERPs) are usually obtained from passive subjects. We examined the effect of a delayed motor task using a contingent negative variation S1-S2 paradigm. The alarm signal S1 was amyl acetate (200ms) delivered towards the olfactory cleft without airflow change. The imperative signal S2 was a sound (500 Hz), added to white noise through earphones. During S2 (2.6 s after S1), the subject pressed a button in one hand to indicate he just perceived the odorant or a button in the other hand if he did not perceive it. Ninety of such sequences and 90 sequences with S2 but without S1 were randomly presented with a variable time interval of 7.5s ±25%. Recording sites were Fp1 and Fp2 vs. linked ear lobes (mouth breathing, closed eyes).

In most normosmic subjects, a slow contingent negative variation (CNV) appeared between S1 and S2. It was usually preceded by a positive peak (analogous to P2) and, rarely, by an early negative peak (possibly N1). CNV was absent when S1 was not delivered. Differences in the CNV time course were observed among the subjects. Patients lacking olfactory bulbs did not show odorant triggered CNV.

We believe that the present odorant triggered contingent negative variation (OTCNV) is a form of olfactory event related potentials (OERPs) which is useful as a marker of the activation of the olfactory system. The absence of signal in patients suffering bilateral olfactory bulb agenesis suggests little contribution, if any, of the chemoreceptive nasal trigeminal fibers.

We are examining the usefulness of this method in a clinical context (bilateral obstruction of the olfactory clefts, cranial trauma etc.).

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Ligand-Specific Induction of Endocytosis in Taste Receptor Cells

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We demonstrate for the first time uptake of dye by endocytosis in receptor cells in vertebrate taste buds. The region investigated was the anterior 3 mm of the vomer, the tongue, and the lips of juvenile brown trout (less than 10 cm body length) that hold around 600 taste buds. Exposing the oral cavity to L-cysteine, a stimulant with acceptance ratio of 90 % together with a fluorescent styryl dye (FM1-43) at +20°C, induced staining of 175 receptor cells in 145 taste buds. The staining was punctuated and the whole cell was rapidly stained. Exposure to FM1-43 alone caused staining in about 40 receptor cells in 30 taste buds. Exposure of the oral cavity to L-cysteine and FM1-43 at +2°C, or after pre-exposure to nocodazole, which disrupts microtubules, caused a substantial reduction in the number of receptor cells stained. Texas Red dextrans 3 kD and 40 kD, too large to enter the cells by ion channels, were also taken up by the taste receptor cells. These observations indicate that the dyes are taken up by taste-specific induction of endocytosis, and further that the endocytic vesicles are transported towards the cell soma.

Texas Red dextran was found to be a deterrent taste substance, giving an acceptance ratio of 6 %. In fish, first exposed to a stimulant (L-cysteine + FM1-43) and subsequently to a deterrent (Texas Red dextran), stained receptor cells were observed in 209 taste buds. Of these taste buds, 97 had only FM1-43 stain (yellow) and 67 had only Texas Red stain. 45 taste buds had both yellow and red cells but none of these cells were co-stained. These findings indicate that taste receptors for attractants and deterrents may be expressed in the same taste buds, but in separate receptor cells.

The method applied in the present study opens for a variety of experimental avenues for studies of the organization and function of chemosensory organs.

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Topic 30: Olfactory Transduction

The Physiological Role of Ion Channels Activated by Hyperpolarization and Cyclic Nucleotides in Mouse Vomeronasal Sensory Neurons

