Chem. Senses 36: E1–E84, 2011 doi:10.1093/chemse/bjq126

ABSTRACTS

Abstracts from the XXth Congress of European Chemoreception Research Organization, ECRO-2010, Avignon, France

SYMPOSIA ABSTRACTS

Symposium Odorants, Receptors and glomeruli:

The dynamics of the peripheral olfactory system

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The peripheral olfactory system is often thought of as a static organ, reasonably well developed at birth and maintained through an unusual ability to regenerate new neurons throughout life in the peripheral tissues and in the interneurons of the olfactory bulb. In fact the system is in a surprisingly dynamic condition, with new receptor genes being turned on and off and proliferation occurring at very different rates through out the life span of the organism. Not only is the epithelium, and perhaps also the bulb, growing for a considerable period postnatally, but it continues to change in crucial ways, both functionally and structurally, throughout life in response to environmental and other factors.

Mitral cell time of origin determines position and cortical connections

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Odorant receptor maps in mammals, constructed by the glomerular coalescence of sensory neuron axons in the olfactory bulb and the convergence of the apical dendrites of mitral cells into single glomeruli, is essential for proper odor information processing. However, how this map develops is unknown. Using a battery of methods, including markers of cell division in combination with tracers of neuronal connections and *in vivo* imaging, we show that early- and late-generated mitral cell are differentially distributed within the dorsal and ventral subdivisions of the odorant receptor map. In addition, we demonstrate that late generated mitral cells extend significantly stronger projections to the olfactory tubercle than the early-generated mitral cells. Together, these data indicate that the odorant receptor map may be determined initially by the timing and sequence of mitral cells genesis. The relationship of the timing of neurogenesis and pattern of connectivity for the olfactory bulb principal neurons, mitral cells, make it clear that like multi-layer cortices, the cellular segregation and functional organization of the olfactory bulb is developmentally-dependent.

Deconstructing smell

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Humans and other mammals can discriminate a vast array of chemicals in the external world. While most are sensed as odors, others act as pheromones that stimulate hormonal changes or instinctive behaviors. Using molecular genetic approaches, we have searched for receptors that first detect odorants and pheromones in peripheral sense organs and then explored how signals derived from those receptors are organized in the nervous system to generate diverse perceptions and innate responses.

Olfaction targeted

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The mammalian olfactory system is increasingly recognized as an attractive model system for studying the formation of neural circuits during development. An olfactory sensory neuron expresses just one the of ~1000 odorant receptor genes in the mouse genome. Axons of neurons that express a given odorant receptor gene coalesce into a few of the ~1800 glomeruli in the olfactory bulb of the mouse. A mapping problem is thus posed: ~1000 populations of neurons, each expressing a particular odorant receptor gene, must be sorted onto ~1800 glomeruli. A productive approach to study axonal coalescence is targeted mutagenesis of odorant receptor genes, to express axonal markers in neurons expressing a given odorant receptor gene. These experiments have revealed that the odorant receptor is a critical determinant of axonal coalescence into glomeruli. My talk will summarize recent results of our research on odorant receptor gene choice and axonal wiring.

Symposium Gut-brain interactions:

Metabolic regulation of brain reward systems

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In order to maintain normal physiological functions, animals must obtain metabolic fuels from a diverse array of macronutrients. Animals rely primarily on chemoreceptor systems, such as gustation, to establish consummatory preferences among available food sources. Accordingly, brain dopamine systems, which are critical regulators of food intake, display exquisite sensitivity to preferred tastants including most sweet substances. However, evidence is mounting in favor of the hypothesis that these reward-processing neurotransmitter systems are also under the strict influence of metabolic signals generated upon nutrient intake that do not depend on chemosensory processing. We will discuss recent evidence supporting a fundamental role for nutrient utilization, in particular of glucose metabolism, in regulating sugar intake. These findings are

further corroborated by evidence of dopamine release regulation by glucose oxidation rates. The current picture therefore suggests that carbohydrate-specific preferences can develop independently of sweetness or caloric load, an effect associated with the ability of a given nutrient to regulate glucose metabolism and stimulate brain dopamine centers.

Neuronal responses in the nucleus accumbens during hunger and satiety

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In addition to the well known role of the Nucleus Accumbens (NAc) in reward processing, the NAc is also contained in the circuitry involved in the control of feeding; especially the consumption of palatable foods. Previous studies on rats have found that pharmacological manipulations of NAc either increase or decrease food intake and that palatable food intake release dopamine in NAc. Moreover, downregulation of D2 receptors in NAc have been related with compulsive eating behavior. However, the exact role of NAc neurons on ''natural'' freely licking for food remains unclear.

Here, we recorded single neurons in the NAc, while freely-moving rats feed themselves with ''Ensure®'' until satiety. Ensure® is a liquid food supplement with chocolate flavor and that is rich in protein and fat. Importantly, the rats decided on their own volition when to begin and end a ''meal.'' We identified two populations of neurons that responded during feeding: one inhibitory and one excitatory. Firing rate modulations began with Ensure® consumption and was maintained for the duration of the meal and up to 15 minutes beyond. Both excitatory and inhibitory responses did not covary with either the isolated licks or licking clusters, suggesting that these NAc neurons do not encode the ''licking microstructure,'' but rather the ''macrostructure of feeding'' as reflected in the meal size.

These results suggest that in NAc both inhibition and excitation are required to initiate, maintain and complete an effective meal. In summary, we found that the microstructure and macrostructure of licking may use separate neural substrates and that a population of NAc neurons encodes the macrostructure of feeding.

This work was supported in part by CONACYT 78879 and ICYTDF PICDS08-59

The taste organ is a target for orexigenic and anorexic mediators

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Sweet taste perception is important for animals to detect external carbohydrate source of calories and has a critical role in the nutritional status of animals.

Our previous studies demonstrated that the taste organ is a peripheral target for leptin, an anorexic mediator that reduces food intake by acting on hypothalamic receptors. In mice, increase in plasma levels of leptin leads to reduction of sweet taste responses of taste nerve and cells via activation of leptin receptors. In human, the recognition thresholds for sweet compounds have a diurnal variation that parallels variation for leptin levels, with lowest in the morning and highest in the night. Plasma levels of leptin are shown to inversely correlate with circulating endocannabinonds (anandamide and 2-arachidonoyl glycerol), orexigenic mediators that induce appetite and stimulate food intake via CB1 receptors mainly in hypothalamus.

Here, we investigated potential effect of endocannabinoids on sweet taste responses in mice and found that endocannabinoids oppose the action of leptin to act as enhancers of sweet taste. In wild type mice, administration of endocannabinoids enhances behavioral, taste nerve and taste cell responses to sweet taste without affecting responses to bitter, salty, sour and umami stimuli. In contrast, mice genetically lacking CB1 receptors show no such enhancement by endocannabinoids Endocannabinoids, therefore, not only stimulate food intake via central systems but also may increase palatability of foods by enhancing peripheral sweet taste responses.

Reciprocal regulation of peripheral sweet taste reception by endocannabinoids and leptin may contribute to their opposing actions on food intake and play an important role in regulating energy homeostasis.

Supported by JSPS Grant-in-Aid for Scientific research no.18109013 and 18077004 (to: Y.N.)

Gut-brain nutrient sensing in the control of energy homeostasis

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The proximal small intestine is a primary site for nutrient absorption during and between meals. Neural sensors in the gastrointestinal tract also relay information to brain sites in the hindbrain and forebrain important in limiting meal size.

Intestinal lipid infusion elicits the local production of fatty acid derivatives that, in turn, promote satiety and reduce food intake. Intestinally infused lipids also rapidly suppress endogenous glucose production via a sensory vagal neural circuit linking the small intestine to the brainstem, which in turn modulates hepatic glucose production via the vagal motorneuorns supplying the liver.

This circuit provides an early warning system modulating endogenous glucose availability when nutrients are digested during or following a meal. These critical intestinal nutrient sensing areas are not stimulated after gastric bypass, which may account for the rapid and prolonged beneficial effects of this significant weight reduction therapy in treating type 2 diabetes and morbid obesity.

Supported by the Skirball Institute for Nutrient Sensing, NIH RO1 DK 066618, NIH DK 020451

Gut-brain interactions and dopamine release

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Palatable stimuli, such as sucrose, elicit appetitive behavioral changes indicated by a side bias reversal in two bottle test responses and dopamine release in the ventral striatum. Previously we found in Trpm5$^{-/-}$ mice in which functional sweet-taste transduction is absent, ingested sucrose, but not non-caloric sucralose, can produce the same behavioral and neurochemical effects. However, the underlying mechanisms remain unknown. To clarify the contribution of circulating glucose to the post-ingestive effects of sucrose, we performed the same behavioral test with rats and compared oral ingestion with endovenous administration of glucose solutions in the jugular and portal veins. Glucose administered in the jugular vein conditioned robust side-biases albeit at much higher concentrations than glucose administered directly in the hepatic portal vein. Only the changes in glycemia in the portal vein blood after administration of glucose solutions orally were consistent with the behavioral side bias study. Moreover, at the same concentration, glucose administered in the portal, but not jugular vein, increased the relative frequency of spontaneous dopamine release events in the ventral striatum of anesthetized rats, as measured by cyclic voltammetry. We conclude that glycemia levels in the hepatic portal venous system are more relevant for reward-related responses than systemic glycemia.

This research was funded by NIH DC 01065.

Symposium Odour representation in the olfactory bulb:

Odor coding in mammals involves information redistribution but not decorrelation in neural assemblies

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How neural networks encode sensory information is a key question in neuroscience. From insects to mammals, the sense of smell is central to animals' life as it is involved in feeding, mating, predator avoidance but also social interactions or navigation. Regardless of the diversity of purposes, the problem of detecting and identifying odors has found highly conserved solutions among the animal reign.

In all vertebrates and invertebrates, odorant molecules are sensed by a large family of olfactory receptor proteins expressed by sensory neurons. These neurons select only one receptor out of a large possible repertoire, and their axons converge in a receptor specific manner onto segregated anatomical structures called glomeruli in the first brain relay of olfaction [the vertebrate olfactory bulb (OB) or the insect antennal lobe (AL)].

Beyond their input organization, OB and AL also share circuitry organization and functional homology. However, it is still debated whether these first relays use the same neuronal coding principles in all species. In particular, fish and to some extent insects are thought to encode, over time, odorant stimuli through "slow" temporal processes (between 300 ms to 2 s) such as decorrelation of odor evoked firing patterns in the neuronal population. Such process of pattern decorrelation tends to separate over time similar population firing activity evoked by similar stimuli, possibly leading to perceptual discrimination.

In mammals, it is still unknown whether such mechanism exits especially since animals can discriminate between odors in 100-300 ms and since the system has to cope for constantly changing inputs due to the breathing. In this study we addressed this question by analyzing the encoding of odor identity and intensity of simple compounds as well as mixture morphing.

We performed large scale single unit recording using multiple implantable tetrodes, allowing us to record large cell assemblies. We found that at a fast time scale, we did not observed decorrelation but a dynamic evolution of correlation (increase and decrease) matching the inspiration and expiration cycle. As the firing rate of the population also evolved dynamically by increasing and decreasing during inspiration and expiration respectively, the correlation evolution seems to reflect firing rate variation. On a slow time scale, we observed a small decorrelation of activity across respiratory cycles but mostly between the first and second cycles. On the other hand, we found that different cells assemblies convey sensory information at different time period.

In conclusion, in the mammalian olfactory bulb, odor coding does not involve over time decorrelation but rather redistribution of information in different cell assemblies.

Fast simultaneous imaging of mitral cell populations reveals odor specificity of latency patterns

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At the level of the olfactory bulb (OB) odor information is contained in the spike patterns of mitral/tufted (M/T) cells. In addition to the identity of the activated M/T cells, the temporal patterns of their responses are important for olfactory coding (Laurent et al 2001). However, it is still unclear as to which aspects of these spatio-temporal patterns contain the odor-specific information that is subsequently decoded by the OB's target areas. Behavioral studies in vertebrates show that odor discrimination and recognition can be accomplished within 500 ms or even less depending on the task and the species. Attention has thus been directed towards activity features on short time scales, such as instantaneous firing rates, the average firing phase and response latencies; Bathelier et al 2008, Lehmkuhle et al 2006). The latter have been shown to be important coding parameters in other sensory systems. While odor- and concentration-dependent latencies have also been described in the olfactory system, a potential role for odor coding has been discussed controversially (Bathellier et al 2008; Schaefer and Margrie 2007).

In order to elucidate the specificity and reproducibility of odor response latencies, one has to meet two crucial requirements: (1) M/T cell responses need to be measured at a sufficiently high temporal resolution and simultaneously, since only simultaneous recordings can reveal the relative response latencies which may contain the odor-specific information. (2) The similarity of the latency patterns of the M/T cell population under investigation has to be

quantified in order to assess the patterns' reproducibility and specificity under different experimental conditions. Since studies satisfying both requirements are lacking, the role of response latencies in olfactory coding has remained unclear thus far.

We solve this issue by using a fast confocal line illumination microscope and ensemble correlation techniques. The resulting evidence reveals that latency vectors of M/T cells are reproducible and odor-specific. They accurately predict the odor identity on a single trial basis and on short time scales. Latency patterns thus appear to contain all the information higher brain centers would need to identify odors and their concentration.

Bathellier, B., Buhl, D.L., Accolla, R., and Carleton, A. (2008). Dynamic ensemble odor coding in the mammalian olfactory bulb: sensory information at different timescales. Neuron *57*, 586 – 598.

Laurent, G., Stopfer, M., Friedrich, R.W., Rabinovich, M.I., Volkovskii, A., and Abarbanel, H.D. (2001). Odor encoding as an active, dynamical process: experiments, computation, and theory. Annu Rev Neurosci *24*, 263 – 297.

Lehmkuhle, M.J., Normann, R.A., and Maynard, E.M. (2006). Trial-by-trial discrimination of three enantiomer pairs by neural ensembles in mammalian olfactory bulb. J Neurophysiol *95*, 1369 – 1379.

Schaefer, A.T., and Margrie, T.W. (2007). Spatiotemporal representations in the olfactory system. Trends Neurosci *30*, 92–100.

Odor Processing with an experimental model of Olfactory epithelium and bulb

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Artificial olfaction was introduced as a model tool to investigate olfaction properties [1]. Nonetheless, the only analogy between the natural and the artificial system lies just in the selectivity properties of the receptors. The implementation of more sophisticated features such as the large number of receptors and the glomerular layer have been hampered by technical difficulties related to the management of large numbers of simultaneous signals.

As demonstrated in the past, optical imaging is a read-out technique for sensors development that can provide large sensor arrays [2]. On that basis, we recently introduced an artificial olfaction system based on the imaging of a continuous layer of chemical indicators [3]. In this situation an image sensor provides a segmentation of the whole sensing layer in a number of elementary units corresponding to the pixels of the image. Eventually, since it is possible to evaluate the optical properties of every single pixel, each pixel of the image may correspond to an individual sensor. In this regard, even low-resolution images may easily result in thousands of independent sensing units.

In our system a collection of arbitrarily shaped regions of color indicators is illuminated by a controlled light source; the optical characteristics of each pixel of the image are measured by a camera yielding the light intensities in the three channels red, green, and blue. The combination of illumination sequence and camera read-out results in a fingerprint encoding the optical properties of the sensing layer portioned in image pixels. Even a simple classification of these fingerprints assigns each pixel to a class, and each class contains pixels carrying the same color indicator. This behavior resembles the association between ORNs carrying the same chemical receptors into the same glomerulus [4]. On the basis of this analogy it is straightforward to describe the layer of indicators as an artificial epithelium, pixels of the image as artificial olfactory neurons, and the classes provided by the classifier as an abstract representation of artificial glomeruli.

This system thus allows the generation of a complex model of olfaction, including glomerular compartmentalization [5], which is then applied to data generated by the exposure to pure and mixed gases. Results show that such a model enhances the discrimination of pure and mixed odors. Eventually, such a platform, apart from evidencing the similarities between natural and artificial olfactory systems, is also proposed as a practical tool to test olfactory models.

1. K. Persaud and G. Dodds, *Nature* 299 (1982) 352
2. Dickinson et al., *Nature* 382 (1996) 697
3. C. Di Natale et al., *PLoS ONE* 3 (2008) 3139
4. P. Mombaerts, *Annu Rev Neurosci* 22 (1999) 487
5. D. Schild and H. Riedel, *Biophysical Journal*, 61 (1992) 704

Representation of chemical features in the olfactory bulb

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In the vertebrate brain, chemical information is encoded by olfactory sensory neurons (ORNs). ORNs expressing the same receptor protein converge to distinct compartments in the olfactory bulb, the glomeruli, where synaptic contacts with downstream neurons are made. Hence, the activity of one glomerulus reflects the odor response of a population of ORNs with a specific odorant receptor. We aim at characterizing the chemical features of odorants which activate specific glomeruli combining virtual and biological screening approaches in an iterative way.

In biological screening, we first measured the *in vivo* response of identified glomeruli to a set of odorants. Automatic, computer-controlled odorant delivery enabled high-throughput screening. Based on the measured responses, we built predictive models for glomerulus activation based on the physico-chemical properties of the odorants. Then we used these models to filter an odorant database for potential ligands (virtual screening). Subsequently, we assessed the accuracy of our predictions in biological screening. The results from the second biological screening round are incorporated into our models to improve their predictive power. Preliminary results indicate two previously unknown ligands for the ORN under investigation.

We will continue the iterative scheme until we have achieved satisfactory prediction accuracy. Then, we can extract physico-chemical features of odorants which are likely to evoke responses in the glomerulus [1]. We will extend our predictions to as many identifiable glomeruli as possible. Our goal is a feature map which charts the relevance of physico-chemical features for glomerulus responses across the olfactory bulb.

This work is funded by the Deutsche Forschungsgemeinschaft (DFG) within the Priority Programme SPP 1392 ''Integrative Analysis of Olfaction''.

[1] Schmuker M, de Bruyne M, Hähnel M and Schneider, G (2007): Predicting olfactory receptor neuron responses from odorant structure, Chemistry Central Journal, 1:11.

Granule cells in the adult olfactory bulb: from development to function

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Neurogenesis occurs in the adult central nervous systems of a variety of vertebrate and non-vertebrate organisms. The roles that the new neurons play, and the factors that influence their birth and survival, are critical issues that are under intense investigation. Significant progress has been made in understanding the functions of new neurons in the olfactory system, a pathway where neurons are added throughout life in a wide range of species. With the adult mouse olfactory bulb as a model, this presentation will address a series of fundamental questions concerning the role(s) that neurogenesis plays in the normal functioning of the adult olfactory system: How do these regions balance the need for plasticity with the need to maintain already–functional information processing networks? Is neurogenesis in the adult olfactory system a constant, restorative process, or is it flexible, producing different numbers of neurons to certain microcircuits according to an animal's environmental experience? And are new neurons in the adult brain born to perform a particular task not possible for mature neurons, or are they generated as flexible units to undertake whichever role their target structure is in need of most?

Together, the confluence of data from different behavioral analysis suggests a complex dialogue between the environment and information processing in the olfactory bulb circuitry. This presentation will touch on these various topics that are of broad interest to understand how our sense of smell adapts to the ever-changing external word/internal states.

Symposium Bitter taste receptors throughout the animal kingdom:

Introduction

Maik Behrens and Wolfgang Meyerhof

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Based on chemosensory cues animals respond to the detection of nutrients in general with attraction, whereas noxious substances lead to aversive behaviour. Morphologically, this concept is reflected by separate populations of chemosensory cells expressing receptors specialized to the detection of nutritionally relevant molecules, such as sugars, or cells expressing receptors responding to potentially harmful substances. Moreover, even the chemical compounds activating the chemosensory cells in animals as different as fruit flies and mammals are often identical. Whereas this is not too surprising for attractive stimuli like sugars or amino acids, because they are general building blocks of nature, it is rather astonishing that of the numerous structurally diverse compounds humans perceive as bitter some also result in avoidance behaviour in animals including other mammals, non-mammalian vertebrates such as fish, and invertebrates such as fruit flies and worms.

Bitter taste receptor genes are believed to undergo rapid adaptive diversification in response to selective pressure exerted by toxic compounds present in the environments of animal species. However, the habitats of e.g. human and fish are very different, but yet, both species respond to the classical bitter stimuli quinine and denatonium.

The apparent similarity of responses to noxious compounds is even more surprising since: i) the degree of amino acid conservation in the corresponding receptors is often low, both within and between species. ii) The number of receptor genes in different species can be as different as ~50 in frogs and 3 in chicken. iii) A putative common ancestor of vertebrate and invertebrate receptors for noxious substances has not been detected. It seems therefore likely that the task to detect and avoid all/most potentially harmful substances was achieved in the different species in different ways.

The aim of this symposium is to bring together experts working in the field of chemosensory receptors specific for noxious substances (''bitter taste receptors'') focusing on different vertebrate and invertebrate species as well as experts on evolution of chemosensory receptor genes. Ideally, direct comparison of e.g. common or distinct pharmacological properties, expression patterns and gene numbers, etc., will reveal general evolutionary concepts underlying noxious compound perception in animals.

Bitter taste receptors of mammals

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Among the five basic taste qualities the perception of bitter compounds by the corresponding bitter taste receptors (TAS2Rs) is most complex. Depending on the species, mammalian genomes contain about 20 to 40 functional receptor genes belonging to the large family of G protein-coupled receptors that are devoted to the detection of hundreds of structurally diverse bitter compounds. While bitter taste receptors initially were predominantly associated with gustatory recognition of potentially harmful substances, recent evidence identifying TAS2Rs also in respiratory and gastrointestinal systems suggest a broader function in the recognition of noxious substances throughout epithelia exposed to xenobiotics. Within the oral cavity TAS2Rs are expressed in bitter taste receptor cells forming a population of sensor cells capable to detect all bitter compounds with high accuracy. In recent years the successful deorphanization of, in particular human TAS2Rs, shed some light on the question of how comparably few bitter taste receptors suffice to detect such an array of diverse bitter substances. Whereas some hTAS2Rs appear to possess a relatively restricted agonist spectrum, a number of hTAS2Rs and, in particular, hTAS2R10, -R14, and

–R46 are broadly tuned sensors for a multitude of bitter agonists. As the ability to interact with such a large variety of bitter substances requires an architecture of ligand binding pockets combining flexibility with selectivity, the fact that hTAS2Rs possess only a single agonist binding pocket as exemplified by hTAS2R46 is astonishing. Recently, a novel tool to study the function of bitter taste receptors and bitter taste perception per se has emerged in form of a specific antagonist. Intriguingly, much as bitter substances themselves also inhibitors seem to be capable to act on several receptors and even depend on contacts with amino acid residues of the receptor proteins known to facilitate agonist interaction. The new insights into bitter receptor structure, activation, and antagonism open new research possibilities on one hand, and the development of novel applications on the other hand.

Bitter taste sensation in fish

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Vertebrates may share a common taste-sensing system, and small fish can be a useful model for human taste science. Our group has been working on the taste sensing system in teleost fish to get insight into its evolution in vertebrates including humans. For quantitative assay of taste preference-aversion in zebrafish and medaka fish, we developed a novel system using fluorescently labeled foods that enabled to measure food intakes. The use of this system quantified how much the fish dislike denatonium, a synthetic bitter compound, and how much they like amino acids. To elucidate the molecular mechanisms of the bitter taste sensation in these teleost fish, we searched for T2R genes in their genome databases, and identified several T2R genes. Interestingly, teleost T2Rs were phylogenetically diverse and distantly related to mammalian T2Rs. We then investigated the specific expression of the teleost T2R genes in a subset of taste bud cells, and found that the population of T2Rs-expressig cells in zebrafish was significantly smaller than those in rodents and humans. We also demonstrated that, in zebrafish and puffer fish, the T2R-expressing taste bud cells were distinctly divided into several populations which expressed mutually different sets of T2R genes unlike the situation observed in mammals. Calcium imaging analysis of T2Rs in zebrafish and medaka fish using a HEK293T heterologous expression system revealed that zfT2R5 and mfT2R1 responded to denatonium. These results suggest that, although there may be some fish-specific way of discriminating the tastants, vertebrates have a conserved gustatory mechanism of responding to aversive, bitter tastants.

Bitter taste receptors in insects

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Bitter tasting is crucial for insects because it may help them to avoid ingesting noxious substances. While taste perception involves mainly the oral region in vertebrates, taste perception in insects involves sensory organs or sensilla, distributed all over the body, including the legs, the wings and the ovipositor. These taste sensilla generally host few taste neurons and a mechanoreceptor, and they are involved in detecting food stimuli, pheromones and oviposition substrate cues. In *Drosophila*, each taste neuron may express several gustatory receptors chosen amongst a family of 60 receptors. Most available data indicate that *Grs* are expressed in either ''appetitive'' or ''aversive'' taste cells tuned to sugars, bitter substances or contact pheromones. Bitter substances are detected by aversive taste cells which increase their firing rate in the presence of such ligands. Bitter and pheromonal signals may overlap since bitterness can inhibit mating and inhibitory pheromones can inhibit feeding. Bitter and sweet signals may also interact as we observed that alkaloids like quinine or strychnine, inhibit the response of appetitive cells to sugars. Alkaloids may interact directly with other molecular elements of the transduction of sugar-sensing cells. Alternatively, lateral interactions may occur between taste cells at the periphery. The picture that emerges from such observations is that encoding bitterness in insects may involve not only bitter-sensitive neurons but also other taste modalities, suggesting that insects may be capable of discriminating bitter molecules on a finer scale than previously thought. Studying bitter taste in insects is important because of the potential applications of using specific bitter molecules against insects attacking crops or livestock.

Evolutionary dynamics of bitter taste receptor gene repertoires in primates

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Organisms need to respond properly to the ever-changing environment and to alter their own physiological status or behavior. The receptor molecules sensing various external signals, such as rhodopsin and olfactory and taste receptors, play major roles in sensing physical or chemical environmental changes and in taking in such information inside the organisms. The necessity of such receptor molecules for each organism could be variable and heavily dependent on the environment to which each organism has adapted.

Mammals can basically sense tastes of sweet, sour, bitterness, salt, and umami (the taste of monosodium glutamate). Of these five modalities, sweet, bitter, and umami substances are perceived by G-protein-coupled receptor (GPCR) signaling pathways (Wong et al. 1996). Two GPCR families are involved in these pathways: One is TAS1R, which is associated with sweet and umami substances (Nelson et al. 2001; Li et al. 2002), and the other is TAS2R, which is associated with bitter substances (Adler et al. 2000; Chandrashekar et al. 2000; Matsunami et al. 2000). Bitter sensitivity has a particularly important role because many naturally poisonous substances taste bitterness and virtually all animals show an aversive response to such tastants (Hilliard et al. 2004; Ueno et al. 2004; Chandrashekar et al. 2006), suggesting that bitter transduction evolved as a key defense mechanism against the ingestion of harmful substances and repertoire of the genes then could be determined by what each organism eat. Reflecting this, previous studies have demonstrated that the number of *TAS2R* genes in vertebrates varies from several in fishes and chickens to around 50 in frogs, and even in mammals varies from 4 in platypuses to more than 30 in mice and rats (Go 2006; Shi and Zhang 2006, Dong et al. 2009).

In this talk, I show the evolutionary dynamics of *TAS2R* gene repertoire in 10 primates and a tupai in terms of *in silico* genomic searches and discuss possible evolutionary forces for shaping each gene repertoire comparing with other mammalian data. Additionally, population genetic analyses in chimpanzees and Japanese macaques will be represented and discussed the intra-species polymorphisms in terms of bitter sensitivity especially focusing on the genotype - phenotype linkage.

Molecular evolution of the TAS2R38 gene in primates

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The *TAS2R38* gene encodes a bitter taste receptor that responds to a variety of toxic compounds found in plants. It is hypothesized that the main role of the gene is to provide protection against overconsumption. The importance of this role in primates is unclear. It could be that the gene is crucial because plants depend heavily on plants as a source of nutrition. However, primates are also behaviorally sophisticated, which could enable them to avoid consumption of toxic plants by other means. Both of these possibilities should affect the pressures of natural selection upon the gene. We used a molecular evolutionary approach to examine these pressures. We sequenced *TAS2R38* in 40 primate species representing all major taxonomic groups, including the great apes, New and Old World monkeys, and prosimians. We found evidence that mutations have occurred at a high rate in *TAS2R38* in primates. However, the gene appears to be intact in most species. Premature stop codons and frame-shift mutations were found in four of the forty species examined, suggesting that the gene has completely lost its function in only a few cases. In addition, the distribution of variation along the length of the gene showed evidence of clustering. A significant excess of mutations was found in regions encoding external loops of the receptor, which are thought to be important in ligand recognition. Fewer mutations were found in transmembrane regions and inner loops. This suggests that the receptor has adapted to respond to different bitter compounds while retaining a basic functional structure.

Symposium Culture, language and olfactory perception:

The perceptive relationship as a model to highlight cultural diversity in olfactory perception

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This paper aims at showing the cultural variability of olfactory perception. It relies on linguistic data in French, German and English and on ethnographic data in France. Many investigations pointed the existing intercultural differences in the process of perception. These differences appear both in the lexical field and in social aspects (especially norms, values and behaviors in the perception of odors). The researchers dealing with these topics underlined the connection between these factors. However, few models were drawn to understand the relationship from a linguistic or ethnographic point of view. In the long term, we plan to enhance the link between culture and language in a model combining both dimensions.

We shall first deliver a concise linguistic analysis of verbs in the sense of smell in French (*sentir*), German (*riechen*) and English (*smell*). Indeed, we would like to show that a central point in the meaning of these verbs lies in the notion of perceptive relationship, that is to say the nature and quality of the cognitive relationship between the perceiver and the perceived object in the perceptive process. The results will then be faced with ethnographic data to underline that there are different types of olfactory knowledge, (which can be acquired). So, the concept of perceptive relationship may update the theoretical discussion about the cultural generation of smell perception.

The aim of this paper is to shed new light on the matter of smell perception by highlighting the interaction of linguistic and cultural aspects in relation to a physiological background, all of which calls for the construction of an olfactory object. In an epistemological perspective, we shall point how seminal a collaboration between linguists and anthropologists might be in such a frame of a research about cultural diversity in chemical senses.

The language of perception across cultures

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How are the senses structured by the languages we speak, the cultures we inhabit? To what extent is the encoding of perceptual experiences in languages a matter of how the mind/brain is ''wired-up'' and to what extent is it a question of local cultural preoccupation? The ''Language of Perception'' project tests the hypothesis that some perceptual domains may be more ''ineffable'' – i.e. difficult or impossible to put into words – than others. While cognitive scientists have assumed that proximate senses (olfaction, taste, touch) are more ineffable than distal senses (vision, hearing), anthropologists have illustrated the exquisite variation and elaboration the senses achieve in different cultural milieus.

The project is designed to test whether the proximate senses are universally ineffable – suggesting an architectural constraint on cognition – or whether they are just accidentally so in Indo-European languages, so expanding the role of cultural interests and preoccupations.

To address this question, a standardized set of stimuli of color patches, geometric shapes, simple sounds, tactile textures, smells and tastes have been used to elicit descriptions from speakers of more than twenty languages—including three sign languages. The languages are typologically, genetically and geographically diverse, representing a wide-range of cultures. The communities sampled vary in subsistence modes (hunter-gatherer to industrial), ecological zones (rainforest jungle to desert), dwelling types (rural and urban), and various other parameters. We examine how codable the different sensory modalities are by comparing how consistent speakers are in how they describe the materials in each

modality. Our current analyses suggest that taste may, in fact, be the most codable sensorial domain across languages. Moreover, we have identified exquisite elaboration in the olfactory domains in some cultural settings, contrary to some contemporary predictions within the cognitive sciences. These results suggest that differential codability may be at least partly the result of cultural preoccupation. This shows that the senses are not just physiological phenomena but are constructed through linguistic, cultural and social practices.

Disgust at the crossroads of taste and smell: a lexicological comparison of English and French

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After defining and comparing the concept(s) of 'taste' in English vs 'goût' in French in a previous paper, I have started a lexicological study of the link between taste and the sense of smell in English and in French, and the role that they play in disgust.

My work is based on definitions from various dictionaries and a corpus study of literary and non-literary texts. The most significant examples will be developed in this paper. The first findings show that:

- there is a specific verb, *smell*, to refer to olfaction in English, whereas the French verb *sentir* can have many different meanings, making olfaction more abstract, but also maybe more intimate;
- there is a polarity taste - positive *vs* smell - negative, which is obvious for example in the adjectives *tasty* and *smelly;*
- taste seems to be more subject-centred both in English and in French, whereas the sense of smell seems to be more object-centred in French than in English;
- disgust seems to be more strongly linked to the sense of smell than to taste.

Odour Perception and Cognition. A symposium in memory of Trygg Engen:

Is there but one odor space?

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To define an odor space of practical importance for indoor air quality, it may be fruitful to use an iterative changeover between a top down and bottom up approach. The top down approach is used for determining human ability to perceive similarities/dissimilarities among odor qualities of odorous substances, material emissions and/or complex air mixtures. The bottom up approach is used for selecting reference materials for specific odor qualities, which together would cover and define the potential odor space. An iterative top down and bottom up approach would make it possible to build an n-dimensional metric odor space according to a content model or a distance model. For such an odor space to be useful it must be anchored in a set of reference odor qualities, which would define the odor space coordinates for new odorous materials to be tested. The perception experiments confirmed earlier published results, namely that there are very large interindividual differences in similarity (and pleasantness) scales for odor qualities. The main result was still that there is only one common odor space.

Acknowledgements: This research was supported by grants from the Swedish Research Council FORMAS and the EU FP6 NEST SYSPAQ-project coordinated by the Technical University of Berlin, Germany.

Role of experience in measured chemosensory sensitivity

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A half-century ago, Engen showed that odor thresholds declined progressively over days of practice. In 1968, Engen's student Semb, who studied two Ss intensively, noted, "In both Ss thresholds decreased over a log unit in the first six series. These results are similar to those reported by Engen (1960) which illustrated that absolute thresholds to several odorants decreased markedly as a function of practice in the early stages of experimentation." Similarly, Rabin & Cain found progressive lowering of the threshold for odorants over sessions and even within a day of testing. They concluded: "A large sample of participants studied only superficially cannot, therefore, substitute for a sample of whatever size studied more thoroughly." In 1991, Cain & Gent found that, "On average, threshold declined uniformly by 25% per session". In testing participants who agreed to longer involvement, they found even greater declines, 34% with feedback about the correct answer on each trial and 47% without feedback. Results from Laska in 2004 and Laska & Teubner in 1999 offer two more examples, as did Laska & Hudson in 1991. Indeed, virtually no counterexamples exist. The same effect of experience holds across the basic tastes, as Mojet *et al.* found in repeated testing of ten substances. Cain & Schmidt found perception of the flavor of glutaraldehyde to decline with session over testing. Hence, the phenomenon is robust and generic in the broadest sense of the word, just as Engen implied.

In our Archival Data Project, we have sought to obtain true absolute thresholds within a small margin of error. The thresholds lie about 40-fold below typical values and exhibit relatively low variability. The work has entailed: 1) verification of delivery, including its variability, 2) no use of solvents, except water, 3) minimization of adaptation, 4) gathering of psychometric functions, 5) friendly interface between delivery device and subject, and 6) leisurely collection of hundreds of judgments per session for each subject. We reasoned that any effects of experience would occur early in a session and require no "correction." When we looked at individual odorants we saw no consistent effect, but when we looked across many (*viz.*, 24), we found a mild effect. It lasted just briefly and showed itself only for concentrations (dilutions) where subjects showed at least modest ability to detect. This has suggested that gathering thresholds under virtually ideal circumstances can minimize the facilitating effect of experience but that it will probably never go away.

Supported by NIH grants DC05602 and DC002741.

Olfaction and the brain for better for worse: Top-down processing and consequences for health and performance

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Whereas olfaction shares bottom-up processing with other sensory systems, a characteristic feature of olfaction is heavy top-down processing. This matrimony (or distinct two-way communication) between the peripheral olfactory nervous system and higher cognitive brain areas is for better for worse. Thus, whereas it has many advantages in guiding the individual to either approach or avoid certain odorous items, it may also contribute to negative consequences for health and performance. Health problems and reduced productivity due to odorous and pungent substances in the indoor and outdoor environments are considerable. Apart from causing annoyance and weak symptoms in a large proportion of those exposed, certain individuals will develop hypersensitivity to odorous and pungent substances, such as in the sick-building syndrome and idiopathic environmental intolerance to chemicals (also referred to as multipel chemical sensitivity). A psychobiological model is proposed and discussed that offers an explanation for how chemical exposure through mediating and moderating factors may evoke physiological and psychological health effects and poor performance. The model's mediating factors include chemosensory properties of the exposure and health-risk perception, and moderating factors include gender, stress, anxiety, depression, and worry about health effects of modernity.

Acknowledgements: This study was supported by grants from the European territorial cooperation program Botnia-Atlantica, the county of Västerbotten (Sweden), and the Regional Council of Ostrobothnia (Finland).

The pleasantness of odor memory

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Odor memory encompasses two interesting olfactory characteristics: strong odor- emotionality association and weak odor-language association. While naming an odor is a very difficult task, the memory evoked by the odor typically makes one feel emotional and ''brings'' one back to childhood. I will present behavioral and imaging evidence supporting this notion, and discuss aspects of this uniqueness in the context of odor pleasantness as a dominant factor in the above phenomena.

Autobiographical odor memory

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Previous studies on autobiographical memory (i.e., personally experienced events) have suggested that memories evoked by odors are older, more emotional and associated with stronger feelings of being brought back in time than word-evoked memories. However, these studies have exclusively focused on unimodal retrieval cues (i.e., cues pertaining to one modality). Thus the aim of the present project was to investigate autobiographical multimodal retrieval cues (i.e., cues pertaining to several modalities simultaneously). Participants were randomized into one of four retrieval cue conditions (i.e., olfactory, visual, auditory, or multimodal) and asked to retrieve autobiographical memories related to the cues. Retrieved events were dated and rated on five phenomenological dimensions (e.g., pleasantness, the feeling of being brought back in time). Results indicated that autobiographical memories evoked by odors were typically older than memories triggered by pictures, sounds or multimodal cues. Importantly, based on modelling of the age distributions it is suggested that multimodal retrieval is driven to a larger extent by visual and auditory information than olfactory information. Regarding the five phenomenological ratings no differences were observed across the four cue conditions.

Symposium Physiological significance on glutamate signaling from the luminal side of gut directly and/or via vagal afferent to the brain functional changes:

Purpose

Glutamate signaling in the gut recently has been unveiled it notifies the brain about the food intake direct action to luminal cells and/or via gastric vagal afferent, and initiates its digestion and homeostatic control of amino acids by the vagal efferent as well as entero hormone release.

Outcomes – knowledge and action points

Glutamate, the major constituent of protein, serves as the principal signaling molecule for the fifth basic taste, umami, through a cascade of molecular mechanisms not only in the gustatory apparatus but also in the gut. Glutamate receptors have been recently localized in the gut mucosa and the gastric vagal afferents' response to glutamate alone amongst other amino acids and sugars suggest its premier role in the gut-brain cross talk. Blood glutamate level does not change appreciably after dietary intake of protein. Glutamate serves as the principal source of energy for he gut mucosa. Intracellular glutamate from glutamine by glutamine synthetase regulates endocrine and exocrine functions in the gut. Glutamate driven vagal afferents activate number brain areas bringing about efficient digestion, energy homeostasis, satiation and over all food preference. Beyond excitatory neurotransmission, glutamate plays a broad integrative role in health and nutrition.

Experimental studies of food choices and palatability responses of European consumers exposed to the Umami taste

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In the Western world, consumers have only recently learned to discriminate the Umami taste, although they have enjoyed its contribution to the palatability of traditional dishes for centuries. The flavor enhancing properties of MSG and their impact on food preferences and intake have been scientifically investigated in European subjects. By adding MSG to such foods as soups, the amount of salt can be decreased without altering palatability, thus favoring a net decrease in sodium intake. Consumers presented with a novel food most often have to get accustomed to the new taste. Liking and/or preference for a novel food develop with repeated exposures. A study showed that the presence of adequate amounts of MSG accelerates the acceptation of novel foods, which are then consumed in greater amounts. Elderly persons, especially those living in nursing homes, are often reported to have low appetite and insufficient energy intake. In elderly persons housed in a nursing home in the Paris area (n=65, mean age 84 years), the addition of MSG

to nutritionally valuable foods (soups, vegetables, starches) induced an increase of intake of MSG-added foods. Total meal size, however, was not affected, since the increased intake of MSG-containing foods was followed by a decreased consumption of foods served later in the meal, such as desserts. The same observations were repeated in hospitalized diabetic patients. Two groups of 31 patients, paired for age, gender, type and duration of diabetes, were studied at lunch time, in the settings of a hospital dining room, for four consecutive days. One member of each pair had MSG added to foods of good nutritional value (vegetables, low glycemic index starch dishes), while the other member of the pair was served identical foods without MSG. Again, the patients ingested more MSG-containing foods and less of other foods, with the same total meal energy intake, compared to paired controls. These two studies suggested that MSG could be used to facilitate appropriate food choices in certain populations without increasing total energy intake. More confirmations of this property of MSG should be obtained, in clinical as well as healthy populations.

Acknowledgements: Studies were funded by grants from the International Glutamate Technical Committee and by the COFAG (France).

Responsiveness of forebrain glucose-monitoring neurons to intraorally and intragastrically delivered monosodium glutamate

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Monosodium-L-glutamate (MSG) induces a unique - "umami" - taste sensation, and it is also known and broadly used as a culinary flavor enhancer. Recent research provided evidence for the existence of specific glutamate receptors both in the oral cavity and the upper and lower gastrointestinal tract, and various physiological consequences of ingestion of MSG have also been demonstrated.

Although some information is available on umami-associated peripheral and central neuronal activity changes, it is yet to be further elucidated which forebrain regions and what kind of neurons and neuronal systems participate in these above effects. The so-called glucose-monitoring (GM) neurons in the forebrain are known to play important roles in the central regulation of various homeostatic functions.

In order to unravel the possible involvement of these chemosensory neurons in the above glutamate-associated processes, single neuron activity was recorded in the mediodorsal prefrontal cortex (mdPFC), anterior cingulate cortex (ACC), and nucleus accumbens (NAcc) of Sprague-Dawley rats by means of tungsten wire multibarreled glass microelectrodes during:

1) microelectrophoretic administration of D-glucose and other chemicals,

2) intraoral gustatory stimulations by solutions representing the five basic taste qualities,

3) intragastric infusion of MSG, D-glucose, and NaCl.

In the above forebrain structures, about 20-25% of all neurons tested changed in activity in response to the microelectroosmotic administration of D-glucose, i.e. these cells were identified as parts of the central GM neural network. Gustatory stimulations elicited firing rate changes in approximately 1/3 of all cells in these forebrain regions, and the GM neurons, compared to the glucose-insensitive (GIS) ones, were more likely to be influenced by tastes. Most frequently the salty, sour and umami taste stimuli resulted in firing rate changes in the mdPFC, whereas proportion of the sweet and bitter sensitive neurons was comparable to the former in the ACC and NAcc. As a general attribute of the GM units, a majority of the gustatory cells were responsive to more than one tastant in all three limbic structures tested. Characteristic activity changes were identified to the intragastric infusion of chemicals. MSG induced both phasic and tonic firing rate changes in the GM neurons, whereas GIS cells displayed no phasic and only fewer tonic responses to the umami substance.

Our data indicate that both endogenous and exogenous chemosensory signals converge in these limbic forebrain areas, which appear to be particularly involved in gustatory and visceral umami signaling mechanisms. The neurons here, especially the GM ones, process complex chemical information to play significant integrative role in the central homeostatic control.

Supported by: Health Care Scientific Council of Hungary (ETT 315/2006), National Research Fund of Hungary (OTKA K 68431/2008), National Developmental Agency (NKTH-RET-008/2005 MEDIPOLIS), Ajinomoto Co., Inc. (51064/2009), and HAS.

Free dietary glutamate promotes gastric secretion via neuro-endocrine pathways

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In many mammalian species, dietary free L-glutamate (Glu) in the stomach interacts with specific glutamate receptors (T1R1/T1R3 and mGluR1-8) which are expressed on surface epithelial and gastric gland cells (San Gabriel et al., 2007; Nakamura et al., 2010). Furthermore, luminal Glu activates the vagal afferents in the gastric submucosa through the paracrine cascade, led by nitric oxide and followed by serotonin (5-HT), which in turn interacts with 5-HT_3 receptors on afferent fibers (Uneyama et al., 2006). Thus, dietary Glu is able to stimulate both local responses of gastric glands and neuro-endocrine pathways.

To elucidate the role of dietary Glu in neuro-endocrine control of gastro-intestinal phase of gastric secretion, we used models of the small gastric pouches surgically isolated according to Pavlov (vagally innervated) or Heidenhain (vagally decentralized) in mongrel dogs. Secretion in the isolated small gastric pouch was stimulated by infusion of small amount of Glu-free amino acid-rich liquid diet (Elental®, Ajinomoto Co., Inc.; 20 kcal, 20 mL) and/or monosodium glutamate (MSG) into the main stomach. Intra gastric intubation of MSG alone (100 mM, 20 mL) did not change basal secretion in the isolated gastric pouch. However, supplementation of Elental diet with MSG (10-100 mM) caused dose-dependent potentiation of secretion of acid, pepsinogene and fluid both in Pavlov and Heidenhain gastric pouches. Additionally, MSG elevated level of gastrin-17 in peripheral blood. Deafferentation of the main stomach with luminally applied local anesthetic lidocaine (5%, 10 mL) blocked increase of gastric pouch secretion and elevation of gastrin-17 induced by MSG. The effect of MSG was also partially

suppressed by 5-HT_3 receptor blocker granisetron (20 μg/kg, i.v.). In summary, aqueous solution of MSG alone does not affect secretion in the stomach. Instead, MSG becomes a moderate activator of gastric secretion when it is co-applied with other nutrients. Free dietary glutamate at doses not exceeding its common concentrations within dietary foods substantially promotes gastric secretion through neuro-endocrine pathways. Its effect is partially mediated by serotonin. We suppose that supplementing the liquid diet with free glutamate may improve gastric secretory capacity and can be considered for patients with gastrointestinal complications.

Roles of gustducin, taste receptors and transient receptor potential channel M5 in gut

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Gustducin, T1r receptors, Trpm5 channels and other taste signaling elements are expressed in duodenal enteroendocrine and brush cells. T1r receptors and gustducin are present mainly on enteroendocrine L and K cells that express incretins: the insulinotropic glucagon-like peptide 1 (GLP-1) and the gastric inhibitory peptide (GIP). We have found that gustducin knockout mice have post-ingestive deficiencies in enteroendocrine cell secretion of GLP-1 and GIP in response to sugars and fatty acids introduced directly into the gut by gavage or duodenal lavage. Gustducin knockout mice also have disrupted regulation of their plasma levels of insulin and glucose. Dietary sugar and sweeteners also appear to use taste receptors on enteroendocrine cells in a signaling pathway that leads to increased enterocyte expression of sodium-dependent glucose transporter-1 (SGLT1), the rate-limiting step for dietary carbohydrate absorption. T1r3 and gustducin null mice fail to upregulate SGLT1.

Trpm5 is expressed in many chemosensory cells throughout the intestine and its role there has been only partially uncovered. In mouse duodenum, Trpm5 is expressed in a special type of brush cell that produces endogenous opioids and uroguanylin. We have determined that beta-endorphin is secreted into the gut lumen in response to various stimuli mimicking digestive products and this activity depends on functional Trpm5.

The wide spread presence of taste signaling molecules in chemosensory and endocrine cells suggests a commonality to the regulation of nutrition and metabolism through chemosensation.

Modulation of hormone secretion from chemosensory endocrine cells by various agonists and antagonists may explain some of the diseases of overconsumption endemic to affluent countries as well as provide novel treatments for obesity, diabetes and malabsorption.

Acknowledments: Supported by NIH grants DC007399 and DK076248 to BM and DC03055 and DC03155 to RFM.

Physiological roles of dietary glutamate signaling via gut-brain axis for energy expenditure

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Gustatory and visceral stimulation of food regulates digestion and absorbed nutrient utilization. Free glutamate (Glu) release from food induces the umami taste that increases food palatability. Dietary glutamate is the main source of energy for the intestinal mucosal absorption and metabolism, thus, only a trace amount of Glu reaches the general circulation even after the intake of dietary protein and Glu added in foods when umami taste sensation is not sufficient to be palatable. In addition to these roles, we demonstrated a unique gastric sensing system for Glu. Glu is the only amino acid that activates rat gastric vagal afferents from the luminal side possibly via metabotropic Glu receptors on mucosal cells. Functional MRI (4.7T) analysis revealed that luminal sensing with 1% Glu (most preferred concentration) in rat stomach activates the medial preoptic area (body temperature control) and the dorsomedial hypothalamus (basic metabolic regulator), resulting in diet-induced thermogenesis without changes in food intake. Interestingly, rats fed a high fat and high sugar diet with free access to 1% Glu and water showed the strong preference for Glu solution, and subsequently lower fat deposition, weight gain and blood leptin compared with those without Glu. In addition, these brain functional changes were abolished in the case of total vagotomized rats. Total and partial gastric and celiac branches vagotomy induced similar results to each other, suggesting that glutamate signaling should be yielded from the luminal side of the alimentary tract. From these results, we propose that dietary glutamate functions as a signal for the regulation of gastrointestinal tract via gut-brain axis play important roles to contribute to the maintenance of our healthy dietary life.

Symposium Chemoreception in aquatic animals:

Fish olfactory receptor repertoires

Sigrun Korsching

Abstract not communicated

Which olfactory receptor neurons code amino acid stimuli in black bullhead catfish: spontaneously inactive or spontaneously active neurons responding to olfactory stimuli?

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In fishes, olfactory organs contain spontaneously active and spontaneously inactive olfactory receptor neurons (ORNs). To study responsiveness of spontaneously active ORNs we subjected olfactory organs of black bullhead catfish (*Ameiurus melas*) to dechlorinated tap water (~1kiloΩ cm). To study responses of spontaneously inactive ORNs we subjected the olfactory organs to low conductive highly purified water (>18,2 megaΩ cm) that reduced shunting and enabled detection of action potentials from ORNs distant to the electrode.

The ORNs responses to amino acids (N=402) consisted of either a decrease (suppression) or an increase (excitation) in the number of

action potentials that occurred after stimulation. Most spontaneously active ORNs responded to amino acids with suppression (62%), few cells responded with excitation (11%) and 11% of ORNs did not respond to amino acid stimuli. Some ORNs responded with suppression to one and excitation to a different amino acid (16%). In more than 50% of cases (N=225) spontaneously active ORNs responded only once to the same repeated stimuli. Cells that do not respond to the repeated stimuli repeatedly cannot code olfactory stimuli. Very small proportion of spontaneously active ORNs responded to stimuli on a dose-depended manner (8% of suppressions and 4% of excitations). We compared responses of spontaneously active ORNs to pairs of amino acids that bullhead catfish can discriminate and those that they cannot discriminate. Large percentage of different responses to amino acids that catfish cannot discriminate and a large percentage of nearly identical responses to amino acid that catfish can discriminate also indicate that most of the spontaneously active ORNs cannot code olfactory stimuli. Fish olfactory organs also contain ORNs that lack spontaneous activity and respond to amino acids with excitation (Europ. J. Physiol., 459:413–425, 2010). After stimulation these ORNs elicited either phasic–tonic or tonic only activities. The spike frequencies of phasic activities consisted of transient frequencies up to 108 Hz that lasted <450 ms, whereas the tonic activities saturated at action potential frequencies of 17–21 Hz. The durations of tonic activities were dose-dependent over several log units of concentration. Specificities of 44 ORNs were investigated with ten different amino acids tested at 10^{-4}M concentrations. Thirteen ORNs were excited by one amino acid, L-norvaline, 22 additional ORNs were excited by two amino acids L-norvaline and L-methionine and nine ORNs were excited by >2 amino acids. In 29 of 31 neurons responding to >1 amino acid, duration of responses to the most stimulatory amino acid was double compared to that of the second most effective amino acid indicating ORNs' high stimulus specificity. Spontaneously inactive and spontaneously active ORNs that respond to amino acid stimuli dose-dependently code olfactory stimuli.

Olfactory sensitivity to inorganic cations in fish

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Teleosts maintain internal concentrations of physiologically important inorganic ions - principally calcium and sodium - within tight limits, often in the face of large variations in external concentrations. The regulatory mechanisms responsible for this ionic homoeostasis are well studied. However, it is not fully understood how - or even if - fish detect changes in environmental levels. Nevertheless, growing evidence suggests that teleosts have olfactory sensitivity to inorganic cations; fish can 'smell' calcium and sodium. The aim of the current presentation is to review this evidence, suggest possible mechanisms and outline unanswered questions and directions for future research.

Freshwater, estuarine and marine fish show olfactory responses to changes in external [Ca^{2+}], as assessed by electrophysiological recording from the olfactory system. In freshwater fish, the olfactory system responds to increases in external [Ca^{2+}] in a concentration-dependent, but saturable, manner with EC_{50} values close to plasma free ion levels (around 1.0 mM). In marine fish, conversely, the olfactory system responds to *decreases* in external [Ca^{2+}], with IC_{50} values again close to plasma free [Ca^{2+}]. The response seems to be specific for calcium rather than concomitant changes in osmolality or accompanying anion concentration. Immunocytochemistry has shown the Ca^{2+}-sensing receptor in a sub-population of olfactory receptor neurons, suggesting a possible mechanism for olfactory sensitivity to external [Ca^{2+}]. Moreover, agonists of the mammalian Ca^{2+}-sensing receptor (neomycin and Gd^{3+}) also evoke olfactory responses.

Fish also show strong olfactory responses to changes in external [Na^+]. In contrast to calcium, however, the response to changes in external [Na^+] is to increases (*not* decreases), whether in freshwater or marine fish, and shows no tendency to saturate. Nevertheless, the response is specific for Na^+; changes in external osmolality or [Cl^-] do not evoke olfactory responses. Furthermore, olfactory responses to changes in [Ca^{2+}] or [Na^+] seem to be largely distinct. Given the non-saturable nature of the olfactory response to Na^+, it is possible that it is channel-mediated. This, however, has yet to be demonstrated experimentally.

The cellular mechanisms for olfactory sensitivity to external cations obviously need to be established. Changes in external [Ca^{2+}] and [Na^+] may have non-specific effects on the excitability of olfactory receptor neurons. Nevertheless, euryhaline fish maintain olfactory sensitivity to 'conventional' odorants (e.g. amino acids) despite large changes in environmental salinity. How is this achieved? The role of external cations -particularly in the mucus - in olfactory transduction - needs to be clarified.

Finally, how do fish use this olfactory input? To avoid sudden or dramatic changes in external salinity? To activate or modulate the osmoregulatory processes? Or as a means to navigate in a complex environment? These explanations are not mutually exclusive; all may be important, but all need experimental investigation.

Funded by FCT, Portugal: POCI/BIA-BCM/55467/2004

Selective staining of olfactory pathways

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Sensitivity and specificity are fundamental features of the sense of smell and are based upon the unique construction of the olfactory system where thousands of sensory neurones make synapses to a small number of secondary neurons. In the present study, we ask how many sensory and secondary neurons are activated by two different odorants, and if some of the secondary neurons are activated by both odorants. To this end, we use the odor-specific endocytosis in the sensory neurons and the fact that the internalized dextrans are transported in the axons and across the synapses in the glomeruli to stain the secondary neurons. These features provide means for a functional visualization of the pathways engaged in transmitting information about odorants to the brain. The odorants were applied in succession to the olfactory rosettes of crucian carp, first the bile salt taurolithocholate (TLC) together with a dextran conjugated with Alexa 488 (green), then to an alarm substance analogue, hypoxanthine 3-N-oxide (H3NO) together with a dextran conjugated with Alexa 647 (red). The two odorants activated mostly separate sets of ciliated sensory neurons with long dendrites. The axons of the two types of sensory neurons projected to overlapping regions of the olfactory bulb. In spite of the overlapping terminal fields, the two odorants stained separate sets of secondary neurons. The axons, stained red and green lay intermingled in the medial part of the medial olfactory tract, confirming

earlier studies of the projection of ciliated sensory neurons to the secondary neurons (*Chem Senses* 2002, 27:395). There are about a dozen neurons that mediate the presence of each of these odorants. None of the secondary neurons showed co-staining; a feature which strongly indicates separate pathways for these two odorants.

Symposium Olfactory Transduction Proteins and Genes:

More than two decades have passed since the discovery of the first olfactory-unique transduction elements in the ciliary membranes, adenylyl cyclase III, G_{olf} and the cyclic nucleotide gated channel. Since, considerable body of evidence and molecular details have emerged on these and additional components of the transduction machinery of vertebrate olfactory sensory neurons. The last two years have seen a new flurry of activity, with novel genes and coded proteins, as well as novel insights, coming on stage. The session will highlight such advances, in the context of what now appears as a rather complete molecular portrait of chemosensory transduction, downstream from the receptors. Such a session is important as it brings together accurate gene- and protein-based characterization with a large body of functional physiological data on odorant-activated functions. It underscores the unique molecular features that allow for olfactory neuronal signaling, amplification, desensitization and recovery.