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Pheromones are chemical signals released by animals that cause changes in physiology and/or behavior in another member of the same species; some pheromones are detected by vomeronasal sensory neurons located in the vomeronasal organ of Jacobson (VNO). We investigated the properties of hyperpolarization-activated currents (I_h) in sensory neurons from acute slices of mouse VNO. In voltage-clamp studies, I_h was identified by the: characteristic kinetics of activation, voltage-dependence, and blockage by Cs^+ or ZD-7288, two blockers of the I_h, but not by Ba^{2+}. Forskolin, an activator of adenylyl cyclase, shifted the activation curve for I_h of 9 mV to less negative potentials. A comparison of the measured I_h properties in VNO neurons with those of heterologously expressed hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, together with RT-PCR experiments in the VNO, indicate that I_h is due to HCN2 and/or HCN4 subunits. In current-clamp recordings, blocking I_h with ZD-7288 induced a hyperpolarization of 5.1 mV, an increase in input resistance from 4.1 to 5.1 G, a decrease in the sensitivity to elicit action potentials in response to small current injections and did not modify the frequency of action potentials elicited by a 5 pA current step of 3-sec duration. It has been shown that in VNO neurons some pheromones induce a decrease in cAMP concentration, but the physiological role of cAMP is unknown. After application of blockers of adenylyl cyclase we measured a hyperpolarization of 5.1 mV in eleven of fourteen neurons, suggesting that basal levels of cAMP could modulate the resting potential. In conclusion, these results demonstrate that mouse VNO neurons express HCN2 and/or HCN4 subunits and that I_h contributes to setting the resting membrane potential and to increase excitability at stimulus threshold.

The G-Protein γ-Subunit, Gγ8, in the Transduction of the Pheromonal Signal

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G-protein protein coupled receptors are involved in several functions in the peripheral olfactory systems as detection of odorants or pheromones, axon targeting, cell survival. In the VNO there are two types of neurons that differ in their expression of pheromone receptors and G-proteins subunits. Chemosensory neurons of the apical and basal region express high levels of respectively Gαi2 and Gαo protein subunits. Previous observations showed that the G-protein γ-subunit, Gγ8, is mainly expressed in the basal but also in the apical vomeronasal neurons, suggesting that Gγ8 may play a part in the transduction of the pheromonal signal. Here, we have investigated the role of Gγ8 in the vomeronasal system.

CNG Block may Reduce the Olfactory Sensitivity

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In the human history, the flavor and fragrance have been broadly employed not only for inducing the pleasant sense of scent, but also for masking the unpleasant smells. Such dual effects of odorants are explained by the fact that human olfaction receives two opposing effects of excitation and inhibition from odorant molecules. Especially, a unique property of wide-spectrum and low-selective odorant inhibition of the olfactory signal has been employed in the smell-masking industries, such as the usages of spices or the development of perfumes. One possible mechanism explaining this wide-spectrum olfactory inhibition has been shown to be at the sensory receptor cell level. In the present work, we show that olfactory cyclic nucleotide-gated (CNG) channel that is a key element that converts odorant stimuli into electrical signals is sensitive to odorant inhibitions, consistent with the expression of wide-spectrum olfactory inhibition. It was shown that the spectra for human olfactory masking have a positive correlation with those of the CNG channel blockage. In order to examine whether volatile molecules in the air phase can affect CNG channels situated in the mucus, Ringer' solution that was just pre-exposed to vapors was applied during the CNG response. As a result, odorant vapors affected CNG channels after the natural partition of molecules across the air/water boundaries. Cl (Ca) channels that pass more than half of the transduction current were resistant for the odorant suppression. It is interpreted, however, that in the natural odorant response this Cl channel is affected by the reduction of Ca-influx through the CNG channels. Because this signal transmission process includes non-linear boosting, CNG blockage leads to an amplified reduction in the net current. The present work thus suggests that CNG channels switch on/off the olfactory signalling pathway, and that the on/off signals are both non-linearly amplified by the subsequent opening and closing of $Cl_{(Ca)}$ channels. Furthermore, it has been shown that the olfactory cilia where CNG channels are densely distributed are directly exposed to the body-external environments covered by the mucus layer (Takeuchi & Kurahashi, 2008). The olfaction could thus be gain-controlled with volatile chemicals from the outside of the body.