Calcium-activated chloride currents in olfactory transduction

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Olfactory sensory neurons use an amplification mechanism based on a calcium-dependent chloride efflux to detect odorants. The binding of odorants to receptors in the cilia of olfactory sensory neurons activates a transduction cascade that involves the opening of cyclic nucleotide-gated channels and the entry of calcium in the cilia. Calcium activates a chloride current that produces an efflux of chloride ions and amplifies the depolarization. Despite the relevant physiological role played by calcium-activated chloride channels in olfactory transduction, as well as in several other physiological processes, their molecular identity is still unclear. We have previously indicated bestrophin2 as a candidate for being a molecular component of the olfactory calcium-activated chloride channel. Indeed, bestrophin2 is expressed in the cilia of olfactory sensory neurons and its functional properties are rather similar, although not identical, to those of the native channel. However, when we compared wild type and knockout mice for bestrophin2 we did not find any significant difference in the odorant responses. Thus, bestrophin2 does not appear to be the main molecular component of the olfactory channel. Moreover, the physiological role of bestrophin2 in the olfactory cilia is still unknown. Meanwhile, some members of the transmembrane 16 (TMEM16, also named anoctamin) gene family have been suggested to encode for calcium-activated chloride channels. We showed by immunohistochemistry that TMEM16b is expressed in the cilia of mouse olfactory sensory neurons and performed an extensive functional comparison using both inside-out and whole-cell voltage-clamp techniques to record currents in the ciliary region of mouse isolated olfactory sensory neurons and in HEK293 cells transfected with TMEM16b. Our findings support the hypothesis that TMEM16b is a promising candidate to be part of the native olfactory channel, which contributes to the chloride-based amplification in olfactory transduction.

Transduction components in Grueneberg ganglion neurons

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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; this finding suggests an olfactory function of the GG. Chemosensory responsiveness of nasal neurons is based on the expression of olfactory signaling elements including odorant and vomeronasal receptors. To scrutinize the concept that GG neurons may serve a chemosensory function, they were subjected to molecular phenotyping. It was found that a distinct vomeronasal receptor type designated as V2r83 was expressed in the majority of murine GG neurons. V2r83-negative GG neurons were observed to express members of the trace amine-associated receptor (TAAR) family of olfactory receptors.

V2r83-positive but not TAAR-expressing GG neurons were found to be activated by cool ambient temperatures. Attempts to unravel the signaling mechanisms underlying coolness-induced GG responses revealed that the coolness-sensitive TRP ion channel TRPM8 was not expressed in the GG. Instead, coolness-responding GG neurons were endowed with elements of a cGMP-mediated cascade, including the transmembrane guanylyl cyclase subtype GC-G, the phosphodiesterase PDE2A and the cyclic nucleotide-gated ion channel CNGA3. Experiments with CNGA3-deficient mice demonstrated that this ion channel is required for coolness-evoked responses in the GG, indicating that a cGMP pathway is indeed involved in responsiveness to cool ambient temperatures.

Expression of chemosensory transduction elements such as olfactory receptors suggests that the GG, in addition to coolness, might also be activated by odorous cues. In fact, we have recently identified a limited set of defined odorants which induce responses in GG neurons. Responsiveness to these odorous substances was confined to a given subpopulation of GG neurons which were endowed with a number of distinct transduction elements, suggesting that these proteins could be relevant for odor-induced signaling in these cells.

This work was supported by the Deutsche Forschungsgemeinschaft.

A role for the guanine nucleotide exchange factor Ric-8B in olfaction

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We have previously shown that Ric-8B, a putative guanine nucleotide exchange factor (GEF) interacts with Gaolf and can amplify odorant receptor signaling through Gaolf in heterologous cells. We have also shown that Ric-8B is able to interact with another heterotrimeric G protein subunit, the Gg13 subunit, and that Ric-8B enhances targeting of the Gaolf to the plasma membrane in HEK293T cells. These results suggest two possible roles for Ric-8B in the olfactory sensory neurons: Ric-8B may work as an assembly factor that assists in association and targeting of the Golf protein complex to the plasma membrane; and/or Ric-8B may act as a GEF to amplify odorant signal transduction. If this is true, Ric-8B must have an important role in olfactory sensitivity. We are now producing and characterizing Ric-8B knock out mice in order to determine whether and how the olfactory system is affected in these animals. Ric-8B is a highly conserved gene. We are also characterizing the human counterpart for the Ric-8B gene and its expression in different human tissues.

Supported by FAPESP and CNPq.

Genetic variation analysis for the human olfactory transduction universe

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While human olfactory receptor (OR) genomic variability is well-studied, much less is known on human genetic variability in other olfactory related genes. Our focus has been aimed toward two gene categories, collectively termed accessory olfactory genes: genes that mediate OR signal transduction which are expressed in the olfactory sensory neurons (OSNs), and genes involved in OSN development. The long-term goal is to perform association studies between variations in these genes and across-odorant phenotypic variations. An extreme case is congenital general anosmia (CGA), for which we possess a large number of samples. In parallel, we aim at weaker phenotypes, i.e. the 'general olfactory factor', previously reported (Cain & Gent 1991) and recently confirmed in our laboratory in analyses of two large cohorts phenotyped for sensitivity to several odorants. We reckon that this general olfactory sensitivity in a particular individual may be, at least partly, explained by genetic variants of accessory olfactory genes. We perceive general olfactory sensitivity as a quantitative trait, whereas several genes contribute to the apparently continuous characteristic.

For producing a candidate gene list we scrutinized the literature of olfactory transduction and OSN development studies, mostly done in animal models, including mouse gene knockouts. Additionally we applied the Next Generation Sequencing (NGS) method to sequence human olfactory epithelium transcriptome, looking for genes enriched in the sensory tissue. Such transcriptome analyses verified and lead to an augmented list of 150 human olfactory accessory genes. For some genes we see evidence for olfactory-related splice variants, manifested in potential new olfactory specific exons.

Subsequently we produced a catalogue of deleterious variations in olfactory accessory genes, by scrutinizing genomic sources for Single Nucleotide Polymorphisms (SNPs) such as the dbSNP and the 1000 Genomes Project data. We have identified 257 candidate deleterious variations in our accessory olfactory genes. These are akin to our previously reported segregating pseudogenes (SPGs) in ORs, in constituting natural gene knockouts. For example, we have identified 3 frameshift events in human Adenylyl cyclase III (ADCY3), suggesting this gene, previously shown indispensable to olfaction in knockout mice, is perhaps not always functional in humans. Also, intact allele of the olfactory-specific cytochrome P450 (CYP2G1) gene, encoding a potential odorant biotransformation enzyme, is reported as rare in humans however there is no data of its effect on olfaction. In addition we discovered null copy number variations (CNVs) that affect parts or the full sequence of 52 olfactory accessory genes. All of these deleterious variations, as well as 1230 missense variations, can be used in the planned association studies, using the high throughput Illumina Golden Gate SNP assay. The emerging variation map of the transduction and development genes in olfaction may assist in rationalizing the great inter-individual variation in human olfaction, in parallel to studies of odorant-specific deficits.

Symposium Extracting significance from complexity. Odour mixtures' perception from insects to humans & molecules to behaviour:

Unravelling The Mechanisms Of Odour Mixture Perception

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Most smells in our environment be it in the home, garden or in the workplace are complex mixtures often consisting of dozens of odorants in concentrations that are above their detection thresholds. Although instruments can identify the odorants, in most instances they cannot indicate those a human, animal or insect will detect and use in their behavioural response. This review will examine different aspects of odour mixture perception including the strategies that are employed to analyse mixtures emanating from 'static' odour sources such as wine, foods and perfumes and 'active' odour sources as occurs with plumes containing polluting odours and animal and insect pheromones. In particular, the limitations of strategies for analysing and identifying constituents in both types of odour sources will be discussed including how dynamic odour coding and temporal processing of mixture constituents complement or suppress constituent detection and identification[1, 2, 3]. Particularly relevant to the latter phenomena is the ability of the olfactory system to rapidly adapt and recover to minimise lowering the ability of the system to detect, discriminate, identify and respond to a changing odour environment. The mechanisms underlying odour suppression will also be reviewed including the role of competitive mechanisms at the periphery and inhibitory mechanisms at central locations in producing neural maps of 'mixtures' that allow the brain to rapidly identify an 'odour' regardless of whether it emanates from a single odorant or from a complex mixture[4]. The combinatorial tactic used by the olfactory system provides a mechanism for the simplification of odour signatures in the brain and a rapid 'one trial' learning of the identity of foods, predators, trails and territories. Odour suppression will also be discussed from the viewpoint of regulating the perceived intensity of mixtures whilst maintaining sufficient neural input for source identification.

Acknowledgements

I wish to acknowledge Anthony Jinks, Andrew Livermore, Geoffrey Francis, Andrew Eddy and Helmut Panhuber who made substantial contributions to many of the studies that will be discussed. Much of the work conducted in Australia was funded by the Australian Research Council.

1. Laing DG, Francis G. (1989).The capacity of humans to identify odors in mixtures. Physiol Behav, 46, 809-814.

2. Goyert HF, Frank ME, Gent JF, Hettinger TP. (2007). Characteristic component odors emerge from mixtures after selective adaptation. Brain Res Bull, 72, 1-9.

3. Jinks A, Laing DG. (1999). Temporal processing reveals a mechanism for limiting the capacity of humans to analyze odor mixtures. Cognit Brain Res, 8, 311-325.

4. Joerges J, Kuttner A, Galizia CG, Menzel R. (1997). Representations of odours and odour mixtures visualized in the honeybee brain. Nature, 387, 285-288.

Odour mixture coding, processing and learning in the honeybee, *Apis mellifera*

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Natural olfactory stimuli encountered by foraging honeybees are complex floral blends. However, most previous research on olfactory processing in the primary olfactory center, the antennal lobe, has focused on single odorants, showing that local networks within the AL reformat odor representations from olfactory receptor neurons at the input, to second-order (projection) neurons at the output. However, how this reformatting affects mixture representation is still unclear. In the present work, we compared the rules underlying mixture representation at the input and at the output of the AL, using *in vivo* calcium imaging. We systematically measured odor-evoked activity in 22 identified glomeruli in response to four single odorants and all their possible binary, ternary and quaternary mixtures. Using a bath-applied preparation emphasizing input activity, we found that mixture representation follows a strict elemental rule: the more a component activates the AL when presented alone, the more it is represented in a mixture. By carrying out specific staining of a population of projection neurons, we determined how the AL network reformats mixture representation and what advantage this confers for odor discrimination. We show that increased inhibition within the AL leads to more synthetic, less elemental, mixture representation at the output than at the input level. As a result, mixture representations become more separable in the neural olfactory space. Next, we analyzed perceptual relationships among all mixtures and their components, by measuring honeybees' generalization behavior with these odors. Our data suggests that the AL output mixture representation predicts bees' behavior better than the input mixture representation.

Olfactory receptor neuron responses to odor mixture may predict the perceived odor quality

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Single neuron recordings in the rat *in vivo* show the functioning of the olfactory system periphery in conditions as close as possible to physiology. Such experiments have been fruitful approaches to prove the wide molecular tuning of olfactory receptor neurons (ORN)[1,2] and the olfactory receptors (OR) they express and also to address the question of mixture coding. We first showed clear evidences of suppressive or synergistic interactions between molecules[3]. Here, we selected two specific odorant pairs interacting at perception[4] or at OR binding levels[5]. The first pair comprised isoamyl acetate (ISO) and whiskey lactone (WL) and the second pair, Citral (CIT) and Octanal (OCT). Odor pairs were mixed at 50/50 or 1/10 and delivered at various concentrations.

Mainly, WL antagonized the ORNs' response to ISO in a dose dependant manner. However when WL alone was excitatory, synergisms were observed. For the CIT/OCT pair, in most cases, OCT elicited stronger responses than CIT and CIT was suppressive regarding OCT responses, shifting the cell response threshold towards higher concentrations, the ratio changes in mixture also impacted on the interaction strength between the two molecules. The targeted action on threshold may suggest that interactions between CIT and OCT may occur at the receptor pocket level by competition.

Given the complexity of interactions which arise in binary mixtures, in natural mixtures which comprise several tens, hundreds or thousands of compounds, the complexity should drastically increase. Thus, the numerous components or notes which compose natural or synthetic scents will generate specific and complex interactions raising specific levels of activity in a unique neuron assembly. Such a peripheral coding mode would confer to the olfactory system a quasi infinite plasticity. Our results highly support a major role for the peripheral cellular integrative mechanisms.

[1]Duchamp-Viret, P., Chaput, M. and Duchamp, A. Odor response properties of rat olfactory receptor neurons, Science, 1999, 284, 2171-2174.

[2]Duchamp-Viret, P., Duchamp, and A. Chaput, M. Peripheral odor coding in the rat and frog: Quality and intensity specification. *J. Neuroscience*, 2000, 20, 2383-2390.

[3]Duchamp-Viret, P., Duchamp, and A. Chaput, M. A. Single olfactory sensory neurons simultaneously integrate the components of an odor mixture. Eur J Neurosci , 2003, 18, 2690-2696.

[4]Atanasova B, Thomas-Danguin T, Chabanet C, Langlois D, Nicklaus S, Etiévant P Perceptual interactions in odour mixtures: odour quality in binary mixtures of woody and fruity wine odorants. Chem Senses. 2005, 30, 209-17.

[5]Peterlin, Z.; Li, Y.; Sun, G.; Shah, R.; Firestein, S., and Ryan, K. The importance of odorant conformation to the binding and activation of a representative olfactory receptor. Chem Biol. 2008, 15, 1317-27.

Odour mixture processing: from birth to adulthood

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Odours encountered in the environment originate from either monomolecular odorant or most often mixture of odorants. These

odours are usually perceived among others in a chemically complex context. In order to extract rapidly the pertinent information from this environment, the olfactory system can engage an elemental processing in which each component of a mixture is perceived. Alternatively, this sensory system can activate a configural strategy in which a mixture is perceived as an entity, qualitatively different from the odours of the components[1]. The present review will focus on this duality of the olfactory system to process odour mixtures form birth to adulthood and will especially underline the influence of mixtures' composition and organisms' experience. In human adults, configural processing was suggested for mixtures of high complexity, but was also evidenced for binary and ternary mixtures[2]. Configural processing was advocated to support perceptual blending in mixtures eliciting novel odour percepts. Perceptual blending is then likely specific of certain odorants to be mixed and also of specific proportions of the odorants[3]. Additionally, the perception of odour mixtures was found to be under the influence of learning and experience[4]. Indeed, perceptual processing strategy and exposure to single components can influence mixture perception in human subjects[5]. Configural and elemental processing are also functional as soon as the first days of life. At this period of development, analytical processing of key molecules may be essential to perceive odour signals like pheromones, while configural processing could help to distinguish complex mixtures of odorants also linked to the social and/or feeding environment. In this point of view, recent studies in the newborn rabbit[5,6] showed that configural perception of odour mixtures may effectively occur early in life, that it may astonishingly occur for mixtures similar as those blending in human adults, and that such neonatal perception is also dependent of the chemical nature and proportions of the components.

Supported by grants from the Burgundy Region, the EU-ERDF and IFR 92 to GC and TTD.

[1] Kay LM, Crk T, Thorngate J. (2005) Behavioral Neuroscience 119: 726-733.

[2] Le Berre E, Thomas-Danguin T, Beno N, Coureaud G, Etievant P, Prescott J. (2008) Chemical Senses 33: 193-199.

[3] Le Berre E, Beno N, Ishii A, Chabanet C, Etievant P, Thomas-Danguin T. (2008) Chemical Senses 33: 389-395.

[4] Stevenson RJ. (2001) Learning and Motivation 32: 154-177.

[5] Coureaud G, Thomas-Danguin T, Le Berre E, Schaal B. (2008) Physiology & Behavior 95: 194-199.

[6] Coureaud G, Hamdani Y, Schaal B, Thomas-Danguin T. (2009) The Journal of Experimental Biology 212: 2525-2531.

The hedonics of odorant mixtures

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The perceived quality of odorant mixtures can typically be described in terms of its components' qualities. This means that if an odor A and B are mixed, its quality will exhibit some resemblance to its components in proportion to the relative perceived intensity of the components. How about the *hedonics* of odor mixtures? Will a similar averaging procedure be valid also in this case? The literature suggests this is not the rule. Moreover, pleasantness of an odor is strongly dependent on the context which has been shown in both behavioral and imaging studies. These circumstances make the hedonics of mixtures hard to predict, but an attempt is made here.

In a first experiment that investigated binary odorant mixtures of amyl acetate (pleasant), pyridine (unpleasant) and n-butanol (intermediate) we a utilized a *substitution* procedure to mix odorants. This leads to that the mixtures differ only in quality and not intensity from the single components that constitute the mixture. The results suggest that as the stimulus is gradually changed from component A to B over mixtures of the two, the quality but also the pleasantness of the odors changed accordingly. In the second experiment, we used an *additive* procedure to mix the same odorants. This typically leads to that the *intensity* of mixtures is stronger than its components. That is, the mixture differs both in quality and intensity from its components. This time it was obvious that an averaging of components' hedonics could not approximate the hedonics of the mixture.

To model the hedonics of mixtures in this latter case we did the following: First we analyzed the relation between odor intensity and pleasantness in *single* odorants. This relation is well described by a family of 2nd-degree polynomial functions indicating that pleasant odors have an optimal intensity for being maximally pleasant whereas unpleasant odors simply get worse with increased intensity. To model the *mixture* hedonics, we assumed that the mixture hedonics would follow the same family of functions as the single components, as the mixture intensity increases. Second, we assumed that the mixture function should be a weighted average of the functions for the single components along which the predicted mixture hedonics could be determined from the observed mixture intensity. These weights are defined by the relative dominance of one quality of the other observed for each mixture. The accuracy of the model suggests that hedonics of mixtures of single odorants can be predicted. Implications of the model will be discussed.

Acknowledgements: This study was supported by grants from the Swedish Research Council (2005-1779) (to MJO)

Symposium Neuromorphic Olfactory Sensors:

A biomimetic gas sensor array system designed to test computational olfaction models

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The olfactory epithelium of animals is populated with thousands of Olfactory Receptor Neurons (ORNs) that bind different molecular properties of the volatiles depending on the olfactory receptor protein they express These ORNs may express different receptor proteins providing a high degree of diversity. But not all ORNs are of different type since a large number of them express the same receptor protein endowing the ORN population with a high degree of redundancy. These two structural features of the olfactory epithelium play an important role in capturing chemical information from volatiles and conveying it to the following stages of the olfactory system. It is well accepted that receptor diversity mediates odour identification since each odorant elicits a different pattern of

activity across different-type [1]. Grosmaitre et al. [2] have recently found that the response of ORNs expressing the same receptor do not respond in the same way to odour concentration. Thus, odorant concentration becomes encoded as an activity pattern across ORNs of the same type.

This paper presents a chemical sensing system that takes inspiration from the combination of sensory diversity and redundancy at the olfactory epithelium to enhance the chemical information obtained from the odorants. The system is based on commercial MOX sensors and achieves, first, diversity trough different types of MOX along with modulation of their temperatures, and second redundancy including 12 MOS sensors for each type (12x8) combined with a high-speed multiplexing system that allows connecting 16 load resistors. The different load resistors mimic the different sensitivity found by Grosmaitre even in ORN expressing the same olfactory receptor.

The paper will have to main contributions. In the first one, the concentration encoding capacities of the Biomimetic System will be compared to those of the Olfactory Epithelium and in both cases the effect of redundancy and diversity will be explored using information theoretic measures.

In the second part of the paper, clustering techniques in the sensor response space will be applied to identify 'arfificial glomeruli'. The ability of this 'articial glomeruli' to code for odor identity while at the same time encoding odor intensity and improve signal to noise ratio will be presented.

This work has been funded by European Project NEUROCHEM: Biologically Inspired Computation for Chemical Sensing. FP7- Grant 216916

[1] Malnic B, Hirono J, Sato T, Buck L, Combinatorial receptor codes for odors. Cell (1999) vol. 96 (5) pp. 713-723

[2] Grosmaitre X, Vassalli A, Mombaerts P,Shepherd G, Ma, M, Odorant responses of olfactory sensory neurons expressing the odorant receptor MOR23: a patch clamp analysis in gene-targeted mice. Proceedings of the National Academy of Sciences (2006) vol. 103 (6) pp. 1970-1975

Odor recognition framework for evaluating olfactory codes

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One of the key challenges in the attempt to understand fundamental computational mechanisms underlying the functionality of the sense of smell is to elucidate the neural representation of key stimulus features. It still remains unclear how the olfactory system conveys the information about odor identity and intensity to facilitate robust recognition in natural environments.

This study is concerned with the examination of different hypothetical early olfactory coding strategies from the perspective of the discriminative power of the resultant secondary representations. In particular, it was aimed to evaluate a recently proposed concept of fuzzy interval coding of odor concentration in juxtaposition with concentration-saturating representations within the pattern recognition framework in a range of odor discrimination scenarios. To this end, olfactory data, including both single odorants and their mixtures, synthesized using a reduced model of early odor information processing in the layer of olfactory receptor neurons and the olfactory bulb in vertebrates was classified with support vector machines and connectionist neural network approaches. The use of machine learning techniques provided a link with artificial olfaction and the discussion of various learning and classification scenarios conveyed in this work was found relevant to the development of a modern electronic nose.

In conclusion, despite that no significant differences in the average performance of neural networks and support vector machines were reported, the latter classifiers produced the most consistent results and facilitated a fast model selection process. In terms of olfactory coding, the outcome of the extensive evaluation demonstrated that the fuzzy interval approach clearly favored concentration dependent odor recognition allowing for reliable classification of odor identity and detection of its concentration range. The specificity of this coding strategy though rendered the resultant representation less conducive to generalization. In this respect, conventional concentration-saturating codes had more universal characteristics in the recognition tasks examined here. More biologically plausible scenarios are expected to reveal other relevant properties of these coding schemes.

Stereotyped firing response patterns in moth antennal lobe neurons: from experiments to models

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Pheromonal communication in moths constitutes an exceptionally favorable model system for studying neural circuits and odor-guided behavior. In this study, we report experimental analysis and computational modelling on the activity of pheromone-sensitive neurons in the antennal lobe of the moth *Agrotis ipsilon*. Dual extracellular recording experiments were conducted using unique odor puffs and pulsed stimulations with the complete pheromonal blend. The large majority of the recorded neurons exhibited a multiphasic response consisting in an excitatory phase (E1) followed by an inhibitory phase (I) and a long tonic excitation (E2). The second group of neurons exhibited a pure tonic excitatory response. For multiphasic neurons, the duration of E1, but not of I, depends on stimulus concentration and duration. These neurons were able to follow pheromone pulses up to several Hertz. Moreover, their responses (E1 phases) were very precise and reliable over repeated trials, which was not the case for monophasic neurons. These results indicate that only multiphasic neurons are adapted to detect the fine structure of odor plumes. We then developed a biophysical neuron model capable of reproducing and explaining the stereotyped E1/I/E2 response. Finally, we discuss functional implications of the different phases of the neuron responses in the stereotyped surge-and-cast behaviors of male moths and how our knowledge about biological neural processes can influence the design of artificial olfactory systems such as electronic noses and olfactory robots.

Analysis of Exhaled Breath for COPD monitoring based on E-NOSE/SPME System

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Over the last few years, the non-invasive analysis of exhaled breath has emerged as an innovative tool with potential for diagnosis of several conditions. Novel sensor and volatile sensing technology allow quantification for many potential biomarkers near the patient's bedside.

Because of the potential impact on health care, Lung Cancer, Stomach Cancer, Tuberculosis, and Chronic Obstructive Pulmonary Disease (COPD) are selected target diseases. Among the diseases, COPD is a chronic inflammatory lung disorder that is considered one of the leading causes of morbidity and mortality worldwide, and predicted to become the third most common cause of death and fifth most common cause of morbidity, evaluated as disability adjusted life years, in the world by the year 2020.

We are developing an intelligent sensor system for monitoring of COPD patients combined semiconductor metal oxide based sensor module, pattern recognition subsystem and non-invasive sampling of volatile emitted from a patient into a high intelligent sensor system that allows the rapid processing of these samples. It is capable to assist early and rapid diagnosis of changes in state of a patients, and aid decision marking by medical personnel in the treatment of such patients.

In this congress, we present results obtained from exhaled breath analysis for COPD patients using array based gas sensing system incorporating an automated SPME (Solid Phase Micro Extraction) desorption system to enable the system to be used for clinical validation. Aiming to increase the sensitivity, SPME pre-concentration is used for sampling of headspace air and the response of sensor module to variable concentration of volatiles emitted from SPME fibre is evaluated. The GC(Gas Chromatography)/MS(Mass Spectrometry) is used to determine potential bio-markers at its concentration levels, sampling can be based on SPME from direct measurement from exhaled breath. PCA(Principal Component Analysis) and RBFN(Radial Basis Function Network) results give indications that COPD patients may be discriminated from normal peoples at the early stage of diagnosis. The microbial laboratory analysis using clinical samples taken from patients verifies the performance of system.

This work was supported by Converging Research Center Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2009-0082185, 2009-0093683) and PMI2(Prime Minister's Initiative Education 2) fund ref.146 managed by the British Council.

Development and test of a large array of conductive polymers sensors resembling the redundancy of the biological olfactory system

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It seems that the sensitivity and the selectivity of the mammalian olfactory system are enhanced by both the redundancy and the massive convergence of the olfactory receptor neurons (ORN) to the olfactory bulb (OB). As a verification, a large array of polymeric chemical sensors and its own read-out electronic circuit have been built in the frame of the FP7 funded European Neurochem project.

The resistive sensors have been arranged in a topological matrix configuration having m rows and n columns in order to reduce the number of the connection lines between the array and the read-out circuit.

The whole sensor array was fabricated on a borosilicate substrate by means of photolithographic processes, which allowed to build it in a square with side of 2.1 cm, comprising the pads necessary to bond the die to the scanning board[3].

Conductive polymers were selected as sensitive materials because of their broad and overlapped specificity to different volatile organic compounds[1,2]. A few of them were repeatedly deposited by means of ink-jet printing and airbrush deposition. Hence, a high degree of redundancy has been implemented.

A first set of conductive polymers was selected to be deposited by a Piezoelectric Dimatix DMP 2831 printer (FujiDimatix Inc.).Some of them are commercially available, others were synthesised in our laboratory.

In recent years, inkjet printing has gained much attention as a patterning method for conductive polymers. It is easy to carry out, compatible with various substrates, non-contact patterning, low cost and fast. Furthermore, different configurations can be obtained with a very high grade of precision, without using masks and without wasting material. However, not many conductive polymers have been printed until now because of the restrictive features of the ink.

Another set of conductive polymers was selected to be deposited on the substrates by airbrushing.

A layer of hard baked SU8 photoresist was spin-coated on the array and patterned by photolithographic masks in order to obtain well defined sensitive areas and to reduce the cross-talk among adjacent elements.

A custom delivered system has been used to test the sensor array with different analytes.

Sensor data has been processed using multivariate analysis and the results will be shown in the final work.

This research is being supported by a grant from the European Community's Seventh Framework Programme (FP7/2007-2013) NEUROCHEM.

[1] K.C. Persaud,. Materials Today, 2005, 8(4): p. 38-44.

[2] M. Cole, N. Ulivieri, J.Garcia-Guzmàn, J.W. Gardner, Microelectronics Journal 2003, 34(9), p:865-875

[3] R. Beccherelli, E. Zampetti, S. Pantalei, M. Bernabei and K.C.Persaud, Sensors and Actuators B: Chemical, 2010, 146(2): p.446-452.

Symposium Offensive odours - Chemistry, Behaviour, Development and Language:

Introduction

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Modern humans are supplied with sensory detection/integration systems that have adapted over several-millennia from exposure to natural and culturally-shaped environmental conditions. However, rapid environmental change, pollution, technological/industrial innovations, world-wide exchange of goods, and massive migratory movements confront human communities with unfamiliarity in terms of space, food, and ways of sociality in urban confinement. Here, we will focus on a particularly pervasive, yet often overlooked sensory aspect of quality of life, nasal chemoreception. Due to increasing mobility, more and more humans have to face unprecedented smells. These range from immigrants or refugees collected in populated camps and receiving culturally-unforeseen foodstuffs, to the urban commuter exposed to anthropogenic smells in public transportation, or to the infant that is given foods that are mismatched with expectations gained from previous experience.

New odorous substances are being generated every day, for instance primarily for their impact in food or cosmetics, or as by-products of innovative building or packaging materials, colourings and many other materials concerning our daily lives. If these products and materials are associated with unfamiliar smells we often speak of ''off-odours'', using the term in the classical way for a smell that disturbs us. Aversive reactions to these situations may be induced by novelty and an inability to judge if an unfamiliar smell might be associated with harm. Also, often people are not even able to adequately communicate what they perceive. This is, of course, to some extent a cross-cultural, but also a developmental phenomenon.

Work in the area of smell is mainly focused on the positive aspects of smell. Development and optimisation of food odorants, as well as pleasant smells to be used in body care are hot topics. Accordingly, negative smells are often not directly addressed by characterisation of the formation process but more often by covering bad smells with pleasant ones (as realised by indoor scenting, sprays, etc.). However, such strategies might also induce considerable annoyance. Recently, with the development of novel analytical methodologies, offensive smells can be addressed in a more targeted manner. Based on the molecular-chemical characterisation of the odour-inducing substances and improvement of the underlying technologies, novel concepts can be developed to support human wellbeing in a chemosensorially attractive or, at least, neutral environment.

This symposium will unite chemists, behavioural biologists, psychologists and linguists in an attempt to cross perspectives about the nature of aversion to smells, their impact on everyday life, their development, and their description in language, as well as the underlying molecular-chemical principles involved.

Unconditional response to offensive smells: what we learn from newborns' reactions

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Years after years, the accumulation of results permits us to better understand the development of human food preferences during the lifespan. The foetal and neonatal periods appear to be very important in this process. Neonates are attracted to volatile compounds being present in the amniotic environment (Marlier et al., 1997). Women who consumed garlic, alcohol, anise, or carrot flavour in late pregnancy produce offsprings that manifest positive responses towards these odorants, for durations ranging from hours, days to months (Hepper, 1995; Faas et al., 2000; Schaal et al., 2000; Mennella et al., 2001). After birth, the exposition to an artificial odorant (camomile) spread on the breast at each breastfeeding episode starting from birth induces a preferential orientation of 3-4 day-olds newborns for it. Further, the preference for the camomile, in comparison to another artificial odorant, stays stable during at least two years (Delaunay-El Allam et al., 2006).

As a résumé, these results could lead to the idea that all odorants inevitably become attractive upon positive exposition, e.g. in the context of feeding. However, recent results show that the hedonic evaluation of some compounds seems not to change substantially with experience.

Recently, application of GC-Olfactory analyses of the volatile compounds of the key fluids of the young human' environments show that amniotic fluid (AF) contains AND and human milk (HM) AND and But A. Further, AND is present in AF and HM at a higher concentration than the newborns' capacities would require to detect it. Now, in spite of a long familiarization to AND during foetal life and a regular associations of AND and But A with breastfeeding, these two compounds are persistently highly negatively evaluated. Comparing the responses of newborns and adults in reaction to saturated solutions of androstenone (AND) or butanoic acid (But A), it appears that these compounds remain mainly negatively evaluated between these two periods of life. The frequency of negative reactions to AND is lower in adults than in newborns (42.3% vs. 74%; Chi^2 test; $p<0.05$). However, this decrease appears to be low with regard to the exposition time of foetus and newborns to this compound. More surprising, the frequency of negative reactions to But A increases between newborns and adults (62.5% vs. 100%; Chi^2 test; $p<0.01$).

So, contrary to Engen who maintained that ''odor preferences are absent at birth and acquired with age'' (1982), the present studies about the newborns' reactions teach us that some preferences seem not possible to be acquired. So, some odorants seem to be unconditionally offensive.

Delaunay-El Allam (2006) Infant Behav Dev, 29:308–21.
Engen (1982) The perception of odours, p. 130.
Faas (2000) Alcohol, 22:7–17.
Hepper (1995) Int J Prenat Perinat Psychol Med, 7:147–51.
Marlier (1997) C R Acad Sci, 320:999–1005.
Mennella (2001) Pediatrics, 107:1–6.
Schaal (2000) Chem Senses, 25:729–37.

Molecular-chemical characterization of off-odours and their formation pathways - indicators for potentially harmful processes?

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Off-odours are defined as sensory impressions, which are not characteristic for a specific food [1]. Consumers evaluate such odours as unpleasant and in many cases as potential indicators for harmful physiological effects, relating off-odour in most cases to spoilage, deterioration effects or toxic or other harmful contamination of the food. Accordingly, food companies are highly aware of this problem, so that production processes and storage conditions of foods are continuously otpimised and audited to ensure highest possible quality. As a result, off-odour development was minimised and economic losses were reduced. However, empiric instead of systematic approaches were applied in most cases to erase the problem, and off-odours are still observed sporadically in several foods.

In previous investigations, a number of compounds have been identified that originate from e.g. microbial sources or packaging materials, just to name a few [3, 4]. However, recent research in this field has shown that the issue of off-odour formation is quite more complex and does in a number of cases not simply relate to formation or contamination with offensive odorants. Often, odour changes are induced in the foods due to decreases of positive aroma contributing compounds e.g. resulting from chemical breakdown or simply evaporation or changed partitioning within the food matrix. These changed profiles can then be interpreted by the consumers as negative due to their profile that is divergent from what is expected.

As a consequence, off-odour characterization requires, in some cases, a comprehensive approach involving techniques such as gas chromatography/olfactometry (GC/O) and stable isotope dilution techniques [2] together with sensory characterization of the respective off-odour problem. Combination of these techniques offers the possibility to relate the odour profile of the sample under investigation with the respective positive control. Based on the observed differences, either on molecular or compositional basis, avoidance strategies can be developed.

The lecture will give a brief overview about different types of off-odours and will present examples of repeatedly occurring cases in foods. Related off-odorant sources and pathways will be discussed.

[1] DIN 10950-1:1999-04. Sensory test – part 1: terms (in German). Beuth Verlag, Berlin, 1999.

[2] Grosch W. Evaluation of the key odorants of foods by dilution techniques, aroma models and omission. Chem. Senses 2001, 26, 533-545.

[3] Chatonnet P, Dubourdie D, Boidron JN, Pons M. The origin of ethylphenols in wine. J. Sci. Food Agric. 1992, 60, 165-178.

[4] Spadone JC, Takeoka G, Liardon R. Analytical investigation of Rio off-flavour in green coffee. J. Agric. Food Chem. 1990, 38, 226-233.

Material off odours in the built environment - annoying or harmful?

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Keeping the indoor environment healthy and comfortable is a special challenge for builders and building product manufacturers. Most technical materials and building products release volatile organic compounds (VOC), often odours, into the environment and may have an influence on people present. Although building products have to fulfil certain requirements regarding their VOC emission behaviour before they are allowed to be used, e. g. limits regarding presence of compounds or limits regarding concentration, there are still cases where people complain about being annoyed by odours or even feeling sick in indoor environments, often in brand new homes or after renovation of existing homes. Common smell perceptions can be associated with solvents from wall paints, floor glues or silica sealings, which usually disappear after a couple of weeks because odour causing compounds evaporate over time. Certification authorities as well as manufacturers are aware of this problem. The emission behaviour of building products is usually tested 3 and 28 days after production to take this evaporative decline into account and manufacturers try to replace volatile solvents by less volatile ones. Still sometimes odours remain over weeks and months and do not seem to decline. One cause could be wrong use of materials / material combinations or ignoring instructions e. g. drying times before the next material application step of multi-layer floor or wall constructions. Such cases are an exception and they can be easily avoided. But there are also cases where the odour is a given (undesired) material property originating from the raw material or generated during the production process. Identification of smell causing compounds and their source is a problem of routine emission measurements because such odour-active compounds have very low odour thresholds (< 1 $\mu g/m^3$ air) and are emitted at very low concentrations only. Although odour evaluations with naïve or trained panels are discussed as certification criteria for building products, the failing of a product in odour evaluation does not help in finding the cause of smell. For finding starting points for odour optimisation the structure of off-odour causing compounds has to be known. Therefore odour analysis methods from food and perfume flavour chemistry (gas chromatography - olfactometry) have been applied to off-odourous building products. After identification of the off-odour compounds the whole production process from the raw material to the final product is investigated to localize the source and enable avoidance, removal or reduction strategies. Off-odourants identified in building products causing ill feelings in indoor environments are very variable. Some are common food odorants but are somehow misplaced as building product odourants. Others are of clear synthetic origin and their unknown and unnatural smell rouses fear of being harmful – mostly unfounded.

Sensory and linguistic aspects of offensive odors: how to describe and communicate about unpleasant odors

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In communication about sensory experiences words are used to describe the individual perception to explain it to a dialog partner. As the experience of sensing food is accessible only for the person itself it stays open whether the partner in the conversation perceives the same. As a consequence, untrained people using their own words need to clarify the meanings of their terms in communication. Furthermore, naïve respondents are not able to describe especially negative or unexpected taste or smell perceptions properly. This is observed, e.g. at the Fraunhofer IVV in communication with their

clients, for musty, mouldy or other negative off-flavors. In contrast, in sensory science trained panelists use defined descriptors to express what they perceive when judging food. The meaning of each descriptor is identified beforehand, e.g. in group discussion. Interestingly, in many cases reference material is used to clarify descriptors, i.e. on a non-verbal level.

Possible strategies in language to establish meaning and how it is possible to differentiate perceptions in communication are outlined. Practical examples based on the German language are presented and discussed from a sensory and linguistic perspective.

Does the quality of an odour matter to the brain? Insights from functional neuroimaging studies

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The perception of a smell is an integration of various sensations (olfactory, trigeminal, tactile, thermal, as well as gustatory sensations). Information from the olfactory receptors in the nasal mucosa is transmitted to the olfactory bulbs. In the olfactory bulb, the axons of the olfactory receptor neurons synapse with dendrites of second-order neurons in the olfactory system (mitral and tufted cells) forming discrete glomeruli. All areas receiving a direct projection from the olfactory bulbs constitute the secondary olfactory cortex, consisting of the anterior olfactory nucleus, the olfactory tubercle, the piriform cortex, parts of the amygdala (periamygdaloid cortex, anterior and posterior cortical nuclei, nucleus of the lateral olfactory tract) and a small anteriomedial part of the entorhinal cortex. From secondary olfactory cortex, information is transmitted to several other parts of the brain, including orbitofrontal cortex, agranular insular cortex, additional subnuclei of the amygdala, medial and lateral hypothalamus, medial thalamus, basal ganglia, and hippocampus. These areas have been referred to as tertiary olfactory regions. Most of these areas are not specific for processing of olfactory stimuli and show activation by other sensory inputs as well. This complex network of brain areas provides the basis for odor-guided regulation of behavior, feeding, emotion, autonomic states, and memory. The relationship between odorant structure and odor quality has been a focus of olfactory research for more than 100 years, but still remains poorly understood. Animal studies suggest that topographical representations of odorant structure in olfactory bulb as well as in the piriform cortex form the perceptual basis of odor quality. Earlier neuroimaging studies have indicated that orbitofrontal regions and the amygdala play an important role with reference to the hedonic qualities of an odor. Lately, it has been shown that the posterior part of the piriform cortex may represent a key structure for quality encoding of odors. However, it seems that sensory-specific information about odor quality is not static within the human brain but can be rapidly updated by mere sensory exposure. This experience-dependent neural plasticity parallels behavioral improvements in odor perception, emphasizing the role of learning in shaping neural representations of odor quality in the human brain.

Offensive versus pleasant odours: analytical techniques to characterise physiological and behavioural responses

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Investigations into sniffing are prevalent in the chemosensory literature and it has long been established that the natural sniff is the optimum method for humans to sample odorants [1, 2]. It is inherent knowledge that humans alter inhalation patterns in response to fresh exposure to an odour. The nature of the response depends on the odour quality in question. A pleasant smell generally elicits a tendency to sample the novel odorant more frequently and with more vigour than routine inhalation behaviour. By contrast, an unpleasant or offensive smell is often assiduously avoided by reducing the nasal intake flow.

Such behaviour may be characterised by analytical means. Simple measurements of nasal pressure (i.e. pressure variations directly at the nostril) provide an effective parameter to trace both inhalation frequency and vigour [3], which can be correlated with the character and concentration of the sampled odour. But how do we know what occurs directly inside the nose? Concentration patterns may be monitored within different regions of the nose using an on-line mass spectrometric approach. By utilising a fine canula connected directly to a mass spectrometer, odorant concentration distributions within the nose may be established. For example, we have investigated the intra-nasal concentrations of diacetyl (2,3-butanedione) directly inside the nostril and at the olfactory cleft using proton-transfer-reaction mass spectrometry (PTR-MS [4]). By asking test subjects to vary their inhalation behaviour it was possible to monitor differences in the amount of diacetyl reaching either of these regions.

We have also conducted simple physiological tests with random odours (of unknown character and intensity to the participants) to investigate instinctive odorant sampling behaviour according to odour perception. Here, nasal pressure intensity and frequency data were used to indicate a positive or negative response to the respective compounds and was thereafter compared with the hedonic rating.

Such techniques provide effective tools to further our understanding of olfactory perception. As with any field of research, this applied approach complements the manifold diverse and complex techniques being utilised in olfactology, such as theoretical and physical simulation models, nasal flow pattern assessments, and other psychophysical methods.

The authors acknowledge the support of Fraunhofer IVV in conducting these investigations.

[1] D.G. Laing, Identification of Single Dissimilar Odors is Achieved by Humans With a Single Sniff, Physiol. Behav., 37 (1986) 163-170.

[2] J. Mainland, N. Sobel, The Sniff Is Part of the Olfactory Percept, Chem. Senses, 31 (2006) 181-196.

[3] B.N. Johnson, C. Russell, R.M. Khan, N. Sobel, A Comparison of Methods for Sniff Measurement Concurrent with Olfactory Tasks in Humans, Chem. Senses, 31 (2006) 795-806.

[4] A. Hansel, A. Jordan, R. Holzinger, P. Prazeller, W. Vogel, W. Lindinger, Proton-Transfer Reaction Mass-Spectrometry - Online Trace Gas-Analysis at the ppb Level, Int. J. Mass Spectrom., 149/150 (1995) 609-619.

Symposium Cerebral Imaging in Taste and Olfaction:

Body odors of newborns activate neuronal regions associated with reward in women

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Olfactory signals are prime mediators of mother-infant bonding in mammals, and they have been shown to be linked with maternal attitudes and behavior in our own species as well. Human mothers are indeed highly attentive to their infants' odor cues, but although we have good understanding of the neuronal network supporting mother-infant olfactory recognition in various mammals, to date no such information exists for humans.

The present study is a first attempt at delineating the neural network underlying the processing of infant odor properties by women. Using functional magnetic resonance imaging (fMRI), we measured the pattern of cerebral activation of first-time mothers and nulliparous women while smelling the body odor of unfamiliar 2 day-old newborn infants. Both groups of participants did perceive infants' body odor as equally pleasant, intense, and familiar. Smelling an infant's body odor provoked significant activity in neostriatal areas, the hippocampus, and the insular cortex. The cerebral activation of mothers and nulliparous women differed, however, within these areas. Mothers demonstrated higher activity bilaterally within the caudate nuclei compared to nulliparous women.

This study reveals that body odors from 2 day-old newborns produce activation in reward-related cerebral areas in women, regardless of their maternal status. Recent mothers show, however, stronger infant odor-elicited activation in the caudate nucleus, suggesting that they may be more sensitive than nulliparous women to the reinforcing value of their offspring odor qualities. This suggests that body odors might act as a catalyst for reward learning mechanisms involved in the development of bonding.

The gustatory cortex and multisensory integration

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The central gustatory pathways are part of the brain circuits upon which rest the decision to ingest or reject a food. The quality of food stimuli, however, relies not only on their taste but also on properties such as odor, texture and temperature. We will discuss recent evidence in favor of the hypothesis that the central gustatory system, in particular its cortical aspect, functions as an integrative circuit where taste-responsive regions are also under the influence of somatosensory and olfactory stimulation. Gustatory pathways can also be modulated by the internal state of the body, with neuronal responses to nutrients varying according to fluctuations in physiological parameters. In general, rather than being restricted to function as the receptive fields of peripheral taste transduction, central gustatory pathways seem to operate as a multisensory system broadly tuned to intra-oral stimuli of biological significance.

Neural dynamics of taste intensity perception in humans

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Understanding the neural dynamics in the human brain is an important issue. Brain imaging techniques such as fMRI and PET are well adapted to non-intrusively observe the brain activity in living humans but they do not precisely reflect the rapid time course of the neural activity. To assess the brain neural dynamics, MEG is much more adapted. It offers the possibility to localize the activated cortical areas and to follow the kinetics of activation with a time resolution compatible with brain events.

An important feature of the taste sensation is the perception of the stimulus intensity. The brain mechanisms underlying this function are presently largely unknown. What are the cortical areas involved in this process?

We already showed (Kobayakawa et al. 2008) that the amplitude of the initial neural activity in the primary gustatory cortex is correlated with the concentration of the tastant rather than the perceived intensity. In the present study, we therefore extended our observations, taking into account the timing of activation in several brain areas.

Eleven participants were stimulated with four concentrations (30mM, 100mM, 300mM, 1M) of NaCl (duration of the stimulation was 400 ms) while recording the MEG activity using a 64 channels whole head device at a sampling rate of 250Hz. Stimulations were presented at an interval of thirty seconds. The subjects were instructed to indicate the intensity of the perceived saltiness, from 0 to 5, using fingers. After calculation of the average data for forty stimulations, the root mean square (RMS) value was calculated for all channels. Every 4 ms, the correlation between the RMS amplitude and the perceived intensity was calculated.

We found three latencies (around 120, 400 and 800 ms after the stimulus onset) at which the RMS amplitude was significantly correlated with the perceived intensity. From the topographic analysis of the magnetic fields we found activation in the primary gustatory cortex at approximately 120 ms, as expected from other studies, in the frontal cingulate gyrus and the hippocampus at 400 ms and in the prefrontal areas, and again in the primary gustatory cortex, at 800ms.

These spatio-temporal data reveal that the perception of the taste intensity is not statically processed by only one cortical area, but corresponds to a dynamic interplay between the primary gustatory cortex, the hippocampus and the frontal cingulate (both linked to memory function) and prefrontal area (involved in evaluation and calculation tasks).

Kobayakawa et al., Representation of salty taste stimulus concentrations in the primary gustatory area in humans. Chemosensory Perception, 1(4), 227-234 (2008).

fMRI response to reward value of chemosensory stimuli in the human brain

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Globally, obesity has increased to epidemic proportions, with profound health consequences. The abundance of highly palatable, high calorie food may contribute to obesity, which has increased in prevalence, particularly in certain age groups, yet not everyone becomes obese. Physiological states of hunger or satiety influence behavior relating to the reward value of taste stimuli. We have used fMRI to investigate factors which moderate brain response to chemosensory stimuli, while manipulating physiological state, quality and nutritional value of chemosensory stimuli in individuals with different characteristics. Data were acquired using a 3T GE Research Scanner at the UCSD Center for Functional MRI. Stimuli were delivered through tubing to the oral cavity, using a computer-controlled stimulation device. An event-related paradigm allowed for examination of specific neural events in response to positive and negative stimuli. After swallowing, participants performed a magnitude estimation task using a joystick. ANOVA was conducted with ROIs (e.g., Amygdala, OFC11, OFC 47, Nucleus Accumbens, and the Caudate Head, Body and Tail) to compare differences in activation in response to pleasant or unpleasant and nutritive and non-nutritive stimuli in hungry vs. satiated states across individuals. We also conducted exploratory group analyses and functional connectivity analyses. Subject characteristics, the nature of the stimuli, and the physiological state significantly influenced brain response, suggesting the potential for plasticity in the cortical activation in response to chemosensory stimuli with positive and negative reward value. More precise knowledge of individual variability in plasticity of brain response to chemosensory stimuli, particularly in reward areas under the conditions of hunger and satiety, has significant potential for understanding food palatability, intake and obesity.

Supported by NIH Grant R01 AG04085 from the National Institute on Aging to C.M. We would like to thank the UCSD Center for fMRI and the members of the SDSU Lifespan Human Senses Center.

Workshop: Species differences and similarities in Taste:

Introduction: Species differences in taste

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The differences in the genes that determine taste have renewed the interest in species differences in taste. These differences affect the results and conclusions, which can be drawn from all research methods used in taste as well as on practical applications of these results. On one hand there are several examples of neglecting species taste differences ranging from attempts to create rejection with denatonium benzoate to liking with aspartame. On the other hand, the commonalty between species has widened our understanding of taste tremendously. However this is possible only if the results are evaluated with phylogenetic and dietary differences in mind. A present study by Li et al. (In press) presents a striking demonstration of this. Results of alignments of T1R2 and T1R3 sequences in ten primates show that the degree of conservation of these two proteins reflects their known phylogenetic relationship (Li pers. com.). This introduction and then the workshop will present and discuss some of these.

Vertebrate chemosensory evolution: quality coding, conservation and diversification

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There has long been an interest in establishing a relationship between the chemistry of chemosensory stimulus compounds and perceptual quality. An important insight coming from discoveries of GPCR olfactory receptors (OR), taste receptors (TR) and ion-channel taste receptors is the discrete nature of chemosensory detection and coding. There is no single dimension of smells or tastes but as many dimensions as there are molecular receptors. Analogies between chemical senses and color vision prove artificial. Current numbers for human receptors impart 424 stimulus chemistries, 34 for tastes and 390 for odors. Various species have orthologous receptors, distinct receptors, and missing receptors due to pseudogenization depending on survival value of stimulus detection in their separate ecological niches (Shi & Zhang, 2009). There are no whale OR. Particular T2R bitters such as cycloheximide and Na^+-specific tastes are rodent specific and absent in humans. When the Na^+-specific taste is inhibited, a second taste of NaCl emerges in rodents. However, strikingly similar taste and odor quality coding strategies are conserved throughout the mammalian radiation.

There are independent coding channels for tastes and odors that do not perceptually cross adapt. The 5 taste channels associated with human taste receptors (30 T2R converge) proved easier to identify physiologically and behaviorally. Channels for sugar and salt tastes are carried separately in sucrose-best (across mammalian orders) and NaCl-best (specific to rodentia) neurons of the chorda tympani nerve. Sugar and salt tastes also have distinct channels for humans that are 100% identified when cross-adapted. Although more difficult to demonstrate physiologically, humans also have distinct, independent coding channels for odors such as vanilla (vanillin) and rose (phenethyl alcohol [PEA]), odors for which control identification remains 95% after cross adaptation.

Each taste and odor rapidly self-adapts within a few-seconds. Identification of salt and sugar applied as mists drops by 25% and vanilla and rose sniffed once or twice drops by 50% (Frank et al 2010). Dynamic chemosensory coding is defined by the consequences of this rapid adaptation on identification of components in mixtures presented afterwards. Distinct tastes and odors are mutually suppressed in mixtures (Laing et al, 2002). In mixtures of NaCl & sucrose or PEA & vanillin, identification dropped for salt taste and rose and vanilla odors. But the suppressed tastes and odors emerged following selective adaptation to approach control identification of the individual compounds. Odors also emerged after adapting all but one component of more complex mixtures (Goyert et al, 2007; Fletcher et al, 2010 ECRO Poster).

Rapid selective adaptation and mutual mixture-component suppression are likely conserved across species. They manipulate effective intensity to promote emergence of characteristic qualities of transient chemical stimuli in natural settings. (Supported by NIH grants DC004849 and DC004099).

The rules of encoding in the taste system revealed by complementary neurophysiology and functional neuroimaging

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Complementary neurophysiological recordings in macaques and functional neuroimaging in humans show that the primary taste cortex in the rostral insula and adjoining frontal operculum provides separate and combined representations of the taste, temperature, and texture (including viscosity and fat texture) of food in the mouth independently of hunger and thus of reward value and pleasantness. One synapse on, in the orbitofrontal cortex, these sensory inputs are for some neurons combined by learning with olfactory and visual inputs, and these neurons encode food reward in that they only respond to food when hungry, and in that activations here correlate with subjective pleasantness and with individual differences in and cognitive modulation of the hedonic value of food. Information theory analysis shows a robust representation of taste in the orbitofrontal cortex, with an average mutual information of 0.45 bits for each neuron about which of 6 tastants (glucose, NaCl, HCl, quinine-HCl, monosodium glutamate, and water) was present, averaged across 135 gustatory neurons. The information increased with the number of neurons in the ensemble, but less than linearly, reflecting some redundancy. Although some neurons were sharply tuned to individual tastants, the average encoding was quite distributed. Because each neuron responded to different combinations of taste, oral texture, olfactory and visual inputs, sensory-specific satiety can be computed in the orbitofrontal cortex by an adaptation mechanism with a time-course of minutes.

Attention to the pleasantness of taste stimuli produces selective enhancement of processing in the orbitofrontal and cingulate cortex processing stream, and selective attention to the intensity of stimuli produces selective enhancement of processing in the insular cortex and connected areas, leading to a biased activation theory of selective attention in the taste system that has implications for psychophysics, industry, and appetite control.

Grabenhorst,F. and Rolls,E.T. (2010) Attentional modulation of affective vs sensory processing: functional connectivity and a top down biased activation theory of selective attention. Journal of Neurophysiology 104 Epub 14 July.

Rolls,E.T. (2005) Emotion Explained. Oxford University Press: Oxford.

Rolls,E.T. (2010) Taste, olfactory, and food texture reward processing in the brain and obesity. International Journal of Obesity Epub 3 August.

Rolls,E.T. and Grabenhorst,F. (2008) The orbitofrontal cortex and beyond: from affect to decision-making. Progress in Neurobiology 86: 216-244.

Rolls,E.T. and Deco,G. (2010) The Noisy Brain: Stochastic Dynamics as a Principle of Brain Function. Oxford University Press: Oxford.

Rolls,E.T., Critchley,H.D., Verhagen,J.V. and Kadohisa,M. (2010) The representation of information about taste and odor in the orbitofrontal cortex. Chemosensory Perception 3: 16-33.

Differences in bitter taste receptors and behaviours in species and sub-species of primates: Identification of non-taster Japanese macaques for a specific bitter taste

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In mammals, bitter taste is mediated by *T2R* gene family members. Since T2Rs are directly involved in the interaction between mammals and their dietary sources, these genes likely evolved to reflect regionally specific diets during mammalian evolution. Human *T2R* genes (h*T2Rs*) have been observed to be polymorphic, however, polymorphisms in other wild animals has not been investigated so far. In order to elucidate the evolutional process of bitter taste recognition, we started genotyping of bitter taste receptors of individual primates living in the Primate Research Institute, Kyoto University. As a result, it has been revealed that there are lots of Single Nucleotide Polymorphisms (SNPs) in T2Rs. Behavioral test showed the presence of specific bitter-taste insensitive monkeys whose specific T2R is disrupted. These data demonstrate the presence of model monkeys useful for bitter taste sensitivity, receptor expression, and neuronal processes. In addition to furthering analysis of molecular properties, in cooperation with the global COE program, we are constructing the genetic database of the individual captive primates in the institute.

Acknowledgments

This work was financially supported by Global COE program A06 and by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (2137009) and grants from the Takeda Foundation for Science and the Suzuken Memorial Foundation to H. I.

Labeled lines and taste of compounds in cattle

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Taste fiber recordings in different species increasingly indicate that taste fibers in all mammalian species from functional points are grouped clusters and that each of these clusters give rise to a particular ingestive behavior which must be characterized as fairly stereotypic, such as avoid intake or stimulate intake. At the same time nucleotide and amino acid mapping of the two receptors that are linked to the most stereotypical ingestive behavior, the hedonically positive T1Rs and the negative T2Rs show increasing heteromorphism between species, so that, for example, within the primate order the percent identity between human and marmoset has decreased with close to 20%. However, knowing what a species likes and dislikes, and using phylogenetic relationships as between human and marmoset, we can identify

the cluster that triggers intake and rejection respectively, but how do we identify these taste fiber clusters in a species, such as cattle, with a completely different diet and chemically undefined food?

ORAL COMMUNICATIONS

ORAL SESSION Human Chemoreception:

High Prevalence Of Quality-Specific Taste Disorders In Children: Explaining the neural mechanisms is not so easy

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Otitis media (OM) or middle ear disease, is the most common reason a child is brought to their General Practitioner in the industrialised world[1], accounting for 18% of visits. In Australia over 90% of Aboriginal children exhibit evidence of OM by 30 months of age[2] indicating that almost every Aboriginal child experiences OM. In contrast about one-third of non-Aboriginal children experience OM.