Visualization, Manupilation and Recording of Nanotube Olfactory Cilia

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Olfactory signal transduction is conducted at very fine cell compartment expressing nanotube structure (100 nm diameter). Up to this point, physiological experiments treating such fine structure are very limited, obviously because of technical limitations. Problems were mainly situated in (a) visualization of this thin structure without fixations, (b) manipulation of substances in the highlighted area and (c) simultaneous recoding from the living cilia. To overcome such difficulties, we employed a combined technique of the patch clamp and photolysis of caged compound under fine visualization of nano-scale structure with the laser-scanning confocal microscope (LSM). To understand the nature of cytoplasmic second messengers and the transduction channels (CNG, Cl(Ca)) on the single cilium, cilia were loaded with both caged compounds (either cAMP or Ca) for photolysis and Lucifer yellow for fluorescent visualization.

When the local area (ca. 1 um length) of cilium loaded with caged cAMP was illuminated, the cell showed an inward current response exceeding a hundred pA of current, presumably generated by the high density CNG & Cl(Ca) channels, expressing a high signal amplification to the local ciliary excitation. At the same time, linear summation of small currents was observed with local weak illuminations. With the mapping, it was confirmed that transduction channels are present along entire cilium. Also, responses induced by two different parts within the single cilium were independent, when monitored with adaptation. Based on these observations, we discuss about the real-time biochemical behavior of enzymes, second (& third) messengers and ion channels within the nanotube olfactory cilia in relation to the signal amplification, adaptation, masking and olfactory manipulation.

Topic 31: Behavior

Characterization of Protein Vomeronasal Stimuli Involved in the Predatory Behaviour of Thamnophiine Snakes

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The vomeronasal organ of snakes is involved in many essential behaviours such as territory recognition, partner courtship or prey detection. Its role in the predatory behaviour is particularly crucial in the Thamnophiine snakes. These garter snakes eat preferentially fishes, amphibians and eartworms. The vomeronasal organ allows them to detect and attack preys through perception of stimuli located in the cutaneous mucus of these preys. The vomeronasal organ of garter snakes, well-developed, is located at the base of the nasal cavity which is distinctly separated. It although possesses a connection with the oral cavity through the nasopalatine duct. It has the appearance of a paired, tubular structure with a crescent-shaped lumen. The concave side of the lumen is lined with receptor neurons. These neurons are responsible of the perception of vomeronasal stimuli. Currently, less is known about the chemical nature of these stimuli. However, studies of the predatory behaviour of garter snakes led to the identification of three phagostimulating compounds. These vomeronasal stimuli are all proteins. One, called ES20 (electric shock 20 kDa) and characterized by Halpern et al., is derived from electric shock-induced earthworm secretion. ES20 is chemoattractant for *Thamnophis sirtalis*. The two others were recently isolated in our laboratory. They are derived from the cutaneous mucus of the frog *Rana temporaria* and are characterized as Parvalbumins α et β. These proteins, known to be essentially present in the muscular and nervous tissues, are chemoattractant for *Thamnophis marcianus*. In the present study, we investigated the identification and characterization of proteineceous chemoattractants for *Thamnophis marcianus* and *Thamnophis sirtalis* in the cutaneous mucus of different species of amphibians and fishes known to be eaten by these snakes. Immunological, proteomic and behavioural approaches show that parvalbumins are presented in the cutaneous mucus of each studied prey and are involved in the chemoattraction of cutaneous secretion. Before the study on *Rana temporaria*, extracellular localization of parvalbumins has never been reported.

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Dogs are able to Recognize Insect Individuals by Odour

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Police in many countries uses dogs specially trained to identify humans by individual scent. The most common method of this identification is scent line up for matching the human odour collected on a cotton material to the odour sample of the person suspected. We examined how effective dogs can use their sense of smell to identify insect individuals.

We used the Russian canid hybrid originating from an initial hybrid between a Siberian Husky and a Golden Jackal bred in the Aeroflot airline security. Six dogs were trained to discriminate an initial scent from others. Experiments were performed in a special room used only for scent identification training and line-ups. Insect species tested are the cockroach *Nauphoeta cinerea,* crickets *Acheta domesticus* and *Gryllus bimaculatus,* and the beetle *Pachnoda marginata*. The scent from each individual live insect was collected on cotton sheets in a separate plastic container. The scent line-up consisted of 12 glass jars arranged in a circle, each containing a cotton sheet with scent collected from one insect. A dog received the initial scent (collected from one of 12 insects) and was asked to match it to one in the line-up. In all 20 experiments dogs successfully recognized the target insect individual among 12 conspecific individuals of the same sex and age reared in the same cage.

Individual recognition by odour was reported in mammals, birds, malacostracan crustaceans and social insects (ants and bees). Our experiments show that in some orthopterans and beetles individuals differ in their odour, and it remains to be tested if these insects are able to recognize each other individually.

Volatile Compounds in the Faeces of the Mouse

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Male mice, *Mus musculus*, establish and maintain their territory by marking it with urinary droplets, deposited at particular sites, from which urinary odorant molecules are released in the environment and sensed by other mice. The possibility exists that also odorant molecules released from other body fluids or faeces are involved in chemical communication among mice, but up to now no thorough chemical analysis has been carried out on them, therefore we sought to identify volatile substances potentially involved in chemical

communication from the most abundant of these bodily products. Faecal pellets were collected from different mice and stored at -20°C until analysis. Upon thawing, volatile molecules were collected using SPME (Solid Phase Micro-Extraction) and analyzed via GC/MS according to established protocols. Different groups of mice were analyzed: adult males, castrated males and females differing in their estrous cycle phase. Males and estrous females presented the largest variety of compounds. Ten of the identified molecules were common to all groups, for example pentadecanol, indole and isopropyl-palmitate. Other molecules were specific for each group: 3-octen-2-one was found only in castrated mice, while four molecules were unique to males and other six to estrous females. These results suggest that some of the volatile molecules released from faeces are dependent on gender and hormonal status, and can conceivably convey information about the sexual identity of the releaser to the other mice.

Chemical Signals May Play a Critical Role in Reproductive Isolation of Closely Related Mus Species

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The Mus musculus s. lato species group includes closely related taxa at different stages of divergence: sympatric species *Mus musculus – M. spicilegus*; *M. domesticus – M. macedonicus*; *M. domesticus – M. spretus*; parapatric taxa which hybridize in zones of contact *M. musculus – M. domesticus – M. castaneus*; and allopatric species *M. spicilegus – M. macedonicus*; *M. spicilegus – M. sprertus*. As a result the *M. musculus* species group has served as an excellent model group in studies of microevolution (Sage et al., 1993). To study the role of chemical cues in reproductive isolation of closely related *Mus* species we used three basic approaches: behavioral, hormonal and immunohistochemical. We used standard two and four preference tests as well as habituation-dishabituation tests. In all tests individuals of sympatric *M. musculus – M. specilegus* and allopatric *M. macedonicus – M. specilegus* discriminated con- and heterospecific odors. Both males and females investigated significantly ($p < 0.01$) longer opposite sex urine samples of conspecifics versus heterospecifics. Males responded to exposure of estrous female samples of conspecifics with elevated plasma testosterone level ($p < 0.01$). However we did not observe plasma testosterone response in males when heterospecific female urine was used. In males of different species Fos-immunoreactivity was recorded in main olfactory bulb (MOB), accessory olfactory bulb (AOB) and in vomeronasal organ (VNO) epithelium in response to stimulation with urine samples from receptive con- and heterospecific females. In *M. domesticus* in response to stimulation with conspecific receptive female urine we observed Fos-immunoreactivity in V1R and V2R zone. Using the very same design of experiments in *M. specilegus* we observed Fos-immunoreactivity in V1R zone only. There were no activated cells in VNO receptor epithelium in response to stimulation with receptive heterospecific female urine. Our data support the hypothesis that chemical cues play an important role in reproductive isolation of closely related *Mus* species.