OM can cause taste disorders. The latter occur because the chorda tympani (CT), which innervates the anterior tongue, is vulnerable to damage from OM pathogens as it passes through the middle ear enroute to the brainstem[3]. Damage from OM can reduce the number of taste papillae and buds on the tongue and cause taste disorders resulting in obesity or anorexia.

Given the threat of taste disorders to the health of children, Laing and Armstrong (unpublished) conducted a screening study of taste function in 297 Aboriginal children and showed that a high number (8.2%) had taste disorders. Accordingly, the present study was undertaken to confirm the finding and to compare the prevalence in 432 9-12 year old Aboriginal and non-Aboriginal Australian children. Using a taste identification test involving 5 concentrations of sweet, sour, salty and bitter tastants and water as stimuli, the results indicated a prevalence of quality-specific taste disorders in 12% and 7.9%, respectively, of the Aboriginal and non-Aboriginal participants. Given that WHO define a prevalence of >4% in a population as a massive public health problem, there is an urgent need to determine the consequences for the children.

The quality-specific nature of the taste disorders, raises the question of how such specificity occurs from the indiscriminate damage to the CT arising from OM pathogens which are almost certainly the cause for Aboriginal children. Currently, mechanisms for specific ageusia have been reported for PROP (bitter), MSG (savoury) and citric acid (sour) and were attributed to receptor cell related events, none of which can be linked to indiscriminate damage of the CT in the middle ear. No mechanism has been reported for sweet ageusia, the most common disorder found here. Although it is difficult to envisage the mechanisms underlying the loss of specific qualities here, the specific nature of the losses may provide further insights to the neural coding of taste stimuli.

1: Forer M, Murtagh J (1995). Aust Family Physician, 24, 2093. 2: Morris P S, et al (2005). BMC Pediatr, 5, 27.3: Gedikli O, et al Laryngoscope 2001, 111, 724.

Taste sensitivity increases under conditions of adaptation and inhibition

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Sensory systems must adjust their overall responsiveness to changes in ambient stimulation to maintain function. For instance, adaptation to background stimuli increases a system's differential sensitivity, its ability to detect differences in physical intensities among various stimuli. Since sensory systems utilize myriad transduction mechanisms and adapt in various ways, it is not clear how differential sensitivity is maintained. We investigated whether perceptual and receptor-based increases in differential gustatory sensitivity would occur when responsiveness was reduced by inhibition rather than adaptation. We determined the changes in the position and shape of the psychophysical function as well as the degree of differential sensitivity (Weber Fractions) to sucrose under conditions of sweetener adaptation or topical sweetness inhibition.

Our results show that adaptation and inhibition both cause strong increases in differential sensitivity to suprathreshold sucrose concentrations, despite their different effects on cellular and psychophysical functions. Weber fractions decreased under conditions of adaptation (by 37%) and in the presence of an inhibitor (by 63%) indicating large increases in sensitivity. The differences in the psychophysical functions obtained for adaptation and inhibition indicate that increases in perceived sensitivity are due to reductions in systemic activation regardless of the physiological basis of this mechanism.

Supported by NIH/NIDCD DC02995 (PASB)

Evaluation of taste-aroma and taste-taste interactions with relevance to bitterness perception and bitterness masking potential

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Bitterness is a basic taste property which significantly influences the flavor and liking of many foods. Foods with intrinsic bitterness or bitterness developed during the production process or over a certain storage period are often rejected by many consumers. Therefore, the understanding of key molecules with bitter taste and their formation pathways are essential. The use of taste modifying molecules in order to manage the bitterness perception is another approach (1). Molecules such as homoeriodictyol (HED) isolated from *Eriodictyon ssp.* can decrease the bitterness perception in many applications (2). Taste modifiers potentially interact with other taste-active molecules. The understanding of such interactions increases the sensorial performance in applications. As an example, the flavanone sterubin, which is usually present in certain *Eriodictyon* extracts besides HED, shows moderate masking activity as a neat compound. In contrast, the taste-taste interaction of mixtures of both molecules might lead to lower masking activity towards caffeine. We will also show taste-aroma interactions with special focus on

bitterness. Molecules such as vanillin or 2,3-pentanedione have been evaluated.

Taste modification is a complex phenomenon not yet fully understood at the receptor or signal transduction level. From a practical point of view, the behaviour of taste modifiers in the food matrix environment must be considered to understand their function and effectiveness.

1. Ley, J. P., 2008. Chemosensory Perception 1: 58-77

2. Ley, J. P.; Krammer, G.; Reinders, G.; Gatfield, I. L.; Bertram, H. J. 2005. J. Agric. Food Chem. 53: 6061-6066.

3. Keast, R. S. J., P. A. S. Breslin, and G. K. Beauchamp. 2001. Chimia 55:441-447

4. Kurobayashi, Y., Y. Katsumi, A. Fujita, Y. Morimitsu, and K. Kubota. 2008. J. Agric. Food Chem. 56:512-516.

Natural salt taste enhancing peptides – sensomics-based discovery, human psychophysics, and ENaC activation

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The detection of sodium is of great importance to humans, it is crucial for the ionic homeostasis of the internal environment. However during the past decades the evidence of an association between high dietary salt intake and the development of cardiovascular disease has strongly increased. To disclose new strategies to produce food products with reduced sodium levels, but unaltered salt taste intensity, various attempts have been made to discover salt taste enhancers. While in rodents the amiloride-sensitive, Na^+-specific epithelial sodium channel (ENaC) as well as an independent, amiloride-insensitive transduction mechanism are proposed to be involved in salt detection, the knowledge on the molecular mechanisms underlying human salt perception is rather fragmentary.

The application of a sensomics approach on commercial protein hydrolysates assisted by an *in vitro* screening, based on the functional expression of the ENaC in *Xenopus laevis* oocytes, led to the identification of a series of peptides which enhance the saltiness of pure NaCl solutions as well as a that of a savory model broth up to 20%. For the unambiguous evaluation of the salt taste enhancing activity of fractions and individual peptides, a two-stage human sensory procedure was developed combining ranking and isointensity experiments. Quantitative analysis using hydrophilic liquid interaction chromatography coupled to tandem mass spectrometry verified the natural occurrence of high levels of these salt taste modulating peptides in various food products and protein hydrolysates. Extended human psychophysical analysis of single peptides demonstrated a structure-dependent effect on their salt taste enhancing activity. These data as well as the impact of single peptides on the ENaC-mediated sodium currents in oocytes will be presented.

Reference: Stähler, F.; Riedel, K.; Demgensky, S.; Neumann, K.; Dunkel, A.; Täubert, A.; Raab, B.; Behrens, M.; Raguse, J.-D.; Hofmann, T.; Meyerhof, W. A role of the epithelial sodium channel in human salt taste transduction? Chemosensory Perception (2008), 1, 78-90.

Discovery platform for odorant potentiators and blockers

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We developed methods to test millions of cells to produce panels of cell lines functionally expressing native, full length odorant receptors from multiple species. A subset of prioritized receptors was screened using a library of volatile compounds and resulted in the identification of modulators including novel agonists, potentiators and blockers. Interestingly, potentiators of some receptors performed as blockers of other receptors. This data is being used to guide human sensory evaluation to determine composite effects of identified modulators for blockade of malodors as well as enhancement of desirable fragrances. Data from native taste receptor cell based assays suggest that reliance on native, full length receptors will be critical for accurate decoding of the human olfactory system. This will maximize reliability for effective compound discovery. Data on the Chromovert® technology used to produce functional native odorant receptor cell lines and high throughput screening results will be presented.

Chemosensory communication of anxiety – An emotion regulation study

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In most vertebrates, responses to danger include the release of chemical signals inducing stress adaptations within conspecifics. In humans, chemosensory anxiety signals are potent elicitors of emotion: They enhance withdrawal related motor behavior, and alter emotion related neuronal activity. Furthermore, they reduce the perceptual acuity for visual safety cues, whereas they enhance it for visual cues of danger.

Emotion regulation has strong clinical implications, and several studies have yet addressed the usefulness of reappraisal in the regulation of negative emotional states. Just recently, we have shown that the voluntary regulation of emotions elicited by non-social disgusting odors is possible, but more difficult than the voluntary regulation of comparable visual cues. In the present study, it was aimed to test whether emotions can be regulated in response to social chemosensory cues.

Therefore, 20 non-socially anxious and 20 high socially anxious female students viewed fearful facial expressions presented in the context of chemosensory anxiety signals, or a sport control

stimulus, while emotion regulation was assessed with ratings and the eyeblink startle-reflex. The chemosensory stimuli were sampled from 20 healthy male students donating sweat from both axillae for 90 minutes each, using cotton pads within an anxiety (waiting for a subjectively important oral examination) and a sport control condition (ergometer training). In four counterbalanced blocks participants had to either enhance or down-regulate their emotions in response to the facial expressions. During each trial the faces were presented twice for two seconds: Before and after a visual regulation instruction. Beginning 0.5s before the onset of the face, the Chemosensory stimuli were presented for 2.5s using a constant-flow, 5-channel olfactometer. Startle-probes were presented at three different positions during the trials.

Independent of emotion regulation, the participants rated their emotional response towards the faces as more negative when they were presented along with a chemosensory anxiety cue, as compared to a sport-control cue. Moreover, on a behavioral level, high socially anxious individuals showed larger startle magnitudes towards the pictures presented in the context of chemosensory anxiety cues than the non-anxious individuals. However, results indicate, that independent of chemosensory context, emotion regulation was successful in both groups: When they had to down-regulate, participants rated their own emotional experience as neutral while they felt more negative, and more aroused when they had to enhance their emotion. Moreover, both groups showed smaller startle-responses when they had to down-regulate their emotions, in comparison to the enhance condition. The present results are in accordance with previous research, and show for the first time that emotion regulation towards chemosensory cues of anxiety is possible. Furthermore, they support the notion that high socially anxious individuals are especially sensitive towards chemosensory anxiety signals.

This study was supported by the German Research Foundation (DFG).

Congenitally anosmic adults report less maternal care than normosmics: a retrospective questionnaire study

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Olfactory cues are known to play a significant role in the social interactions of vertebrates and humans are no exception. Human neonates are able to learn the odor signature of their mothers and to orient towards them (Cernoch and Porter 1985; Schleidt and Genzel 1990; Schaal, Marlier et al. 1998). Mothers are more attracted to newborn infant body odors than nonmothers (Fleming, Corter et al. 1993) and are able to recognise their infants based on their odors following very little experience with them (Porter, Cernoch et al. 1983; Schaal and Porter 1991). This reciprocal olfactory-based communication might substantially assist in the process of mother-infant bonding. However, the effect of chemosensory information in mother-child interactions was rarely studied. One research interest is to study mother-infant bonding in individuals with inborn absence of smell. Thus, the aim of the present study was to investigate the consequences of congenital anosmia for the formation of mother-infant bond, as assessed retrospectively. Twenty-eight congenitally anosmic patients (18 - 42 years) and twenty-eight normosmic controls (18 - 42 years) participated in the study. They were administered the German versions of the 25-item Parental Bonding Instrument, to assess their recollections of parental care and overprotectiveness during the first sixteen years of life, and the 21-item Beck Depression Inventory to assess depressive symptoms. Statistical analysis revealed a significant difference in the maternal care dimension ($F(1,54) = 8.62$, $p = .005$, $r = .37$), with congenital anosmic participants reporting less marternal care than controls. No difference was found in maternal overprotection, paternal care or overprotection. No correlation between maternal care and the depression score, educational status or age was found. The results of the present study indicate that olfaction plays a crucial role in the formation of mother-infant bond and that retrospective perception of maternal care may be adversely affected in congenital anosmia.

Cernoch, J. M. and R. H. Porter (1985). "*Recognition of maternal axillary odors by infants.*" *Child Dev 56(6): 1593–1598.*

Fleming, A. S., C. Corter, et al. (1993). "*POSTPARTUM FACTORS RELATED TO MOTHERS ATTRACTION TO NEWBORN-INFANT ODORS.*" *Developmental Psychobiology 26(2): 115–132.*

Porter, R. H., J. M. Cernoch, et al. (1983). "*Maternal recognition of neonates through olfactory cues.*" *Physiol Behav 30(1): 151–154.*

Schaal, B., L. Marlier, et al. (1998). "*Olfactory function in the human fetus: Evidence from selective neonatal responsiveness to the odor of amniotic fluid.*" *Behavioral Neuroscience 112(6): 1438–1449.*

Schaal, B. and R. H. Porter (1991). "*MICROSMATIC HUMANS REVISITED - THE GENERATION AND PERCEPTION OF CHEMICAL SIGNALS.*" *Advances in the Study of Behavior 20: 135–199.*

Schleidt, M. and C. Genzel (1990). "*The significance of mother's perfume for infants in the first weeks of their life.*" *Ethology and Sociobiology 11(145): 154.*

Nasal sniffing enables communication and environmental control for the severely disabled

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Locked-in syndrome (LIS) is a condition of quadriplegia and anarthria resulting from damage to descending corticospinal tracts at the pons level, thus sparing the forebrain-based faculties of cognition and emotion. LIS patients cannot speak nor write and they depend on assistive technology and augmentative communication devices (ACD) for interaction with others. We present a novel concept of ACD based on sniffing, that can serve patients with LIS and similar conditions. Sniffs are precisely controlled sensory-motor acts that depend in part on enervation of the soft palate by at least three different cranial nerves, the 5th, 9th, and 10th. In that this enervation is cranial and distributed, we hypothesized that it may be relatively spared following injury. We present the case of a 51 year old female patient with LIS following a ventral pontine infarction. Nasal pressure was monitored with a nasal canula connected to a MEMS pressure sensor followed by a USB data acquisition system. Training software provided bio-feedback in the form of an on-screen "flame" the user had to "put out" by shifting nasal

pressure as instructed by on-screen commands. Changes in nasal pressure were then used to generate a code that allowed manipulation of an on-screen courser. After 21 days of training, the latency to volitional nasal-pressure shift decreased from ~50 to 2.3 (+/-1.14) seconds (p<.001). Following training, the patient could use the device to write text at a rate of 3 (+/-1.5) letters per minute. This allowed the patient her first meaningful post-stroke communication, and she now uses the device regularly. Because of its distributed cranial enervation, soft palate control may provide an avenue to device-control for patients with severe disability resulting from diverse pathologies.

Molecular mechanism of salt taste

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NaCl is an important component of the environment and it varies in concentration among different environmental niches. NaCl is essential for homeostasis and physiological functions in many organisms. However, the molecular mechanism of NaCl detection is not well known. In mammals, the epithelial Na^+ channel (ENaC) has been shown to be involved in NaCl detection. But there is also an ENAC channel independent salt taste mechanism. We use *C. elegans* to study the mechanism of NaCl taste. Previous studies in *C. elegans* identified five genes involved in NaCl chemoattraction. These are *tax-2* and *tax-4* (cyclic nucleotide gated (CNG) channel subunits), *tax-6* and *cnb-1* (calcineurin A and B subunits) and *ncs-1* (neuronal calcium sensor). Analysis of these mutants in our assay, in which we expose the animals to a very steep NaCl gradient, showed reduced chemotaxis to NaCl. However, we found that these mutants still showed significant attraction to higher NaCl concentrations.

By analyzing the behaviour of double mutants, we found that chemotaxis to NaCl involves two genetic pathways. The first pathway involves two mitogen activated protein (MAP) kinases, *nsy-1* and *sek-1*, and three genes that have been previously characterized, *tax-2*, *tax-4* and *tax-6*. The second pathway involves *tax-2*, another CNG channel subunit, *cng-3*, the G show("a") α protein *odr-3*, the TRPV channel subunit, *osm-9,* and the guanylate cyclase, *gcy-35*. We used cell specific rescue of the mutant genes, laser ablation of specific neurons and neuronal calcium imaging to find out where in the neuronal circuit of *C. elegans* these genes function. Thus far, the involvement of the main salt sensing neurons, ASE, has been confirmed. In addition, we found that the ADF neurons also play a role in chemoattraction. Finally, we have performed a synthetic genetic screen to identify additional genes that play a role in NaCl chemotaxis. We found 6 mutants that have completely lost all NaCl taste response and 23 mutants that showed reduced chemoattraction to NaCl.

We are currently characterizing the behaviour of the identified mutants. We are SNP-mapping (single nucleotide polymorphism) and deep sequencing to identify the genes affected in these mutants.

ORAL SESSION Receptors:

Zebrafish crypt neurons express a single V1R-like olfactory receptor

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Crypt neurons constitute the third type of olfactory sensory neurons. Unlike the extensively studied ciliated and microvillous neurons, our knowledge about them is very limited, not even the olfactory receptor genes they express are known so far. We recently identified a novel olfactory receptor family of six highly conserved G protein-coupled receptors, the v1r-like ora genes (Saraiva and Korsching, Genome Res. 2007). We report here that a single member of this family, ora4, is expressed in nearly all zebrafish crypt neurons, as defined by S100 immunoreactivity. None of the other ora genes are found in this neuronal population. Accordingly, no co-expression of ora4 with any of the remaining ora genes was observed with double in situ hybridization, consistent with the monogenic pattern of expression characteristic of olfactory receptor genes. Furthermore, several lines of evidence suggest the absence of any other olfactory receptors such as ORs, TAARs and the V2R-like OlfCs in crypt neurons. These results suggest that the entire crypt neuron population selects one and the same olfactory receptor gene for expression. This coding strategy is radically different from the expression of large olfactory receptor gene families in both ciliated and microvillous neuron populations. Thus, we have identified a more restricted mode of expression than the well-known 'one neuron – one receptor' rule. This 'one cell type – one receptor' mode is familiar in the visual system, with rhodopsin as the sole light receptor of rod photoreceptor cells, but up to now has not been suspected to occur in the olfactory system.

We began to characterize the signal transduction in crypt neurons. Among 26 genes comprising the family of heterotrimeric G-protein alpha subunits in zebrafish (Oka et al, PNAS 2009), only the expression pattern of the Gi1b gene resembles that of the ora4 gene. Indeed, ora4 and Gi1b were found to be co-expressed using several combinations of immunohistochemisty with in situ hybridization, suggesting that ORA4 receptor may signal through Gi1b.

Our identificaton of the olfactory receptor and a candidate G alpha protein provides the molecular starting-points necessary to study crypt neuron signaling and function.

Genetic basis for variability in bitter taste perception

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Taste is an inherent characteristic of food organoleptic properties, and therefore plays a major role in food preference and behavioural attraction/aversion. For example, bitterness perception is often synonymous with displeasure, even for a human baby only a few days old, consistent with the role of bitter taste as a warning sensor that protects us against intoxication. Bitterness perception differs, however, across individuals. Besides environmental influences and individual differences such as age and gender, genetic variability represents a major determinant for the observed perceptual differences. The most famous example concerns the bitterness of PTC and thioamides. In these cases perceptual differences in the population

are directly linked to genetic polymorphisms in one of the ~25 members of the bitter taste receptor (TAS2R) gene family1,2. The aim of this work was to investigate the extent of genetic variation as cause for inter-individual differences in bitter taste perception.

To this end we sequenced the coding region of all 25 functional human TAS2R genes in a sample of the European population and determined the haplotypes specific to these individuals. The encoded receptor variants were expressed in a heterologous in vitro expression system. In parallel, we assessed the individuals' sensitivities to cognate bitter compounds for various TAS2Rs.

For the individual's genetic diversity, numerous coding single nucleotide polymorphisms were seen in nearly all receptor genes. A smaller number of TAS2R gene loci harboured major mutations such as indels and copy number polymorphisms. The in vitro receptor assays revealed that receptor variants differed frequently in their sensitivity to bitter compounds. This was paralleled by our in vivo measurement, which demonstrated that differences occurred in the individuals' sensitivities to the same compounds.

Taken together our results suggest that genetic variability accounts for functionally distinct TAS2Rs and perceptual differences in the population.

1. Kim, U. K., Jorgenson, E., Coon, H., Leppert, M., Risch, N., and Drayna, D. (2003) Science 299, 1221-1225.

2. Bufe, B., Breslin, P. A. S., Kuhn, C., Reed, D. R., Tharp, C. D., Slack, J. P., Kim, U. K., Drayna, D., and Meyerhof, W. (2005) Current Biology 15, 322-327.

An analysis of the gustatory receptor genes of the insect pest, Epiphyas postvittana (Tortricidae, Lepidoptera)

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Gustatory receptor (GR) genes were detected in a partially complete genome sequence of the Lightbrown Apple Moth (LBAM), Epiphyas postvittana, a polyphagous pest of fruit crops in Australasia, Europe and North America. The search utilised GR sequences from the Silkmoth, Bombyx mori, a specialist feeder, and to date, the only lepidopteran for which a comprehensive list of GR sequences has been published (Wanner and Robertson, 2008). A minimum of putative 151 genes were identified, comprising 9 CO2 receptors, 20 sugar receptors, 6 Dm43a orthologs and 116 bitter receptors. This represents an expansion of all receptor types compared to the Silkmoth.

A preliminary phylogenetic analysis was carried out. All 65 silkmoth genes have orthologs in the LBAM genome and only a single grouping of LBAM genes lacks a Silkmoth ortholog. Several groups of LBAM bitter receptors show dramatic expansion relative to the Silkmoth. The possible relationship between GR gene expansion and feeding behaviour will be discussed. Initial studies of 48 selected putative GRs were analysed by quantitative PCR. Two thirds of these receptors were highly expressed in the heads of 4th instar larvae.

Wanner, K. W., Robertson, H. M. (2008) The gustatory receptor family in the silkworm moth Bombyx mori is characterized by a large expansion of a single lineage of putative bitter receptors. Insect Mol. Biol. 17: 621-629.

I acknowledge the contribution of Drs R. Newcomb, R. Crowhurst and D. Begun in genome sequencing and bioinformatics

The antennal transcriptome of a moth

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Nocturnal insects such as moths are good models to decipher the molecular mechanims of olfaction since these organisms mainly use their sense of smell to understand their environment and to communicate with conspecifics. In addition, since herbivorous moths group devastating species for agriculture, a better understanding of the olfactory mechanisms could lead to the identification of original targets to perturb this sensory modality and thus the insect impact. We will describe an Expressed Sequence Tag (EST) project on male antennae of the noctuid pest model *Spodoptera littoralis,* that allowed us not only identifying candidate genes involved in odour/pheromone detection but also characterizing the antennal transcriptome. More than 20 000 ESTs were sequenced from a normalized library and we identified 9030 unigenes expressed in antennae. 6544 were annotated based on BLAST analyses and gene prediction software identified 6736 ORFs. The unigenes were compared to the *Bombyx mori* (Lepidoptera) proteome and to ESTs derived from Lepidoptera transcriptome projects.

The distribution of unigenes among GO terms revealed enrichment of specific pathways in the antennae. In particular, we identified a large number of candidate genes involved in odour and pheromone detection and turnover, including 31 candidate chemosensory receptor (CR) genes, among them candidate pheromone receptors (see abstract from Montagné et al.), and several chemosensory ionotropic receptors (see abstract from Olivier et al.). Due to a pronounced intra- as well as inter-specific sequence diversity, insect CR-encoding genes are usually identified using complete or partial genomic databases. Using *S. littoralis*, we thus established the use of transcriptomic sequencing for the identification of CR-encoding genes in a species for which no genomic data are available. Interestingly, we also highlighted the presence in antennae of genes potentialy involved in non-olfactory functions, such as genes encoding

defense-related elements involved in xenobiotic and pathogen protection, hormone/biogenic amine receptors and circadian clock components.

The availability of this antennal transcriptome is thus a valuable resource for odour and pheromone detection studies in insects, but also for investigation of the molecular bases of other antennal functions.

3. Jordt SE, et al. (2004) Nature 427:260-265.
4. Bandell M, et al. (2004) Neuron 41:849-857.
5. Bautista DM, et al. (2006) Cell 124: 1269-1282.
6. Salazar H, et al. (2008) Nat Neurosci 11: 255-261.
7. Voets T, et al. (2004) Nature 430: 748-754.
8. Caterina MJ, et al. (2000) Science 288: 306-313.
9. Jordt SE and Julius D (2002) Cell 108: 421-430.
10. Kang K, et al. (2010) Nature 464: 597-600.

Key role of the capsaicin receptor TRPV1 as a mustard oil chemo-nociceptor

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The capsaicin receptor TRPV1 and ankyrin-rich TRPA1 cation channels function as polymodal sensors in nociceptive nerve fibers [1,2,3,4]. Mustard oil (MO, allyl isothiocyanate) and cinnamaldehyde (CA) are plant-derived irritants whose stimulatory effects on nociceptors are thus far thought to be solely mediated by TRPA1 [3,5]. In contrast, we found that both compounds have a bimodal effect on TRPA1, producing block at high concentration. Importantly, we found that, in contrast to the established dogma, both compounds reversibly stimulate TRPV1, although MO is more potent than CA. Activation of TRPV1 by MO occurs in a reversible membrane-delimited manner and is not mediated by residue C157, which is essential for the action of other electrophilic compounds such as allicin [6]. Both MO and CA act as gating modifiers of TRPV1, inducing a negative shift of the voltage dependence of channel activation, which is reminiscent of the mechanism of action of the canonical agonist capsaicin [7]. Furthermore, we show that MO-induced activation of TRPV1 share structural determinants with capsaicin. Notably, MO and CA strongly cross sensitize with heat in TRPV1 activation, which may explain, at least in part, why these compounds induce TRPV1-dependent heat hyperalgesia *in vivo* [4,8]. Comparison of the incidence and kinetics of MO effects on sensory neurons isolated from wild type and *Trpa1* KO, *Trpv1* KO and double *Trpa1*/*Trpv1* KO mice revealed clearly identifiable TRPV1-mediated responses. Finally, behavioral experiments in mice yielded that TRPV1 contributes significantly to aversive responses to MO in forced-drinking and open-field exploration assays.

Our results demonstrate that TRPV1 is a crucial mediator of the noxious effects of MO, and prompt for a re-evaluation of the putative role of TRPA1 and TRPV1 in experimental models of MO-induced nociception and neurogenic inflammation. Our data may also help understanding other important phenomena, such as the relative contribution of these channels to the perception of pungent spices in mustard, wasabi and cinnamon and to mechanisms of directed deterrence that shape the co-evolution of multiple plant and animal species [9,10].

1. Caterina MJ, et al. (1997) Nature 389:816-824.
2. Story GM, et al. (2003) Cell 112:819-829.

On the role of mitochondrial calcium in mouse olfactory sensory neurons

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Ionized calcium is key to a variety of sensory signaling pathways. In olfactory sensory neurons (OSNs), the chemical energy of odorant receptor - ligand interaction is translated into an electrical signal by opening of cyclic nucleotide-gated (CNG) channels and Ca^{2+} iinflux into the cilia. This primary event triggers a number of secondary cellular responses that ultimately shape an individual neuron's sensory output. Therefore, cytosolic Ca^{2+} concentrations are tightly controlled.

Here, we investigate a functional role of mitochondria in shaping the odor-mediated Ca^{2+} response in OSNs. Using both genetically engineered mice that express a Ca^{2+}-sensitive photoprotein associated to the inner mitochondrial membrane and a new dedicated bioluminescence microscope (LV200, Olympus), we establish a novel imaging approach to selectively record Ca^{2+} signals in OSN mitochondria at high temporal resolution.

We show that mitochondria play a vital role in olfactory Ca^{2+} signaling, controlling both primary and secondary Ca^{2+} pathways. When Ca^{2+} sequestration by mitochondria is pharmacologically inhibited, the distinct time course of the odor-mediated cytosolic Ca^{2+} signal is significantly changed in both the OSN dendritic knobs and somata. Moreover, we report activity-dependent mitochondrial translocation to dendritic compartments upon odor stimulation. On-going electrophysiological recordings from identified OSNs aim to reveal the functional consequences of mitochondrial perturbation on the odor-mediated electrical output signal.

Ion channel properties of the Drosophila odorant receptor protein Or83b

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Odorant signals are perceived by binding of odor molecules to odorant receptors (ORs) that belong to the G-protein-coupled receptor (GPCR) family. Odor-binding to ORs activates stimulatory G-proteins thereby enhancing cAMP production that gates cyclic nucleotide-gated ion channels and depolarizes the odorant receptor neuron. Insect OR proteins are 7-transmembrane segment proteins as GPCRs but they lack any sequence similarity to other GPCRs

and show an inverted orientation in the plasma membrane. Insect ORs form heterodimers composed of an odor-specific OR protein and an ubiquitously expressed chaperone protein as the Drosophila Or83b. As previously shown, odorant stimulation of OR protein couples expressed in HEK293 cells activates nonselective cation currents via both an ionotropic and a metabotropic pathway (Wicher et al., Nature 452 (2008) 1007). Expression of Or83b alone provided functional ion channels insensitive to odorants, but directly activated by cyclic nucleotides. Patch clamp recordings in the inside-out configuration revealed that a fraction of Or83b channels is constitutively active even in the absence of cyclic nucleotides. The sensitivity of Or83b to cyclic nucleotides considerably exceeds that of cyclic nucleotide-gated ion channels. The EC_{50} for current activation by cAMP was just 1 nM. However, as current production developed slowly the activation mechanism is different from the gating known from ionotropic receptors.

This study was supported by the Max Planck Society.

ORAL SESSION Coding and Regulations:

Processing of Complex Host Blends in the Manduca Antennal Lobe

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Natural odor cues are often multi component blends and animals typically perceive their olfactory environment as a complex mixture. In insects, the initial representation of odors occurs in the first olfactory center of the brain, the antennal lobe (AL). The resultant neural representation of an odor mixture may either retain simple single-odor information of blend components, or reveal mixture-specific interactions due to processing in the AL network.

Using a novel multicomponent stimulus system we performed intracellular recordings of AL neurons in an attempt to determine the degree and spread of non-linear interactions as well as decipher the cellular aspects of blend response. 82% of the individual in vivo recorded projection neurons (PNs) and local interneurons (LNs) showed non-linear spike frequencies in response to the blend versus its individual compounds. Multicomponent responses exhibited an array of interactions including suppression, hypoadditivity, and synergism. Moreover, we found that a single morphological type of neuron can exhibit diverse blend interactions including different response profiles.

Our results indicate high levels of across-fiber patterning within the antennal lobe creating a highly combinatorial, non-linear process for coding complex host blends in *Manduca sexta*. To assess the network as a whole, additional Ca2+-imaging studies in the AL were applied to reveal how the unique blend identity is encoded.

Towards unraveling the olfactory code in *Mus musculus*: combinatorial encoding of age-, sex-, and genotype-specific olfactory signature by major urinary proteins (MUPs)

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Major urinary proteins (MUPs) of the house mouse form a large group of highly polymorphic acidic isoforms with molecular masses of 18-20 kDa. MUPs transport volatile hydrophobic ligands in aqueous media and various MUP fractions have different affinity to several pheromones (Armstrong et al., 2005). Thus, a specific set of MUPs creates a unique olfactory bouquet, which provides valuable olfactory information about individual characteristics of the animal (Hurst, 2009). Moreover, MUPs by themselves can encode essential information about sex, social rank, and individuality of donors (Chamero et al., 2007). However, fine molecular mechanisms of olfactory coding by MUPs remain to be elucidated.

Using electrophoresis in polyacrylamide gel (PAGE), we examined patterns of MUPs expression in laboratory mice of different genotypes, CBA/LacY and C57BL/6JY, which belong to two main lineages (Cheetham et al., 2009). Quantitative evaluation of eight MUPs fractions with different electrophoretic mobility (A-H) in urine samples from animals of these strains revealed that each genotype is characterized by a specific combination of individual fractions, which might reflect a genetically programmed pattern of *Mup* genes expression. Moreover, we found that the proportion (ratio) of different MUP fractions, rather than their concentrations, plays a key role in encoding essential individual information.

Our data on genotype- and sex-specific ratios of principal MUP fractions (A, C, D, E) in both lines may provide new insights into mechanisms of genetic and epigenetic regulation of *Mup* gene cluster, which are responsible for expression of specific bouquets of MUPs. We propose that various volatile pheromonally active ligands represent ''letters'' of the chemical ''language of communication'' in *Mus musculus* and that, correspondingly, specific combinations of MUPs efficiently organize these ''letters'' into readable ''words'' that encode sex, age, and genotype differences (Novikov et al., 2009).

Supported by Russian Foundation for Basic Research (projects 02-04-49273, 04-04-63050, 07-04-01762).

Armstrong S.D. et al., 2005. Structural and functional differences in isoforms of mouse major urinary proteins: a male-specific protein that preferentially binds a male pheromone. Biochem. J. 391:343–350.

Chamero P. et al., 2007. Identification of protein pheromones that promote aggressive behaviour. Nature. 450:899–902.

Cheetham S.A. et al., 2009. Limited variation of the major urinary proteins of laboratory mice. Physiol. Behav. 96:253–261.

Hurst J. 2009. Female recognition and assessment of males through scent. Behav. Brain Res. 200:295–303.

Novikov S.N. et al., 2009. The pattern of major urinary proteins (MUPs) expression during postnatal ontogenesis of the laboratory mouse depends on genotype and sex. Russian J. Dev. Biol. 40:204–211.

RNAi-mediated dissection of olfactory behavioral response profiles of odorant binding proteins in *Drosophila melanogaster*

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Chemosensation in Drosophila is mediated by large multigene families of chemoreceptors, including olfactory receptors, gustatory receptors, and odorant binding proteins (OBPs). The latter are highly divergent soluble proteins, which in the antennae and maxillary palps are secreted into the aqueous perilymph where they are thought to facilitate transport of hydrophobic odorants to olfactory receptors on the chemosensory membranes of olfactory sensory neurons. Although some OBPs have been functionally implicated in pheromone responses and host plant selection, their functions in general odorant recognition are thus far poorly characterized. To assess molecular response properties of OBPs, we have systematically suppressed the expression of 17 OBPs by crossing a tubulin-GAL4 driver line to lines from the Vienna Drosophila RNAi Center collection that express RNAi corresponding to individual *Obp* transcripts under UAS promoters inserted in the neutral *PhiC31* integration site. RNAi expression was enhanced with a UAS-GAL4 enhancer and real time qRT-PCR showed effective suppression of target RNAs in the GAL4-UAS hybrid offspring. We measured behavioral responses to a spectrum of odorants, and different odorants revealed altered behavioral responses in subsets of lines in which *Obp* gene expression was suppressed. Moreover, the response profiles showed sexual dimorphism. Our results demonstrate that our approach can delineate odorant response profiles of OBPs and confirm that interactions between general odorants and OBPs are combinatorial. Furthermore, our results show that males and females experience the chemosensory environment through different expression patterns of chemoreceptors.

Supported by NIH grant GM059469.

Role of Glycine receptors during postnatal neurogenesis

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Neurogenesis persist in the brain after birth in two main regions, the dentate gyrus of the hippocampus and the subventricular zone (SVZ). The SVZ produces interneurons for the main olfactory bulb. We have shown recently that expression of neurotransmitter receptors, for example glutamate receptors, were responsible for migration, proliferation and survival of migrating neuroblasts (Platel, Dave et al. 2008; Platel, Dave et al. 2010). The GABA receptor, has also been well studied and has been shown to control many aspect of neurogenesis (Bordey 2007; Ge, Pradhan et al. 2007). This effect of GABA is due to the fact that the chloride gradient is different in immature neurons (higher intracellular chloride concentration) compare to mature neurons leading GABA receptors to be depolarizing. During embryonic development another Chloride permeable receptor, the glycine receptor had been shown to become depolarizing because of this chloride gradient flint (Flint, Liu et al. 1998).

We are studying the role the glycine receptor in the context of postnatal neurogenesis. Using calcium imaging on acute slices, we show that glycine receptors start to be expressed in few immature neuroblasts in the SVZ and by the time they migrate in the rostral migratory stream of the olfactory bulb, most of the neuroblasts express it. Activation of this receptor by glycine or taurine lead to an increase of intracellular calcium that is not blocked by DAPV or bicucculine but completely blocked by the glycine receptor antagonist strychnine. We are studying the role of this novel signaling on proliferation, migration, survival and integration of newborn neurons.

Bordey, A. (2007). "*Enigmatic GABAergic networks in adult neurogenic zones.*" Brain Res Rev **53**(1):124–134.

Flint, A. C., X. Liu, et al. (1998). "*Nonsynaptic glycine receptor activation during early neocortical development.*" Neuron **20**(1):43–53.

Ge, S., D. A. Pradhan, et al. (2007). "*GABA sets the tempo for activity-dependent adult neurogenesis.*" Trends Neurosci **30**(1):1–8.

Platel, J. C., K. A. Dave, et al. (2008). "*Control of neuroblast production and migration by converging GABA and glutamate signals in the postnatal forebrain.*" J Physiol **586**(16):3739–3743.

Platel, J. C., K. A. Dave, et al. (2010). "*NMDA receptors activated by subventricular zone astrocytic glutamate are critical for neuroblast survival prior to entering a synaptic network.*" Neuron **65**(6):859–872.

The influence of L-felinine on reproduction in mice and rats

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Chemosensory detection may be an important aspect of predator avoidance strategy for many mammals. In our earlier studies we examined the influence of predator chemical cues derived from feral cat urine on reproductive output of rodents: rats, mice and voles. Animals responded to predator chemical cues with reduced litter size and skewed sex ratio. The reduction in litter size in rodents exposed to predator urine was attributable to suppressed progesterone levels affecting the implantation of embryos. Exposures of mice *Mus musculus* to urine from feral cats *Felis catus* under semi-natural conditions significantly affected survivorship of offspring. Manipulations with the diet of predator and non-predator urine donors revealed the key role of sulfur-containing compounds. Removal of sulfur-containing compounds virtually eliminated the activity of urine. A number of active compounds from predator scents have been detected and tested under laboratory and semi-natural conditions. In current study we examined the influence of potential precursor of Felidae family pheromone L-Felinine on reproductive output in House Mouse and Norway rats. Felinine is a unique sulfur-containing amino acid found in the urine of domestic cats and select members of the Felidae family (Rutherfurd et al., 2002).

Cotton balls soacked with L-Felinine(0.05% w/v, 0.05 ml, US Biologicals) were placed directly into home cages of mice and rats each other day during all period of gestation. Exposure to felinine affected sex ratio in mice (n=40, $p < 0.001$) and rats (n=36, $p < 0.001$) in favour of males. By the day of weaning. in control groups of animals average weight of pups was significantly ($p<0.001$) higher then in exposed to felinine. Both, mice and rats showed seasonal sensitivity to L-Felinine.

Supported by RFBR 10-04-01599

Transition from sea to land: Olfactory function and adaptations in land hermit crabs

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Within anomuran land crabs (Coenobitidae) various degrees of terrestriality have evolved, but all species have marine larval stages. The ability to recognize and discriminate chemical cues in the environment is essential for most animals, and thus a successful transition from aquatic to terrestrial life raises dramatically new demands on the chemosensory system. Hence, these land crabs represent an excellent opportunity to investigate the effects of the transition from sea to land on the olfactory system. We aim to explore the olfactory abilities of the Coenobitidae and to understand how they have evolved as adaptations to the terrestrial environment from a functional and evolutionary perspective. Previous behavioural studies have shown that coenobitids do make use of their olfactory system on land (Rittschof & Sutherland, 1986; Stensmyr et al., 2005) and recent neuroanatomical studies indicate superb processing capacities of their olfactory system (Harzsch & Hansson, 2008). However, virtually nothing is known about what type of chemical compounds are detected. In order to identify this, we used electroantennogram (EAG) recording techniques in two *Coenobita* species, *C. clypeatus* and *C. compressus*. We established antennular responses to several amines and carboxylic acids, i.e. chemical groups known to induce foraging in aquatic crustaceans, as well as to a few aldehydes, whereas several other odorants tested, typically used as foraging cues by insects (e.g. esters, ketons, alcohols, aromatics, other aldehydes), yielded no response. Interestingly, different chemical groups resulted in opposite EAG-responses (i.e. depolarization vs. hyperpolarization); a pattern that also appears for the terrestrial robber crab *Birgus latro* and the marine hermit crab *Pagurus bernhardus*. The behavioural relevance of some of the physiologically active compounds was verified in a two-choice bioassay. Here we found that, although not attractive in itself, water vapour was critical for both natural and synthetic odorants to induce behavioural responses, since crabs were only attracted to, or repelled by, odorants when presented in combination with water. Thus, although these terrestrial crustaceans seem to have evolved a sense of smell functioning in air, our results indicates that their olfactory system is restricted to particularly water-soluble molecules as stimuli, and that water is critical to induce behavioural responses, possibly because of the animals' aquatic origin. Possible explanations for this are discussed.

Supported by FP7-PEOPLE-2007-2-1-IEF, the Swedish Research Council for Environment Agricultural Sciences and Spatial Planning and the Max Planck Society.

Harzsch S, Hansson BS, 2008. *BMC Neurosci.* 9, 58.

Rittschof D, Sutherland JP, 1986. J. Chem. Ecol. 12, 1273-1284.

Stensmyr MC, Erland S, Hallberg E, Wallén R, Greenaway P, Hansson BS, 2005. Curr. Biol. 15, 116-121.

Plasticity of the chemosensory repertoire in *Drosophila melanogaster*

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Adaptation to changes in the environment requires plasticity in the expression of genes that underlie phenotypic plasticity. Chemosensation is critical for survival, and is mediated by large families of chemoreceptor proteins, whose expression must be tuned appropriately to changes in the chemical environment. Using customized cDNA microarrays, we showed that chemoreceptor genes that are clustered in the genome undergo independent transcriptional regulation at different developmental stages and between sexes. Expression of distinct subgroups of chemoreceptor genes is sensitive to reproductive state and social interactions. Furthermore, exposure of flies only to odor of the opposite sex results in altered transcriptional regulation of chemoreceptor genes. These genes are distinct from those that show transcriptional plasticity when flies are allowed physical contact with same or opposite sex members. We analyzed covariance in transcript abundance of chemosensory genes across all environmental conditions and found that they segregated into 20 relatively small, biologically relevant modules of highly correlated transcripts. This finely pixilated modular organization of the chemosensory subgenome enables fine tuning of the expression of the chemoreceptor repertoire in response to ecologically relevant environmental and physiological conditions. In addition to analyzing transcriptional abundance, we used a high sensitivity liquid chromatography tandem mass spectrometry procedure for relative quantification of antennal soluble proteins from the same fly populations reared under corresponding conditions. We detected 366 proteins with high confidence, including 14 odorant binding proteins and two antenna specific proteins. Our results show that plasticity at the level of the antennal proteome is less evident than the observed extensive variation in transcript abundance of chemosensory genes. Nonetheless, 29 proteins showed differences in abundance under different conditions and between the sexes, including one odorant binding protein. A comprehensive systems genetics approach will ultimately enable us to achieve an integrated understanding of phenotypic plasticity in the Drosophila olfactory system in term of endophenotypes (transcriptome and proteome) as well as chemosensory behavior.

POSTERS ABSTRACTS

Methods for Intrinsic Optical Imaging of Human Olfactory Epithelium

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Intrinsic optical imaging allows monitoring neuronal activity by capturing activity-related changes in blood oxygenization, blood flow, and vascular and metabolic processes. This method has been used extensively to map olfactory responses in the rodent olfactory bulb. The human olfactory epithelium presents an unusual opportunity in the form of CNS bipolar sensory neurons that are accessible through the nose.

To probe olfactory coding at this level, we developed an apparatus for optical imaging in awake non-anaesthetized humans. Key to successful intrinsic optical imaging is a complete lack of motion.

With this in mind, we constructed a non-invasive dentist-chair-mounted stereotactic device that prevents head-motion. Linked to the stereotactic device is a jointed lockable swivel arm culminating at a sub-millimeter manipulandum. Mounted on the manipulandum is an imaging camera (Adimec1000, Adimec, Eindhoven, The Netherlands) fitted through an optical filter-housing to a custom-built zoom-fitted 2.7mm ridged endoscope (Richard Wolf, Germany). The endoscope is also coupled to an interchangeable light source (for initial endoscopy: Wolf 5121 Auto LP, Richard Wolf, Germany, for functional imaging: IntraLED 2020+, Volpi AG, Switzerland). The imaging data is collected using a dedicated acquisition system (Imager3001, Optical Imaging Inc., New York, USA). Odorants are delivered using a 40-chanel MFC-based computer-controlled olfactometer with no non-olfactory cues associated with the alternation between odorant presence and absence. Imaging data, odorant control, physiological parameters such as heart rate, nasal air flow, and overall respiration, as well as subject psychophysics, are all computer-linked to a common time-line. In a pilot study we delivered 15 0.5-second events containing the odor Bornyl acetate (1:1000 in PEG 50 ml total, 1:2 in clean air) and 15 0.5-second events containing clean air, both at 6LPM, 37 degrees C, and 90% humidity. Imaging resolution was ~4 microns per pixel, and data was collected at 25Hz.

For initial pilot analysis, we considered the entire middle turbinate as a single pixel (~5x10mm), and data was temporally binned into 10-frame segments. To account for signal variation related to motion and other sources of unwanted variance, we first computed the mean optical signal during odor and no-odor that was obtained from the lateral walls of the nostril where no olfactory receptors are expected. We then subtracted this baseline from the mean response in the middle turbinate, and then subtracted the no-odor from the odor response. This revealed a hemodynamic-type odor-induced response with a peak at 5 seconds. Although this is an encouraging initial indication of a response recorded directly from human sensory neurons, several sources of variance need be considered. In this presentation we will discuss the influence of variables such as optics (endoscope selection), acquisition wavelength, lighting source, subject behavior (sniff/no-sniff), post-processing motion correction, and analysis techniques.

Olfactory perception via both orthonasal and retronasal routes is associated with respiratory activities

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Respiration is essential for smell perception. Odor molecules reach olfactory receptors by inspiration, and odor information projects directly to limbic structures not through the thalamus. Odor stimuli thus induce respiratory changes, and simultaneously induce emotion and memory recognition via stimulation of olfactory-related limbic structures.

We previously analyzed electroencephalograms (EEGs) and respiration simultaneously in normal subjects while testing for detection and recognition of odors. We assumed that if perception or feel odor depend on inspiration, we could be found the inspiration-related potential during olfactory stimuli in the averaged EEGs potentials triggered as inspiration onset. We found that 9–12-Hz cortical rhythms are phase-locked to inspiration during odor stimulation, in a process referred to as inspiration phase-locked alpha band oscillation (I-α).

To identify the generator of this rhythmic cortical potential, we used a dipole tracing method of a scalp-skull-brain head model (SSB/DT), which enabled us to detect generators of event-related potentials in the order of milliseconds. The genesis of I-α were identified in olfactory-related areas including the entorhinal cortex (ENT), hippocampus (HI), amygdala (AMG), and orbitofrontal cortex (OFC)[1]. Such olfactory perception is said to occur via the orthonasal olfaction route. Another route is the retronasal olfaction route, in which odor molecules from food or liquid are drawn up into the nose from the nasopharynx and reach the nostrils. In this study, we investigated the link between respiration phase and retronasal olfactory perception. EEGs and respiratory flows (separately measured with mouth and nose) were simultaneously recorded during stimulation of subjects' tongues with liquids of chocolate, sucrose and water. The percentage of subjects correctly identifying the chocolate taste was higher when subjects were asked to breathe through the nose than when they were breathing through the mouth. In the averaged EEGs triggered by the onset of expiration measured from the flow through the nose, a 8-12-Hz oscillation was observed. Generators of this potential were found in the left ENT, HI, AMG and OFC in the order of milliseconds after expiration onset[2].

Perception of retronasal olfaction is dependent on expiration, and combining retronasal olfactory information with gustatory information and somatosensation enable us to identify flavors when drinking and feeding.

1. Masaoka, Y, Nobuyoshi, K & Homma I (2005) Inspiratory phase-locked alpha oscillation in human olfaction: source generators estimated by a dipole tracing method. Journal of Physiology, Vol. 566(3), 979-997. 2. Masaoka, Y, Satoh, H, Akai, L & Ikuo Homma (2010) Expiration: the moment we experience retronasal olfaction in flavor. Neuroscience Letters, 473, 92-96.

Training the inter-nostril localization ability of olfactory chemicals

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In vision and audition, the pair of receptor organs is crucial for spatial orientation and localisation of stimuli in the 3-dimensional environment. Whether the pair of receptor organs in olfaction fulfils the same function of localizing odor sources is not yet clear. Is is widely accepted that mixed trigeminal-olfactory chemicals can be accurately localized when applied passively to one of the nostrils. Chemicals with a predominant olfactory component seem, on the contrary, hard or impossible to localize in passive stimulation procedure.

Purpose of this study was to investigate whether the ability of subjects to localize an olfactory stimulus delivered passively to one of the two nostrils would improve under training. Fifty-two healthy, normosmic women aged between 18 and 30 years participated in 6 to 7 sessions. Two groups were created: 27 subjects followed an olfactory lateralisation training protocol using two chemicals known to selectively stimulate the olfactory system (hydrogen

sulphide and phenylethylalcolol). The second group performed "brain jogging". Before and after- training subjective intensity and lateralisation ratings were recorded. Additionally electrophysiological parameters after stimulation with the two odorants were recorded at the end of the training. Comparisons between trained and non-trained subjects suggested an improvement of localisation scores in subjects specifically trained for this. Amplitudes N1 and P1N1 were found larger in subjects that performed smell training. Consequently, the ability to localize passively applied olfactory stimuli can be trained; further on training influences the amplitudes of the early oERP components possibly as a result of increased attention towards the olfactory stimuli.

Processing differences between monorhinal and birhinal odor mixtures

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We investigated whether a mixture of two odorants is perceived differently if presented as a mixture in one nostril (monorhinally), versus the same two odorants in separate nostrils (birhinally). In parallel, we investigated whether the different types of presentation give rise to differences in event-related potentials (ERP).

Twenty-four healthy persons, 12 men and12 women, between the ages of 18 to 35 years participated in the study. Using the Sniffin' Sticks threshold test, all participants were screened for threshold differences between both nostrils separately. We excluded those with threshold differences over 2 points between the two nostrils (Gudziol et al, 2006). We compared single odorants A or B monorhinally, mixtures of A and B monorhinally, and simultaneous presentation of A and B birhinally. The odorants used were eugenol (A) and l-carvone (B). The stimuli were presented, using a computer-controlled air-dilution olfactometer (OM6B; Burghart instruments, Wedel, Germany), in a constant flow of odorless and humidified air of controlled temperature (250 ms stimulus duration; 80% relative humidity; total flow of 7 L/min; 36 degrees C). Participants were asked to rate the composition of the stimuli on a visual analogue scale. Hence, they rated to which extent they perceived a single odorant, A or B, or a mixture. The participants also rated the overall intensity of the stimuli. Additionally, ERPs for each type of stimuli were recorded.

Analyses focused on the differences between monorhinal and birhinal mixtures, in regards to the psychophysical ratings as well as the ERPs. The results of the psychophysical ratings show that birhinal mixtures were perceived as more intense than monorhinal mixtures. In addition, there was no difference in the perceived quality of mono- and birhinal mixtures. The analyses of the ERPs show that there were significant differences for the latencies of P1 and N1at Cz, Pz, and Fz between mono- and birhinal mixtures, with shorter latencies for birhinal mixtures. For the latencies of P2, as well as the amplitudes, no significant differences were observed.

The results could be interpreted in terms of a functional additivity of the two nostrils – two nostrils are more effective than one nostril. This is indicated by faster latencies and higher intensities of birhinal mixtures. The results also suggest that mixing odorants peripherally yields the same perceptual quality as mixing them more centrally. P2 latencies and amplitudes did not differ between mixture types, which suggest that a more central process is responsible for the perceived quality of mixtures.

Gudziol, V., Lötsch, J., Hähner, A., Zahnert, T., & Hummel, T. (2006). Clinical significance of results from olfactory testing. The Laryngoscope 116(10): 1858-1863.

Funded by Stiftelsen Lars Hiertas Minne and the Swedish Research Council.

Is the Olfactory Epithelium Tuned to Olfactory Perception?

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The principal axis of human olfactory perception, odorant pleasantness, has been linked to a physicochemical axis of odorant structure (Khan et al., 2007). We set out to ask whether electro-olfactograms (EOlfG) recorded directly from the human olfactory epithelium are tuned to this axis (PC1 of physichochemical space), or in turn better reflect known chemical families. We chose three pairs of odorants, pleasant (pl) and unpleasant (upl) from 3 distinct functional groups (Aldehydes: Butanal (upl) and Hydroxycitronellal (pl); Esters: Cresyl Acetate (upl) and Amyl Acetate (pl); Alcohols: Octanol (upl) and Geraniol (pl)). The odorants were diluted with PEG to a maximum vapor pressure of 0.01mm/Hg at 25̈C outside the olfactometer, and further diluted with heated (36̈C) humidified (80%) air inside the olfactometer to obtain equated perceived intensities ($F(54,5)=1.26$, $P>0.29$). An overall flow of $6_{l/min}$ and maximum partial odorant flow of 3l/min were kept. ISI=45 s, Stim. Dur.=0.5 s, 5 events per condition, Sampling rate=1kHz. Subjects held their breath 1.5s before stimulus onset and 3s thereafter. Following each trial subjects rated pleasantness and intensity.

Current results (n=13): Maximal response amplitude (subject means) was obtained for Octanol (977μV, may involve a trigeminal component) and minimal for Butanal (154μV). A larger EOlfG area under the curve (AUC) was observed for the unpleasant odorant in the Aldehydes and Alcohols, and for the pleasant odorant in the Esters. Multi-descriptor chemical analysis revealed high positive correlations between Edge Adjacancy Indexes and AUC ($r>0.94$, $p<0.005$) and a link between Radial Distribution Functions (RDF) ($t(76) < -21.9$, $p < E\text{-}10$) and AUC. Notably, RDF was also related to odorant pleasantness ($t(76) < -2.06$, $p = 0.042$). In other words, although there was no simple relation of EOlfG AUC to odorant pleasantness, these results suggest correlations between AUC, molecular compactness, and odor pleasantness. Conclusive analysis depends on data obtained from additional subjects and odorants.

Olfactory perception and motion sickness

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The motion complexity of modern vehicles may cause several symptoms as nausea, headache, postural discomfort and unsteadiness that are classified within the broad definition of motion sickness (Golding, 2005). Whereas the scientific community renews efforts to develop some behavioural and pharmaceutical techniques to suppress motion sickness, this problem is not resolved yet. Interestingly, several authors proposed that unpleasant odors could contribute to motion sickness (Glaser, 1959; Turner and Griffin, 1999; Fessler and Arguello, 2004). However, the relation between olfactory system and motion sickness has not been previously investigated. For this purpose, subjects were recruited among healthy and non-smoker women fulfilling Golding's (1998) Motion Sickness Susceptibility Questionnaire (MSSQ). Subjects were divided in two groups: (1) subjects who are very sensitive to motion sickness (N=10), (2) subjects who are not sensitive to motion sickness (N=10). We compared with usual psychophysics methods the olfactory sensitivity (i.e. threshold testing with the odor of n-butanol), and the self-ratings of intensity, familiarity and hedonic valence of a panel of seventeen odorants. Our results showed: *(i)* a poor olfactory sensitivity in subjects sensitive to motion sickness (Intergroup T-test, T=2.63), *(ii)* that motion sickness sensitive subjects judged the odor of leather as more unpleasant than the other group (Intergroup T-test, T=-3.53), *(iii)* motion sickness sensitive subjects judged the odor of petrol as more unpleasant (Intergroup T-test, T=-3.46) and more familiar than the other group (Intergroup T-test, T=2.43).

The present results pointed out some differences on olfactory perception relative to motion sickness susceptibility. Herz (2005) highlighted that the emotional context in which an odor is encountered could influence odor hedonic perception and odor-related behaviour. Thus, we could proposed that subjects who are very sensitive to motion sickness judge odours of leather and petrol as unpleasant because it remind them the bad experience of motion sickness in vehicles, in particular in cars. In this way, we could explain the poor olfactory sensitivity of these subjects as a defence reaction to contend to motion sickness. Although several points of our results need to be deeper explore, this study provided support for new techniques to help motion sickness sensitive subjects, based on olfactory perception.

Fessler D.M.T., and Arguello A.P. (2004). The relationship between susceptibility to nausea and vomiting and the possession of conditioned food aversions. Appetite., 43, 331-334.

Glaser E.M. (1959). Prevention and treatment of motion sickness. Proc. R. Soc. Med., 52 (965), 21-28.

Golding J.F. (1998). Motion sickness susceptibility questionnaire revised and its relationship to other forms of sickness. Brain Res. Bull., 47(5), 507–516.

Herz R.S. (2005). Odor-associate learning and emotion: effects on perception and behaviour. Chem. Senses, 30 (1), i250-i251.

Turner M. and Griffin M.J. (1999). Motion sickness in public road transport: passenger behaviour and susceptibility. Ergonomics., 42(3), 444-461.