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Socializing Increases Club Cell Number in Crucian Carp

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Club cells in the skin of cyprinid fish are assumed to contain "schreckstoff" that releases alarm behavior. Accordingly, they are also denoted alarm substance cells (ASC). In a North-American cyprinid, the number of ASC has been found higher in lakes with than without predator fish. We questioned if such an increase in ASC could result from exposure to alarm signals, released over time by predators labelled by prey odors?

Crucian carp (*Carassius carassius*) develops a deep body in the presence of predator fish; an inducible defense attributed to alarm signal exposure. By exposing crucian carp to conspecific skin extract, we wanted to test if: 1) the number of ASC increases, and 2) any correlation exist between body depth increase and number of ASC.

We raised crucian carp for 7 weeks in groups of four, or alone, and exposed them to either conspecific skin extract or skin extract of brown trout (control: does not possess ASC). In addition, all combinations of raising method and exposure type were duplicated, supplying fish with either a low or a high feed ratio.

Independent of rearing type (group or single), body depth and condition factor increased in crucian carp with the size of their feed ratio, but no differences were found from exposure to conspecific skin extract. The number of ASC per mm of epidermis increased with feed ratio, and almost a 10-fold increase in number of ASC was found as a maximum. Thickness of epidermis increased with increased condition factor, as did the number of ASC in relation to epidermis thickness. An unexpected finding was that epidermis thickness, and thereby ASC, increased significantly more for fishes raised in groups than for fishes raised alone.

We conclude that alarm substances in cyprinid fish, confirmed present when introducing extracts on day-1, may not be the chemical signals inducing body depth increase in crucian carp. However, increased availability of food, resulting in an increased condition factor, allows the fish to invest in more ASC. In addition, there seems to be a hitherto unknown awareness of conspecific proximity, expressed in an increased production of ASC. We speculate that this increase may also be induced by conspecific chemical signals.

Artificial Rearing Retards Taste Learning Abilities in Adolescent Rats

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The acquisition of taste aversions and preferences are considered to be primitive and robust forms of learning, which have been demonstrated as early as the prenatal life. We have studied the effect of artificial rearing on taste neophobia and taste aversion learning in 35-43 day-old rats.

Forty five male Wistar rats were assigned to three groups: a control maternally reared group (Ctrl; n=12) and two artificially reared

groups either on a standard adequate diet (Stand; n=14) or on an n-3 deficient diet (Def; n=14). Animals were allowed to drink a cider vinegar solution (3%) during two morning daily drinking sessions. Additionally, a control maternally reared group (n = 5) was not exposed to the taste solution. All groups were also subjected to taste aversion conditioning. Fifteen minutes after drinking a sodium saccharin (0, 1%) solution, they receive i. p. lithium chloride (0.15M; 1% b. w.) injections. Learned aversions were assessed in a one-bottle test.

Maternally reared, but not artificially reared, rats exhibited taste neophobia, with reduced vinegar solution intake compared with the water baseline intake. For both maternally reared groups, those previously exposed to vinegar solution and those who were not, showed similar taste aversions, evidenced by a reduced intake of the saccharin solution during the one-bottle test relative to the conditioning session. Saccharin aversions were not evident in those artificially reared groups.

The results suggest that the absence of critical developmental cues provided by maternal care may have retarded taste learning abilities. Further work will be required for understanding the nature of the specific artificial rearing effects on the taste learning brain circuits' development.

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Suppression of C-FOS Expression in the Amygdala Impairs the Retrieval of Taste Potentiated Odor Aversion