Influence of information-manipulation of the same odor stimulus on cardiovascular response (2) -using subliminal affective priming method

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In ECRO 2004 and 2006, we introduced intermittent presentation method of short-duration odor to evaluate the cognitive effects on odor adaptation / habituation and evaluated these cognitive effects using electrophysiological techniques such as electro-encephalograph (EEG) and magneto-encephalograph (MEG). In ECRO 2009, we further introduced a method to measure cardiovascular responses following odor presentation under a similar intermittent presentation method of short-duration odor. So far we have obtained results showing that the intermittent presentation method is suitable to detect both cognitive and physiological effects of information-manipulation on the same odor stimulus through minimizing adaptation to the odor. In this study, we investigated effects of "implicit" (unconscious) information-manipulation of the same odor stimulus on its perceived intensity and pleasantness using subliminal affective priming method. Although we have accumulated the results that "explicit" odor information-manipulation is effective in changing subjective and physiological responses to the odor, some lines of evidence suggests deteriorative effects of these "explicit" information-manipulation due to our cognitive suppression. Thus, this study aimed to investigate whether "implicit" odor information-manipulation was effective in terms of changing subjective odor evaluation.

Sixty-five participants including 30 males and 35 females (Mean=19.95, SD=1.41) took part in this study. The odor used as a target stimulus was anise seed oil, unfamiliar odor to the Japanese people. As priming visual stimuli, 20 pictures of faces with "happiness" or "disgust" expression, that were made originally in our laboratory, were used. A priming visual stimulus, a face with either "happiness" or "disgust" facial expression, was presented subliminally (below threshold) together with a target odor stimulus. Each participant experienced a whole series of different priming visual stimuli with identical facial expression (either "happiness" or "disgust" facial expression). The priming (facial expression) and target (odor) stimulus were presented for 4 sec with 8-sec inter-stimulus interval (1 trial) through DIY olfactometer with a similar changeover mechanism as Kobal-olfactometer, and 20 trials were carried out in each experimental session. After the experimental session, the participants were required to evaluate perceived intensity and pleasantness of the odor stimulus. As results, individuals who were presented subliminal "disgust" faces (the negative group) perceived the odor as less intensive as compared with those who were presented "happy" faces (the positive group) only in female participants. The effects of affective information on odor pleasantness, however, were not significant.

These results suggest that implicit affective visual information presented together with the target odor stimulus influenced participants' evaluation of the odor stimulus on the perceived intensity, suggesting significant subliminal affective priming effects on subjective odor evaluation.

How do Italian and Korean consumers perceive the same food? The case of *Perilla frutescens*

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Perilla frutescens is a plant widely used for therapeutic as well as food purposes in Asian countries, especially in Japan and Korea. It contains several volatile components (mainly perillaldehyde and perillaketone, according to the chemotype) which significantly influence its sensory quality and pharmacological activities. Perilla leaves are strongly aromatic with a mint-like flavour and therefore suitable for food preparations. In many Asian countries, the leaves are used as spice, cooked as postherbs or fried and combined with fish, rice, vegetables and soups. Despite its interesting properties, this plant is completely unknown in European countries. Since Perilla might be suitable for Italian food preparation, in the present experiment we decided to assess how this product, which is familiar and accepted in the Korean culture, is perceived and appreciated by Italian consumers. To this purpose, 77 Italian (30 males and 47 females, age: 22-61) and 31 Korean consumers (16 males and 15 females, age: 20-62) were recruited and invited to take part in two different tests.

During the first test, the Napping® procedure was applied to compare Italian's and Korean's olfactory ability to discriminate Perilla essential oils from other essential oils which were previously (Laureati et al., 2010) identified as describing the Perilla odor (anise, fennel, almond, mint, cumin, grass). Results evidenced that both groups were able to discriminate the Perilla samples from the other odors but Italian consumers tended to associate Perilla oils to cumin and grassy odors, whereas for Korean subjects the Perilla odor was specific and not related to other odors. In the second test, in order to evaluate how this plant is appreciated when added as ingredient to a food formulation, 3 soft-drinks obtained by the infusion of different Perilla cultivars were produced and evaluated by the two groups of subjects along with other similar, commercial soft-drinks. Results evidenced that both Italian and Korean consumers preferred the commercial soft-drinks, whereas Perilla samples received low hedonic ratings. However, Korean consumers gave hedonic scores which were significantly higher than those of Italian consumers for Perilla soft-drink, thus suggesting that familiarity with the product play a role in food appreciation.

Laureati, M., Buratti, S., Bassoli, A., Borgonovo, G., Pagliarini, E. (2010) Discrimination and characterisation of three cultivars of Perilla frutescens by means of sensory descriptors and electronic nose and tongue analysis. Food Res. Int., 43, 959-964.

Acknowledgements: This research was funded by the Italian Ministry for Foreign Affairs, Ministry for University and Research and University of Milano

Effect of sensory education on categorisation of unknown odors in children

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Spontaneously, unknown odors are categorised by children according to their hedonic values. This study was set up to verify whether a sensory education program, based on the pedagogical method of the "Classes du gout" developed by Jacques Puisais is able to switch this hedonic categorisation strategy into a more olfactory quality based "expert" strategy. The study was carried out in Dijon, France with school classes of children of 8 to 11 years old and with their usual teacher. It was performed using an experimental group of 87 children and a control group of 81.

Children of an experimental group (N=87) participated in 12 sensory education lessons of 90 minutes each. Both the experimental and the control group (N=81) participated in two laboratory measurements: a pre-test before the education period of the experimental group and a post-test just after the education period. The children were asked to categorise 9 unknown odors in 3 groups of 3 without any other information. They also indicated their liking for the odors. Results showed that the sensory education induced a switch in the strategy of classification of unknown odours towards a less hedonic approach.

The protocol was approved by a medical ethical committee and supported by the National Research Agency of France

Selective adaptation allows identification of odors from a mixture of chemically similar compounds

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Smell disorders affect two million adults in the USA, disorders of smell have an impact on quality of life, and, perhaps more importantly, smell is a safety mechanism to inform one of a substance's potentially harmful chemical makeup and plays a vital role in discrimination of flavor (Mann, 2002) Thus, it is important to understand how humans are capable of processing odorous compounds. One mystery in understanding human olfaction is selective adaptation. When as few as 4 different odorants are presented as a mixture, humans cannot recognize any individual odors (Livermore & Laing, 1996; 1998; Mainland & Sobel, 2006). Goyert et al. (2007) expanded mixture research into study of selective adaptation by choosing 4 compounds with distinct odors and showing with initial presentation of a 3-odor mixture, closely followed by presentation of a new mixture with a 4th odor added, the newly added 4th odor could be identified.

The current research used 4 chemical compounds with similar molecular structures and independent, recognizable odors to test if selective adaptation worked for structurally similar, distinctly labeled, chemicals. 3 mM benzaldehyde (cherry), 5 mM maltol (caramel), 1 mM guaiacol (smoke) and 4 mM methyl anthranilate (grape) were used to test 14 subjects. Subjects attended 2 sessions. In experimental sessions, a mixture of 0 to 3 odorants (adapt solution) was presented, then 5s later, the adapt solution plus an "extra" stimulus (test solution) was presented. In the control session, water was the adapt solution.

Results confirmed selective adaptation. An odor was identified with more accuracy when it was the extra component in the test solution compared to when it was an ambient component in the test solution (p=0.007). Extra stimulus identification represents the 7 times each of the 4 odorous stimuli were extra in the test solution. Ambient stimulus identification represents the 12 times each of the 4 odorous stimuli were ambient components in the test solution. All mixture sizes (binary, tertiary and quaternary) show that a component is better identified when extra than when ambient ($p<0.0001$; $p=0.001$; $p=0.0003$, respectively). Thus, even structurally similar compounds are detected in mixtures after selective adaptation.

Although 3 of the chemically similar compounds were readily identified (96-100%) when presented alone after water, methyl anthranilate was appropriately identified 61%, but incorrectly identified as smoke, 32% of the time. Methyl anthranilate may activate 2 of the more that 300 human olfactory receptors (OR) (Glusman et al, 2001), one of which also detects guaiacol. The confirmation of the phenomenon of selective adaptation with chemically similar stimuli may help elucidate OR specificity and suggests an inter-cellular mechanism of selective adaptation.

Supported by the University of Connecticut's School of Dental Medicine)

Water Quality Monitoring By a Multisensors Apparatus

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Assessment of water quality has still an outgoing interest. Water disinfection involve the use of several chemical compounds mainly halocarbons such as bromodichloromethane, bromoform, chloroform and dichlorobromomethane. These compounds, which have a high volatility can be responsible not only of unpleasant organoleptic properties of drink water but can have dangerous effects on health.

In this paper we present the results of a study carried out by using in parallel GC-MS and Multisensors Apparatus in order to verify the suitability to the Multisensors Apparatus to assess water quality.

GC-MS analysis of water has been carried out by previous extraction and concentration of the halocarbons by SPME. The extraction has been made from the head space as well as from the liquid samples by using different SPME fibres. The quantification has been made after calibration.

The VOC monitoring systems are based on core technology comprising an array of gas sensors that exhibit broad selectivity and high sensitivity to a range of volatile organic compounds. Whilst highly sensitive to VOCs, systems also provide immunity from environmental influences, essential for reliable field deployment.

Volatiles are drawn across the sensor array for measurement of VOC content either directly, or via sampling systems optimised for a particular application. VOC monitoring using this approach is therefore non-contact in nature, ensuring effective operation in harsh conditions and minimising maintenance. Our systems are capable of providing pollution event monitoring to ppb levels.

Multisensors System's products combine leading edge technology with industry standard features in a unique and robust package designed for deployment in demanding conditions.

The results obtained show that the Multisensors System is able to detect halocarbons in water at ppb level without any samples handling, this allows to perform in real time monitoring of halocarbons or other pollutants in drink water.

Acknowledgements: Fondazione Carical Potenza for supporting Dr. A. Rubino.

Design of Mobile Robot with Artificial Olfactory System

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There are much interesting to develop intelligent mobile robot that emulates the sense of human being. Most of researches for intelligent mobile robot have been concentrated with hearing and seeing using speech and/or image processing and synthesis techniques last decades. However, it has been difficult to find an intelligent mobile robot with an artificial olfactory function, even if the smelling is an important factor of human being.

The field showed little sign of advanced research before a research group publication in the middle of 90s from Japan. They developed a mobile robot with plural gas and anemometric sensors to search for toxic gas leak location and an origin of a fire at its initial stage and the location of ethanol gas source.

For development of an intelligent mobile robot having an artificial olfactory system (AOS), it is essential to produce an autonomous AOS. The autonomous AOS on mobile robots have a wide variation in operation. The general AOS cannot help depending upon the control and decision of human, but the autonomous AOS on robot have to be designed to be enable to autonomously decide the measurement time, direction and position besides moving direction. Moreover, the AOS on robot, which has small size and fast processing times, is more adaptable for interfacing with intelligent mobile robot.

We have been developed an intelligent mobile robot with an artificial olfactory function to recognize odors and to track odor source location. The mobile robot also has been installed an engine for speech recognition and synthesis, and is controlled by wireless communication. An artificial olfactory system based on array of 7 gas sensors has been installed in the mobile robot for odor recognition, and 11 gas sensors also are located in the bottom and top of robot to track odor sources and to decide the measurement time, direction and position of electronic nose in AOS. Three optical sensors are also included in the intelligent mobile robot, which is driven by 2 D.C. motors, for clash avoidance in a way of direction toward an odor source.

The overall system of intelligent mobile robot is controlled by a host processor and a sub-processor for olfaction and speech signal processing. The intelligent software is provided for odor recognition and tracking of odor source to an intelligent mobile robot. We adapted the Levenberg-Marquardt algorithm based on the back-propagation (LM-BP), having more advantages then conventional multilayer perceptron neural network algorithms for the simultaneous classification and concentration estimation of odors. It could be possible to classify odor classification and to predict concentration levels simultaneously. In addition, the step-by-step progress method is applied to move the remote-control mobile stage iteratively in the direction of the odor source, which is detected by the sensor array.

Bio-signal based emotion evaluation by olfactory display in playing the game and entertainment

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Everyone knows that scent plays an important role in attraction such as computer game and entertainment. It is well-known that pleasant scents induce positive effects for cognitive, psychological, physiological and physical enhancements of people. In particular, previous works found out that peppermint odor could reduce the work loads due to exercise and arouse attention efficiently. Both jasmine and lavender odors also caused significant decreases in heart rate. Following by recent experimental results, we expect odors synchronizing with game contents for exercise induce the increment of exercise time and continuous participation in the game.

Our research interest is in how to quantify emotional experience when engaged with olfactory display technologies, by developing an evaluation methodology for entertainment environments.

This research motivates why we need such an approach and describes the process by which we designed a new evaluative methodology for measuring emotional variation by olfactory display with interactive game technologies.

In this congress, we present emotional variation analyzing results with odor display in the middle of playing the game. For emotional evaluation, we used a fuzzy algorithm to transform GSR, ECG, EMG, and 4 channel of EEG into arousal and valence. Also, we converted variation of bio-signals transformed into arousal-valence to vector components.

We expect these results to contribute increasing interesting combination between olfactory display and game industry.

Towards a bio-electronic nose: sensing volatiles with rodent olfactory receptor neurons

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The olfactory system, with the olfactory epithelium as the sensory component, allows humans and other mammals to detect odorants and volatiles. This olfactory epithelium comprises olfactory receptor neurons, which are believed to each express only one type of olfactory receptor per cell. The receptors have a limited range of odorants that they can detect, based on the structural features of the odorant molecules. In the olfactory receptor neurons, the chemical stimuli of odorants are converted into neuronal signals, by means of a chemo-electrical transduction cascade. Odorant stimulation of olfactory receptor neurons causes calcium entry through cyclic nucleotide-gated channels via a series of signal transduction components, ultimately leading to an action potential.

By reading out these neuronal signals from a culture of olfactory neurons in vitro, a first part of a bio-electronic nose was established. For the culture of these receptor cells, immature neurons, preferably from embryonic origin, are of particular interest as they can differentiate in vitro. Up till now, researchers have mostly used postnatal (newborn to adult) rodents for cultures of olfactory receptor neurons. The gain in receptor neurons was often low because of the high amount of already differentiated neurons in the olfactory epithelium, which are easily damaged during dissection. In this study, a primary culture of E17 embryonic rat olfactory receptor neurons has been established. By means of calcium-imaging techniques with Fluo-4 AM, odor-evoked calcium transients have been observed in the cells. As a control for signal transduction, measurements with cAMP increasing agents proved to also evoke calcium transients. The culture could be maintained for 2 weeks. Immunocytochemical staining demonstrated the presence of neurons in the cell culture.

This paper also presents the first results on the immobilization the olfactory neurons on micro-electrode arrays (MEA) for the electrical read-out of the neuronal action potentials.

Relationship between odor descriptors and molecular structures

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Numerous descriptive words are used to represent odor quality of substances. These words are called odor character descriptors. They give us semantic odor profiles for these substances. In this study, we classified odor descriptors to reveal the underlying dimensions among descriptors using the reference by Arctander (1969)[1]. Moreover, we attempted to identify the differences of molecular structures among classified clusters of odor descriptors. The reference created by Arctander is one of the largest data banks of semantic odor profiles.

Firstly, the dichotomic matrix was created containing 2776 odorous substances (in rows) and 355 descriptors (in columns) from the reference. In this matrix, element (Xij) took the value of 1 when the descriptor (j) was present in odor profile of a substance (i), and took the value of 0 when the descriptor was absent. Secondly, we took out the descriptors on the basis of their occurrences because the least frequent descriptors were useless in statistical analyses. Out of the 355 descriptors, 218 were removed because they had an occurrence of less than 9 and represented only 4.9% of the total cited. The rest of the 137 descriptors were applied to statistical analyses. In order to classify these descriptors, we applied corresponding analysis to the data matrix and performed hierarchical cluster analysis by using component 1-3 obtained from the corresponding analysis. The descriptors were classified into 8 clusters which were named: ''Acid'', ''Sulfur'', ''Green'', ''Fruity'', ''Spicy'', ''Floral'' ''Woody'' and ''Phenolic'' according to the descriptors included in each cluster. For example, Acid cluster included 7 descriptors: acid, acrid, rancid, burnt, irritant, sour and sweaty. Sulfur cluster included only 2 descriptors of onion and sulfuraceous. Phenolic cluster included 6 descriptors: leather, phenolic, tar, medicinal, oakmoss and smoky.

These results suggest that similar descriptors were classified into the same cluster and there were at least 8 dimensions among the 137 descriptors. We examined common structural features of substances included in same cluster to elucidate the relationship between odor descriptors and molecular structures. Carboxyl group was a major feature in Acid cluster. The majority of substances in Sulfur cluster had either sulfide or thiol groups. Phenolic cluster had both

hydroxylic and phenyl groups, which were phenolic compounds. Ester, ether, hydroxylic and ketone groups were common features in the other 5 clusters in terms of functional group. However, each cluster appeared to have distinctive features in carbon skeletons. These findings suggest that there is a hierarchical relationship among odor descriptors, and the database in this study is of some help in understanding the relationship between odor descriptors and molecular structures.

[1] Arctander (1969) Perfume and flavor chemicals (aroma chemicals). Volumes 1 and 2. Allured Publishing Corporation, USA

Towards an understanding of odor blending in odorant mixtures:a pharmacophore approach

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Odors we perceived in our environment are mainly the result of mixtures of odorants. The first step of odor perception is an interaction between these odorant mixtures and olfactory receptors. The corresponding transduced olfactory signals, conveyed by olfactory sensory neurons, are then processed by the brain (Zou et al. 2006). In previous studies, we investigated the perception of a mixture including specific proportions of ethyl isobutyrate (strawberry-like odor) and ethylmaltol (caramel-like odor). This specific mixture was especially judged as more typical of a pineapple odor than the individual components (Coureaud et al. 2008, Le Berre et al. 2008, Coureaud et al. 2009).

Assuming that combinations of activated ORs encode odor qualities and that molecules sharing a same odorant quality possess common structural molecular property (Furudono et al. 2009, Saito et al. 2009), our assumption was that molecule sharing strawberry, caramel or pineapple odor should also share several common structural characteristics.

To test this hypothesis, we used Common Feature Pharmacophore Generation protocol (Catalyst HipHop) implemented in Discovery Studio 2.1 (Accelrys Inc.) to generate a pharmacophore hypothesis in the absence of known receptor(s). The Catalyst/HipHop program (Clement et al. 2000) is a powerful tool that explores the conformational space and derives a pharmacophore based on features that are common to active molecules. In our study, the pharmacophoric features considered are hydrogen bond acceptors (HBA) and surface of accessible hydrophobic regions (HY).

We selected 19 molecules in the Flavor Base 2004 (Leffingwell and Assoc.): 7 molecules described as ''strawberry'', 6 molecules as ''caramel'' and 6 molecules as ''pineapple''. We then performed HipHop Pharmacophore Generation on each subset separately. Two HY and two HBA constitute the best pharmacophore models obtained for ''strawberry'' and for ''pineapple'' subsets, whereas the best ''caramel'' pharmacophore is constituted by one HY and three HBA. A pharmacophore comparison leads to obtain a good mapping of these three pharmacophore models, putting forward a common spatial arrangement of chemical features. This result suggests that the molecules sharing strawberry, caramel or pineapple odor probably recognize a common OR set. An identification of target ORs would be the next step to understand the early biochemical mechanism supporting blending odor mixture perception.

O.O. Clement and A. Mehl, in Pharmacophore perception, Development and Use in Drug Design, O. F. Güner (Eds.), La Jolla, 2000, pp. 69-84; G. Coureaud, T. Thomas-Danguin et al. Physiol. Behav. 95 (2008) 194-199; G. Coureaud, Y. Hamdani et al. J. Exp. Biol. 212 (2009) 2525-2531; Y. Furudono, Y. Sone et al. Chem. Senses 34 (2009) 151-158; E. Le Berre, N. Beno et al. Chem. Senses 33 (2008) 389-395; H. Saito, Q. Chi et al. Sci. Signal. 2 (2009) 1-14; Z.H. Zou and L.B. Buck Science 311 (2006) 1477-1481.

Interpretation of Psychophysics Response Curves Using Statistical Physics and Double Layer Adsorption Model

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Experimental gustatory curves have been fitted for four sugars (sucrose, fructose, glucose and maltitol) using a double layer adsorption model[1]. In the analytical expression of the gustatory response we have introduced at least three physico-chemical parameters intervening in the adsorption process such as the number of molecules per receptor site (n), the density of receptor sites (N_M) and the concentration at half saturation ($C_{1/2}$). This last parameter is a function of the adsorption energy. The model giving the gustatory response versus the concentration of sugars in solution allows the shapes of the experimental curves to be interpreted using established methods in statistical physics.

Development of the expression of the model using statistical physics allowed assigning of a physical meaning to the constants and to facilitate the interpretation of the sweetness perception mechanism at the microscopic level. Understanding of physico-chemical parameters like density of receptor sites, number of molecules per site and the adsorption energy yields a convenient tool for interpretation of the adsorption process and consequently for the characterization of stimulus-receptor interaction.

We give interpretations of results obtained by the fitting of the psychophysical response curves with the bilayer adsorption model which are related to the taste mechanism of four sweet molecules on the basis of numerical simulation. The physico-chemical parameters introduced in the theoretical model and interpreted physically can produce an amount of information associated to the binding of stimulus to receptor site at the equilibrium. The behaviors of the parameters are discussed in relation with each molecule characteristics.

Starting from the double layer adsorption model we determined in addition the adsorption energy of each molecule on taste receptor sites. The use of the threshold expression allowed us to have information about the adsorption occupation rate of receptor site which fire a minimal response at gustatory nerve. Finally by means of this model we could calculate the configurational entropy of the adsorption system which can describe the order and disorder of the adsorbent surface.

[1] Knani, S., Ben Lamine, A., & Mathlouthi, M. (2007). Modeling of the Psychophysical Response Curves Using the Grand Canonical Ensemble in Statistical Physics. Food Biophysics, 2, 183-192.

Adaptive sensor drift counteraction by a modular neural network

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The response properties of chemo-sensors used in e.g. electronic noses vary in time due to internal or environmental factors. Recalibration is often costly or technically infeasible, which is why algorithms aimed at addressing the sensor drift problem at the data processing level have been developed. These falls in two categories: The pre-processing approaches, such as component correction [1], try to extract the direction and amount of drift in the training data and remove the drift component during operation. Adaptive algorithms, such as the self-organizing map [2], try to counteract the drift during runtime by adjusting the network to the incoming data.

We have previously suggested a modular neural network architecture as a model of cortical layer 4 [3]. Here we show how it can quite well handle the sensor drift problem in chemo-sensor data. It creates a distributed and redundant code suitable for a noisy environment and drifting sensors. A feature extraction layer governed by competitive learning allows for network adaptation during runtime. In addition, training data can be utilized to create a prediction of the underlying drift to further improve the network performance. Hence, we attempt to combine the two aforementioned methodological categories into one network model. The capabilities of the proposed network are demonstrated on surrogate data as well as real-world data collected from an electronic nose.

[1] T. Artursson, T. Eklöv, I. Lundström, P. Mårtensson, M. Sjöström and M. Holmberg, Drift correction for gas sensors using multivariate methods, Journal of chemometrics 14 (5-6), 711-723, 2000.

[2] M. Zuppa, C. Distante, P. Siciliano and K.C. Persaud, Drift counteraction with multiple self-organising maps for an electronic nose, Sensors and Actuators B: Chemical 98 (2-3) 305-317, 2004.

[3] A. Lansner, S. Benjaminsson and C. Johansson, From ANN to Biomimetic Information Processing, Studies in Computational Intelligence 188, 33-43, 2009

Taste preferences in Zebrafish juveniles

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The sense of taste helps acquiring and maintaining an appropriate feeding behavior, allowing to select good food and reject deleterious substances. For instance, sweet taste permits the identification of energy rich nutrients, and bitter warns against the intake of potentially poisonous chemicals. Taste impairment (dysgeusia) occurs in several diseases and it can give rise to weight loss through reduced appetite and altered patterns of food intake. On the other hand, obesity in human may be linked to exacerbated appetite for sweet substances, often acquired during childhood. As a consequence of the important role of taste in nutritional behaviour in healthy humans and patients, considerable research work has recently been focused on the mechanisms of development, function and regeneration of the gustatory system.

We have established a behavioural test that will allow us to determine on which principles zebrafish feeding behavior is based and particularly, what can restrict the quantity of food eaten in a given time. Using this assay, we have noticed that taste buds are already functional in 5day-old zebrafish when larvae begin to take food since bitter compounds like denatonium or cycloheximide prevent feeding in larva, consistent with the work of others. This behavioural assay combined with functional data, will help defining developmental aspects of the physiology of taste in vertebrates.

Characterization of ligands for human T1R3 taste receptor

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The sweet taste receptor is a heterodimer of two G-protein coupled receptors (GPCRs), composed of two subunits called T1R2 and T1R3. Each subunit is a member of the class C GPRC, possessing a characteristic large N-terminal domain (NTD) important for ligand binding. It has been shown that mouse NTDs of T1R2 and T1R3 are both able to bind natural sugars and sucralose with distinct affinities and conformational changes (Nie *et al.*, 2005). However the relative contribution of each NTD to the receptor activation remains largely unknown. The lack of knowledge is mainly due to the difficulties to obtain NTD in sufficient amount for biochemical and biophysical studies.

To achieve this goal, we have produced human T1R3 NTD using *Escherichia coli*. The protein was expressed in large quantities as insoluble aggregated misfolded protein, called inclusion bodies. Transferring T1R3 NTD into its native state by in vitro refolding requires screening to determine buffer conditions and suitable additives. For this purpose, we have established a factorial screen to detect folded functional T1R3 NTDs based on intrinsic tryptophan fluorescence quenching by sucralose. Fluorescence and circular dichroism spectroscopy demonstrated that T1R3 NTD is properly refolded and able to bind saccharide compounds with physiological relevant affinities. We developed a fluorescence test based on selective covalent attachment of thiol-reactive dye. Interestingly, we showed that T1R3 NTD is also able to bind numerous sweeteners, suggesting that T1R3 NTD plays an important role in sweetener recognition. The high quantities of NTD T1R3 produced are going to allow to characterize the interactions by microcalorimetry, nuclear magnetic resonance and to determine NTD structure using crystallography.

This work was supported by INRA and Burgundy council (Région Bourgogne) grants to E.M. & M.S.

Molecular and behavioral analysis of gustatory sugar sensitivity in Drosophila simulans wild populations

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Multiple gustatory receptors (GRs) are known to be involved in the sugar sensitivity to different subset of sugars in Drosophila. Among the 8 sugar GRs, Gr5a and Gr64a have been shown to be required for trehalose and sucrose sensitivity, respectively[1, 2, 3, 4, 5]. Other sugar GRs, however, have not yet been shown to contribute to the sensitivity to a specific sugar or a subset of sugars. Through behavioral analysis of threshold sugar sensitivity we found that different wild strains of D. simulans (D.s.), a sibling species of D. melanogaster (D.m.), often show different thresholds to a given sugar, suggesting that the threshold is genetically controlled. On the other hand, Ala218/Thr218 dimorphism of Gr5a found in D.m.[2, 6] were not found in D.s. and were fixed to Thr218. Correspondingly, all D.s. strains are insensitive as in D.m. flies carrying Thr218 allele. Interestingly, strains sensitive to one sugar are not necessarily sensitive to other sugars, suggesting that sugar sensitivities are independently controlled.

Among sugars we tested, threshold concentrations to fructose were the lowest but also divergent among in D.s. strains. Possible variations in the amino residue sequences of the 8 sugar GRs in D.s. wild strains were then analyzed. Combined behavioral and molecular results will be described and discussed here.

1. Dahanukar, A., Foster, K., van der Goes van Naters, W. M., and Carlson, J. R. (2001). Nat. Neurosci. 4, 1182-1186.

2. Ueno, K., Ohta, M., Morita, H., Mikuni, Y., Nakajima, S., Yamamoto, K., and Isono, K. (2001). Curr. Biol. 11, 1451-1455.