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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 conditioning has been referred to taste-potentiated odor aversion learning (TPOA). Because of its robustness, rapid acquisition and long-lasting association of chemical cues with malaise, TPOA is an interesting model for investigating neuronal mechanisms of plasticity. It is well known that the immediate early genes such as c-fos are involved in long-term changes in cellular functioning linked to mnesic processes. Previously, we used the FOS immuno-cytochemical detection to map the brain regions which are activated when TPOA is retrieved. The rats which had received the lithium chloride showed an increased FOS expression in various brain regions including the basolateral nucleus of amygdala (BLA). Some other studies further indicate that among the brain areas that subserve aversion memory, the BLA could to play a critical role. The aim of the present study was to get new insight regarding the role of the c-fos expression within BLA when the rats are submitted to TPOA retrieval 3 days after initial conditioning. We performed bilateral injection of oligodeoxynucleotides (ODN) antisense to c-fos into the BLA 8 hours before the TPOA retrieval induced by odor presentation. By means of immuno-cytochemistry, we observed that the FOS protein expression was reduced in the BLA. In comparison, injection of sense ODN had no effect on FOS expression. The behavioral observations provided evidence of a deep impairment of the TPOA expression when antisense ODN were microinjected into the BLA. In contrast, rats which received bilateral injection of either sense ODN or inverted ODN avoided of the odor. Our data suggest that the FOS mediated signalling pathways within BLA are essential for TPOA retrieval since the antisense ODN method disrupted the rejection of the odor cue.

The Motor Side of Odour

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We used kinematics to explicitly test the influence of olfactory stimuli on a reach-to-grasp movement. Participants were requested to reach towards and grasp a small (e.g., strawberry) or a large (e.g., orange) visual target. They naturally grasp the small target between the thumb and the index finger (i.e., precision grip, PG) and the large target opposing the thumb with all the other fingers (i.e., whole hand grasp, WHG). This experimental task was performed in the absence or in the presence of an odor evoking either a small (e.g., strawberry) or a large (e.g., orange) object that if grasped would require a PG and a WHG, respectively. When the type of grasp evoked by the odor did not coincide with that for the visual target, interference effects were evident on the kinematics of both hand shaping and arm reach movement. Specifically, the levels of synergies amongst fingers decreased and reach duration increased. When the visual target and the object evoked by the odor required the same type of grasp, facilitation emerged and the intrinsic relations amongst individual fingers were maintained. This study demonstrates that olfactory information contains highly detailed information able to elicit the planning for a reach-to-grasp movement suited to interact with the evoked object. The findings offer a substantial contribution to the current debate about the multisensory nature of the sensorimotor transformations underlying grasping.

Purification and Characterization of Recombinant Brazzein Secreted by the Yeast *Pichia pastoris*

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Brazzein is a small (6.5 kDa), heat- and pH-stable sweet protein originating from the fruit of *Pentadiplandra brazzeana* Baillon, a plant found in West Africa. Brazzein isolated from its natural source exists in two forms differing in sweetness intensity. The major form (54 amino acids, ~80%), called pGlu-bra, contains a pyroglutamic acid (pGlu) at is N-terminus, while the minor form (53 amino acids, ~20%), called des-pGlu-bra, lacks the N-terminal pGlu. It has been reported that des-pGlu-bra is twice as sweet as pGlu-bra. Heterologous expression of brazzein in bacteria is complicated by the presence of a pyroglutamic acid (pGlu) in the major form of brazzein. In this present study aimed at defining the structure-function relationship of brazzein, we established an expression system of brazzein using the methylotrophic yeast *Pichia pastoris* under the control of the methanol-inducible alcohol oxidase

(AOX1) promoter. Brazzein was secreted into the extracellular medium using the α-factor preprosequence peptide of *Saccharomyces cerevisiae* without the Glu-Ala-Glu-Ala spacer. We found that brazzein regularly accumulated in the culture medium reaching approximately 40 mg per liter of culture over an expression period of 6 days. After dialysis, the yeast culture filtrate containing recombinant brazzein was submitted to cation-exchange chromatography and two brazzein isoforms were isolated. MALDI-TOF mass spectrometry revealed that the first isoform was pGlu-bra, while the second isoform, called Gln-bra, was assigned to the same molecule with a N-terminal glutamine residue instead of the pyroglutamic residue. 1D NMR spectroscopy revealed that both brazzein isoforms were properly refolded in agreement with their sweetness properties. The efficient production of recombinant brazzein in *Pichia pastoris* should allow mutational analysis and labelling of brazzein with stable isotopes for NMR investigations to understand the relationships between structure and biological function of this sweet protein.