3. Slone, J., Daniels, J., and Amrein, H. (2007). Curr. Biol. 17, 1809-1816.

4. Dahanukar, A., Lei, Y. T., Kwon, J., Y., and Carlson, J. R. (2007). Neuron 56, 503-516.

5. Jiao, Y., Moon, S. J., and Montell, C. (2007). Proc. Natl. Acad. Sci. 104, 14110-14115.

6. Inomata, N., Goto, H., Itoh, M., and Isono, K. (2004). Genet. 167, 1749-1758.

Expression of Tas1R taste receptor subtypes in mammalian spermatozoa

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Spermatozoa undergo a complex sequence of precisely timed events during fertilization. One of these events is a process called acrosome reaction which represents a specialized form of Ca^{2+}-regulated exocytosis and which allows the sperm to penetrate the egg's glycoprotein matrix, the *zona pellucida* (ZP). Acrosomal secretion is initiated when mammalian sperm come in contact with distinct glycoproteins of the ZP coat. This interaction subsequently leads to the release of hydrolytic enzymes from the acrosomal vesicle at the sperm head thereby digesting the *zona pellucida* and thus allowing the sperm to fuse with the oocytés plasma membrane. Although it is known for a decade that oligosaccharide side chains of the *zona pellucida* glycoproteins play a key role in sperm-egg interaction as well as that the increase in Ca^{2+} elicited by the binding of sperm to the carbohydrate residues of the zona coat is triggered by the activation of a pertussis toxin sensitive PLC cascade, the responsible receptor/s on the sperm surface recognizing the egĝs envelope have not yet definitively been identified. Since members of the Tas1R family of taste receptors are well known to specifically detect a broad spectrum of sweet tastants with different chemical properties, ranging from classical carbohydrates to proteins and artificial sweeteners, we addressed the question whether sweet taste receptors might represent recognition molecules for the detection of the glycoprotein rich *zona pellucida* of the oocyte.

In RT-PCR studies with cDNA derived from murine testis we observed amplification products for Tas1R1 and Tas1R3, whereas primer pairs for Tas1R2 only led to an amplification when cDNA from circumvallate papillae was applied. To scrutinize the notion of a selective expression of the Tas1R1 *umami* receptor and the Tas1R3 sweet receptor, genetic analyses were combined with immunohistochemical experiments using coronal sections of mouse testis. Employing specific Tas1R3 antibodies, immunoreactivity was concentrated in differentiating spermatids covering the luminal surface of the seminiferous tubules, an expression profile comparable to the one registered for the Tas1R1 receptor. To verify if the identified taste receptors are also present in mature spermatozoa, immunocytochemical analyses were performed with isolated murine and human sperm. Remarkably, examining immunostaining in the two functional devices of sperm cells, it was found that labeling was not only detectable in the acrosomal region but was also visible in the flagellum. Since the strict compartmentalization of the highly polarized mammalian spermatozoa is supposed to reflect an adaptation to different tasks, the registered subcellular protein localization might indicate a functional role of the identified taste receptors in acrosome reaction. However, the receptors localized in the flagellum might as well be involved in controlling the chemically guided navigation of spermatozoa towards the egg in the oviduct, a biological phenomenon termed chemotaxis.

Mixture interactions in taste sensilla of *Drosophila melanogaster*

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The inhibition of sugar detection by antifeedants in the taste sensilla of insects is a phenomenon which has been known for more than 20 years. Dethier and Bowdan (1989; 1992) showed a decrease in the response to different sugars (glucose, fructose, sucrose) in presence of various alkaloids (quinine, berberine, caffeine) on *Phormia regina*, using the Proboscis Extension Reflex and electrophysiological recordings on the tarsi. However, the physiological mechanisms underlying this phenomenon are still unknown. Different hypotheses can be considered. Sugar inhibition could be mediated by receptors tuned to bitter molecules but located on the sugar cells and having an inhibitory activity. Or the bitter cell could have an inhibitory effect on the sugar cell through an unknown mechanism. We are trying to unravel part of the process involved in the interaction between sugars and antifeedants by studying this phenomenon on the proboscis taste sensilla of *Drosophila melanogaster*, using a combination of pharmacological and genetical approaches.

Dethier VG, Bowdan E. 1989. The effect of alkaloids on sugar receptors and the feeding behaviour of the blowfly. Physiol. Entomol. 14: 127-136.

Dethier VG, Bowdan E. 1992. Effects of alkaloids on feeding by phormia regina confirm the critical role of sensory inhibition. Physiol. Entomol. 17: 325-330.

Purification and characterization of recombinant gurmarin secreted by the yeast *Pichia pastoris*

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Gurmarin is a polypeptide isolated from the leaves of *Gymnema sylvestre*, an Indian-originated tree. This peptide consists of 35 amino-acid residues with an amino-terminal pyroglutamyl residue. Gurmarin is known to selectively inhibit responses to sweet substances without affecting responses to other basic taste stimuli, such as NaCl, HCl, and quinine in rodents. Recent studies have proposed that gurmarin may interact with T1R2/T1R3 sweet taste receptor.

To further understand the structural basis of gurmarin recognition by this receptor, a large amount of purified gurmarin is suitable for biochemical and structural studies. Here we describe the heterologous expression of gurmarin using the methylotrophic yeast *Pichia pastoris* under the control of the methanol-inducible alcohol oxidase (AOX1) promoter. Gurmarin was secreted into the extracellular medium using the alpha-factor preprosequence peptide of *Saccharomyces cerevisiae* without the Glu-Ala-Glu-Ala spacer.

We found that gurmarin regularly accumulated in the culture medium reaching approximately 15 mg per liter of culture over an expression period of 4 days. After dialysis, the yeast culture filtrate containing recombinant gurmarin was purified using anion-exchange chromatography. MALDI-ToF mass spectrometry of purified peptide revealed the presence of 2 isoforms. The first one corresponded to natural gurmarin with an amino-terminal pyroglutamyl residue, while the second isoform was assigned to the same molecule with a N-terminal glutamine residue. Purified gurmarin was characterized using circular dichroism and 1D NMR spectroscopy revealing that it was properly folded and has secondary and tertiary structures. The efficient production of recombinant gurmarin in *Pichia pastoris* should allow mutational analysis and labelling of sweetness-suppressing peptide with stable isotopes for NMR investigations. These approaches will be used to study interactions between gurmarin and rodent sweet taste receptor.

This work was supported by INRA and Burgundy council (Région Bourgogne) grant to M.S.

A screening for genes encoding G protein-coupled receptors expressed in taste papillae

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G protein-coupled receptors with seven transmembrane domains (GPCRs) play an important role in taste detection and physiology. In taste buds, GPCRs located on the apical microvilli of taste receptor cells (TRCs) act as taste receptors dedicated to sensing sapid compounds. GPCRs found on the basolateral membrane of TRCs and surrounding cells are thought to be crucial for cell-cell communication and the peripheral processing of the taste information.

To identify new GPCRs important for taste bud cell function we conducted a selective screening for genes coding for GPCR expressed in tongue papillae. Eleven genes coding for GPCRs belonging to three broad categories were selected as targets to design pairs of specific primers which were used for RT-PCR on a panel of mouse tissues. Amongst the selected targets 5 were class C orphan GPCRs, 4 were fatty acid receptors and 2 were proton sensing GPCRs. RT-PCR results reveal that one gene in each category displays an expression pattern somewhat restricted to taste papillae. Amongst those genes the one coding for Gpr120, a receptor activated by long chain fatty acids and the anticancer agent grifolin, showed a pattern similar to Gpr113, a receptor exclusively found in taste papillae. In situ hybridization experiments confirmed that the mRNA for Gpr120 is confined to taste buds and immunohistochemistry on circumvallate sections indicated that a portion of Gpr120 is present in TRCs. These data expand the repertoire of GPCRs found in taste papillae and suggest that additional GPCRs might be involved in peripheral gustatory processes.

This work was supported by funds from the General Olfaction and Sensing Program on a European Level, the Deutsche ForschungsGemeinschaft and ANR-08-ALIA-20.

cAMP may regulate bitter taste transduction in bullfrog

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The transduction pathway for bitter taste has been proposed that bitter substances activate phospholipase C beta2 (PLC beta2), rising intracellular inositol 1,4,5-triphosphate (IP_3) levels to release Ca^{2+} from intracellular Ca^{2+} stores. On the other hand, it has been pointed out that bitter substances also activate phosphodiesterase (PDE) to decrease the concentration of cAMP in mice taste bud cells. In this study, we verified whether both IP_3 and cAMP contribute to the bitter taste transduction in the bullfrog taste system by measuring effects of PLC inhibitors, adenylate cyclase (AC) inhibitors and AC activators on bitter taste responses. The neural activities to bitter substances were recorded as summated responses. Inhibitors and activators were infiltrated into taste receptor cells by the application them to the tongue surface with gauze for 1 hour. The magnitude of taste nerve response to denatonium was greatly suppressed by 10 microM U-73122, PLC inhibitor, whereas it had no effects on NaCl and $CaCl_2$ responses. The half maximal inhibitory concentration of U-73122 in response to denatonium was 20 microM. 10 microM thapsigargin, Ca^{2+}-pump inhibitor, also suppressed the denatonium response by a factor of 0.3. These results suggest that bitter substances elevate intracellular IP_3 levels to release Ca^{2+} from intracellular Ca^{2+} stores. The magnitude of taste nerve responses to 0.1 mM quinine were increased by factors of 1.7-2.2 after treatments with AC inhibitors, such as 10 microM SQ22536, 10 microM MDL-12,330A and 30 microM 2',5'-Dideoxyadenosine. These inhibitors also increased taste responses to 0.1 mM denatonium, 0.1 mM strychnine and 10 mM caffeine. In

contrast, 10 microM forskolin, AC activator, suppressed the bitter taste responses by factors of 0.6-0.7.

These results suggest that bitter substances cause not only rise of IP_3 level, but also decrease of cAMP level in bullfrog taste receptor cells. cAMP may play some important role to regulate Ca^{2+} signaling in bullfrog as well as in mice. We will discuss about bitter taste transduction in the bullfrog taste system.

References: Yan W., Sunavala G., Rosenzweig S., Dasso M., Brand JG., and Spielman AI., Bitter taste transduced by PLC-beta(2)-dependent rise in IP(3) and alpha-gustducin-dependent fall in cyclic nucleotides. Am J Physiol Cell Physiol 208, C742-C751(2001). Trubey KR, Culpepper S, Maruyama Y, Kinnamon SC, and Chaudhari N, Tastants evoke cAMP signal in taste buds that is independent of calcium signaling., Am J Physiol Cell Physiol 291(2), C237-44 (2006).

Supported by the Open Research Center Project of Saitama Institute of Technology.

Pharmacology and expression of a dye-permeable voltage-gated channel on mouse taste bud cells

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We show here the expression, permeability, and pharmacology of a voltage-gated channel in certain taste bud cells (TBCs) which is known to be permeable to Lucifer Yellow CH (LY), and known to release ATP as a neurotransmitter in response to taste substances. LY labels TBCs immunoreactive to PLCβ2, a phospholipase subtype, but not the TBC subtype immunoreactive to SNAP-25, a SNARE protein. Besides these subtypes, the majority of TBCs per taste bud are non-immunoreactive; however a few of these subtypes are also labelled by LY. Monovalent- and divalent-anion probes with molar mass less than 1200 also label PLCβ2-immunoreactive TBCs and a few non-immunoreactive TBCs, whereas cation probes do not. The number of LY-labelled TBCs per taste bud is decreased by 5 μM DIDS (4,4'-diisothiocyanostilbene-2-2'disulfonate), 1 mM octanol and 10^{-5} M H^+, but not by 10 μM carbenoxolone or 30 μM flufenamic acid. PLCβ2-immunoreactive TBCs and a few non-immunoreactive TBCs generate a TEA-insensitive outwardly rectifying current. DIDS decreases this current in magnitude with IC_{50} of ~0.4 μM in a voltage-independent manner. H^+ also decreases the current magnitude but carbenoxolone does not.

These results show that the LY-permeable channel occurs not only on PLCβ2-immunoreactive TBCs and but also on certain non-immunoreactive TBCs, that the channel generates the TEA-insensitive outwardly rectifying anion current, and that the pharmacology of the channel is different from hemichannels reported. The discussion focuses on the pharmacology and the role of the LY-permeable channel.

Palatability of long-chain fatty acids assessing from the licking behavior of BALB/c mice

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Dietary oils primarily contain triacylglycerol and small quantities of fatty acids, are highly palatable to animals as well as the human. We have examined the short-term (60 s) licking behavior of mice and observed that they exhibited a high licking response to a low concentration of fatty acid, which is comparable to that observed for pure corn oil. This finding suggests that fatty acids contribute to the palatability of dietary oils. We assessed the licking behavior of BALB/c mice to figure out the palatability of various types of long-chain fatty acids. The mice showed high licking response to 1% unsaturated 16- and 18-carbon fatty acids (palmitoic acid, oleic acid, linoleic acid and linolenic acid), low licking response to 16- and 20 –carbon fatty acids (palmitic acid, and arachidonic acid) , and no significant response to saturated fatty acids (stearic acid and arachidic acid) or fatty acid derivatives (methyl linoleate and linole alchol). Additionally, there were differences in the palatability of 18-carbon unsaturated fatty acids at very low concentrations. At fatty acid concentrations of 0.04% and 0.0625%, the mice showed significant preference for linoleic acid and linolenic acid, but not oleic acid, when compared with mineral oil. These results suggest that mice show high licking responses to 16- and 18-carbon unsaturated long-chain fatty acid, carbon chain length, and terminal carboxyl group.

This study was supported in part by the program for the Promotion of Basic Research Activities for Innovative Bioscience, and by Grant-in Aid for Scientific Research from the Japan Society for Promotion of Science.

Identification of agonist interaction sites in the human bitter taste receptor hTAS2R10 through in silico model-guided mutagenesis

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Unlike many other GPCRs the ~25 functional human bitter taste receptors hTAS2Rs) are broadly tuned to detect several chemically diverse compounds. Yet, every single hTAS2R exhibits a unique agonist activation profile indicating that the broad tuning is not simply achieved at the expense of selectivity. This special feature of hTAS2Rs must ultimately be a function of the architecture of agonist binding pockets.

The receptor hTAS2R10 responds to structurally different bitter compounds such as the alkaloid strychnine, the sesquiterpene lactone parthenolide, and denatonium benzoate, a synthetic bitter compound. In order to identify the amino acid residues of the receptor critically involved in selective agonist interaction, we established a 3D-homology-model of the hTAS2R10. When analyzing this in silico 3D model we observed a putative binding pocket located between the upper parts of TM2, 3, 5, 6, and 7. We identified position N92, which is highly conserved in hTAS2Rs and was already shown to influence activation in a number of other TAS2Rs, at the central bottom of the putative binding pocket. Based on the size of hTAS2R10 agonists, we

subjected residues within a distance of 6å from this position to alanine scanning mutagenesis.

Most of the 23 mutated residues affected agonist interaction in subsequent functional calcium imaging analyses performed with strychnine, parthenolide, and denationum benzoate. For two residues we found no significant changes in responses and for seven positions a loss-of-function. Six exchanges resulted in decreased responses for all tested agonists. Additional six positions showed an agonistselective decreased response. One residue increased responsiveness to all agonists tested, and, most interestingly, one amino acid showed an agonist-specific increased sensitivity after mutation.

This position, S85 in TM3, showed an agonist-specific increased response amplitude and decreased EC50 values to parthenolide and parthenolide analogs, whereas the response to strychnine and denatonium benzoate was decreased. We generated an additional specific exchange from serine to threonine and tested several available parthenolide derivatives and –analogs to identify important structural features presented by this group of agonists. We conclude that mutations of most of the amino acid positions predicted to affect agonist interaction by the in silico 3D model indeed changed functional receptor responses confirming the feasibility of our approach.

Comparison of these data with the previously analyzed hTAS2R46 confirmed that ligand binding pockets in TAS2Rs are positionally conserved consisting of residues predominantly located in TM2, 3, 5, 6, and 7, but suggest that the relative importance of residues can differ dependent on the receptor and agonist structure.

Iterative *in-silico* and *in-vitro* tools for exploration of bitterness

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Bitter-taste perception in humans is mediated by 25 GPCRs of the hTAS2R gene family. How can merely 25 receptors detect thousands of structurally diverse bitter compounds, and why are some of the receptors broadly-tuned, while others are capable of binding only a small number of ligands? The structural basis for the bitter taste receptors unique ability to specifically allocate numerous chemically diverse agonists is the focus of the current study.

To elucidate the sites of interaction between the bitter taste receptors and its different agonists, we generate an all-atom 3D model of the receptor. Comparative sequence conservation analysis for each of the binding site positions in the hTAS2R family is then performed to determine which residues may contribute to ligand binding and specificity. Computational docking of this ligand to the receptor is used to evaluate feasibility of the predicted specific interactions and is followed by *in-vitro* assays to confirm the proposed binding mode. This approach was applied to the broadly-tuned hTAS2R14 bitter taste receptor. Functional assays on wild-type vs. mutant constructs confirmed the *in-silico* predicted interactions and corroborated the main predicted binding site, situated inside the trans-membrane bundle. This site is analogous to previously identified binding pockets of other bitter taste receptors, such as the PTC bitter taste receptor (hTAS2R38) which was determined by means of homology modeling and hTAS2R46 which was elucidated by a combination of experimental and computational means, and also to non-bitter GPCRs for which structures were determined experimentally (Rhodopsin, β1 and β2-adrenergic receptors and A2A-adenosine receptor).

The information on bitter compounds and receptors from the literature and from on-going experiments is organized in our database BitterDB. Since there is no free public database which specializes in taste compounds, the aim of BitterDB is to (1) gather all available public data on bitter molecules and their corresponding receptors; (2) characterize bitter molecules and computationally predict additional bitter candidates; (3) predict receptor functional groups involved in ligand binding. Currently BitterDB holds over 300 chemical structures of bitter tastants, and is constantly growing.

The iterative scheme for elucidation of molecular recognition of the bitter compounds by their cognate receptors represents first steps towards *in-silico* bitterness prediction.

Vesicular glutamate transporter 1 positive nerve fibers in rat fungiform papillae

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Glutamate has been proposed to play a role in neurotransmission in the peripheral taste system. Evidence supporting a transmitter role for glutamate in the lingual gustatory papillae includes the expression of vesicular glutamate transporter 1 (VGlut 1), glutamate receptors and glial glutamate-aspartate transporters (GLAST). However little is known about the origin of glutamate and its signaling pathways within rat taste buds.

In the present study, we used a double labeling strategy combining VGlut1 immunoreactivity and (i) neuronal marker PGP9.5, peptidergic marker CGRP and non peptidergic marker IB4, (ii) chorda tympani (CT) and lingual nerve (LN) neuronal tracings to investigate the origin and type of nerve fibres containing glutamate, and (iii) synaptic marker synaptobrevin 2.

We observed (i) that VGlut 1 co-localizes with PGP 9.5 but is absent in CGRP positive nerve fibres and in IB4 positive nerve fibres, (ii) that VGlut1 is present in some CT and LN fibres, and (iii) the presence of some spots of co-localization for VGlut 1 and synaptobrevin 2 within fungiform papillae.

The present findings demonstrate the presence of some CT and LN glutamatergic fibres and suggest that these nerve fibres could be myelinated fibres. Our study suggests moreover that glutamate might be involved in efferent signaling pathways within rat fungiform papillae.

Tingle sensation by a sanshool derivative and its effects on primary sensory neurons

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Szechuan peppers are a preferred spice in some cuisines because of their tingling-numbing and cooling sensations. The tingle agent OH-α-sanshool activates a subset of sensory DRG neurons by inhibiting 2-pore potassium channels. We presently investigated the ability of a sanshool analog, isobutylalkenyl amide (IBA), to elicit tingle sensation in humans and to excite primary sensory neurons from rats. Using calcium imaging, IBA excited ~40% of cultured rat dorsal root ganglion (DRG) neurons of different sizes. Of IBA-sensitive cells, 30% also responded to menthol and/or cinnamaldehyde (CA) and 66% to capsaicin (CAP), with many responding to multiple Transient Receptor Potential (TRP) channel agonists. There was significant self-desensitization to repeated application of IBA. CAP did not cross-desensitize responses to IBA in CAP-insensitive DRG cells. The TRP channel blocker Ruthenium Red did not affect IBA activation of DRG cells but inhibited their activation by menthol, CA and CAP. In human studies a modified time-intensity procedure was used to assess if lingual IBA (0.5%) evokes temporally distinct tingling, pungent, cold and/or numbing sensations. IBA elicited a sensation initially described as tingling and pungent, but after approximately 15 min, as evoking a cooling sensation with a smaller numbing component. Similarly, using a half-tongue, 2-AFC methodology, pretreatment with CAP (10 ppm) or mustard oil (MO; 0.125%) did not cross-desensitize the tingle sensation evoked by subsequent IBA application. The cellular responses elicited by IBA are remarkably similar to the sensations observed in human psychophysical trials. The ability of IBA to excite menthol- and CAP-sensitive DRG cells is consistent with sensory qualities (tingle, pungency, cool) elicited by IBA. The lack of cross-desensitization by CAP or MO suggests that separate populations of IBA-sensitive cells are likely involved in conveying sensations of pungency and tingle.

ACKNOWLEDGMENT: supported by NIH grants DE013685 and AR057194.

The role of TRPV1-dependent chemosensation in alcohol perception and consumption by mice

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Ethanol activates three different chemosensory systems (or modalities): gustatory (taste), olfactory (odors), and trigeminal (chemosensory irritation causing burning sensation). The trigeminal system mediates defensive reactions to potentially harmful substances. Therefore, activation of the trigeminal system by ethanol could evoke aversive responses and restrict alcohol consumption. *In vitro* studies have shown that ethanol activates TRPV1, a mechano-, thermo- and chemosensitive receptor that belongs to the TRP-family vanilloid receptors (Trevisani et al., Nat. Neurosci, 2002, 5:546). The aim of the present study was to evaluate the role of TRPV1 in ethanol perception and avoidance in mice using genetic and pharmacologic approaches.

The study was performed on the 4-6 month old mice of both sexes. We used mice from two inbred strains (alcohol preferring C57BL/6ByJ and alcohol avoiding 129P3/J), and B6.129X1-*Trpv1*^{tm1Jul}/J mice with a targeted mutation of the *Trpv1* gene. Taste responses to ethanol (1.25-20%), sucrose (1-32%), quinine (0.01-1 mM) and capsaicin (1-30 μM) were assessed in the brief-access licking tests using the Davis MS-160 gustometer (DiLog Instruments, Tallahassee, FL, USA) in naïve animals and in animals with LiCl-induced (0.23 g/kg B.W. i.p.) conditioned taste aversion (CTA) to 10% ethanol The brief-access licking test demonstrated no significant differences in responses to ethanol, quinine and sucrose between homozygous knockout (*Trpv1* -/-) and wild-type (*Trpv1* +/+ C57BL/6ByJ) mice. *Trpv1* -/- and *Trpv1* +/+ mice did not differ in generalization of CTA from ethanol to quinine and sucrose. We also assessed generalization of CTA from 10% ethanol to 1-30 μM capsaicin and found that 129P3/J mice perceive similarity between ethanol flavor and capsaicin.

In the pharmacologic experiment, administration of a TRPV1 antagonist, capsazepine (10 mg/kg B.W. i.p.), did not affect licking responses to 1.25-20% ethanol by C57BL/6ByJ and 129P3/J mice. In summary, these data show that mice perceive capsaicin-like sensation from ethanol, but TRPV1-mediated trigeminal chemoreception has only limited contribution to ethanol avoidance by in mice. This suggests that in mice taste and/or olfaction are predominant factors that determine chemosensory responses to ethanol.

Supported by NIH grants R03TW007429, R01AA11028R01 and DC00882

The role of olfaction in perception and consumption of ethanol by mice

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Alcohol (ethanol) evokes sensations of sweet and bitter taste, smell, and somatosensory irritation. The aim of this study was to elucidate whether ethanol odor contributes to ethanol aversion and influences perception of ethanol flavor, and its sweet and bitter taste components. The study was performed with 129P3/J inbred mice, which avoid higher concentrations of ethanol. Two models of experimental anosmia were used: chemical using intranasal application of 5% $ZnSO_4$, and surgical using olfactory bulbectomy. Licking responses to solutions of ethanol (1.25-20%), sucrose (1-32%) and quinine (0.01-1 mM) were evaluated in the brief access tests using Davis MS-160 gustometer (DiLog Instruments, Tallahassee, FL) in naïve animals and after development of LiCl-induced (0.23 g/kg, i.p.) conditioned taste aversion (CTA) to 10% ethanol. In both models, experimental anosmia abolished avoidance of all tested concentrations of ethanol and also reduced avoidance of high concentrations of quinine (0.3-1 mM), but did not affect licking to sucrose as compared to control mice. However, anosmic mice with CTA to 10% ethanol avoided 10-20% ethanol solutions.

This suggests that mice detect non-olfactory (most likely taste) components of 10-20% ethanol flavor, but unconditioned avoidance of these solutions is primarily evoked by ethanol odor. Control mice did not generalize CTA from ethanol to sucrose, but anosmic mice generalized CTA from ethanol to 4-16% sucrose. Both control and anosmic mice avoided solutions of quinine and did not generalize CTA from ethanol to quinine (probably due to the floor effect).

These data demonstrate that unconditioned avoidance of ethanol solutions in 129P3/J mice depends mainly on olfactory perception and that smell of ethanol overshadows taste perception of its sweet component. Our data also suggest that olfaction can contribute to perception of high concentrations of quinine. Animals with induced anosmia are a good model to study taste quality perception of volatile compounds.

Supported by grant R03TW007429 from the Fogarty International Center, NIH.

Just noticeable difference in olfaction is related to trigeminal component of odorants

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Many studies have dealt with the interrelationships between both olfactory and trigeminal systems but a poorly explored question concerns the role of each system in the detection processes, especially in the just noticeable difference (JND). The aim of this study was to investigate variations in JNDs for three odorants in relation to their trigeminal component, i.e. low, middle, high. The results indicated that the higher the trigeminal component, the lower the JND, suggesting a better capacity to perceive intensity changes for pungent odorants than for relatively pure odorants.

Major urinary proteins (MUPs) as a promising tool for analysis of the intermale aggression in mice

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Aggression is one of the major types of social communication between animals: territorial fights, mating, establishment and maintenance of hierarchical ranks are mainly based upon aggressive behavior. It takes place between at least two animals and is realized through a complex exchange of information via several sensory modalities. For mice, like for many other species, the leading sensory modality is olfaction, and much of the information is conveyed by highly specific ligands, pheromones, which have the ability to initiate innate (genetically programmed) behavioral responses. Importance of pheromonal polymorphism in mediating aggression between male mice was established already 35 years ago [1], but genetic bases of these differences are still largely unknown.

Major urinary proteins (MUPs) of the house mouse form a large group of highly polymorphic acidic isoforms with molecular masses of 18-20 kDa. MUPs transport small hydrophobic ligands and various MUP fractions have different affinity to several volatile pheromones [2]. These proteins are encoded by the *Mup* gene cluster, which is mapped to chromosome 4 [3]. Currently, MUPs are believed to be a key component in formation of the individual olfactory signature in *Mus musculus* L. [2, 4].

In 2003 we put forward an idea that genes *Mup, Gus, and Mep1a* form an «aggressive behavior gene network» [5]. *Gus* gene is located on chromosome 5 and encodes beta-glucuronidase (GUS, EC 3.2.1.31), while *Mep1a* is mapped to chromosome 17 and encodes meprin (EC 3.4.24.18), which probably participates in MUPs' degradation process [5].

In the current study we examined this idea in details. Quantitative analysis of plasma testosterone level, MUPs content and GUS activity in voided urine, kidney, preputial and salivary glands in male mice of different genotypes revealed a strong correlation between plasma testosterone level, GUS activity, differential pattern of *Mup* gene expression, and aggressive manifestation. We suggest that ratios and concentrations of MUP fractions of the given combination define behavioral response of the recipient mouse: some MUPs combinations promote aggression, others initiate mating behavior. Taking into account recent developments in the field [6], our model can effectively outline complex chemosensory pathways from specific genes to olfactory mediated physiological responses via sensory neurons of vomeronasal organ. We therefore postulate that MUPs can be used as a fine pheromone tool to dissect aggressive behavior phenotypes.

Supported by Russian Foundation for Basic Research (projects 02-04-49273, 04-04-63050, 07-04-01762).

[1] Kessler S. et al., 1975. Behav. Genet. 5:233-238. [2] Cheetham S.A. et al., 2009. Physiol. Behav. 96:253-261. [3] Bennett K.L. et al., 1982. Proc. Natl. Acad. Sci. USA. 79:1220-1224. [4] Novikov S.N. et al., 2009. Russian J. Dev. Biol. 39:204-211.

[5] Novikov S.N., 2003. Dokl. Biol. Sci. 391:318-321. [6] Chamero P. et al., 2007. Nature. 450:899-902.

Females of two subspecies of mice differ in their urinary volatile profile

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The segregation of two or more subspecies of mice sharing the same area is based on the specificity and efficacy of the mate recognition system. In the last years, the sexual mate preference and the recognition system that allow a correct mate choice between two subspecies of the European house mouse was investigated with behavioural, chemical and genetic studies. The data supported an involvement of urine chemical composition in mate recognition and choice, resulting in subsequent mating segregation. These studies put a particular attention to the differences in odorant pattern of the male mouse subspecies: males of two subspecies and their first-generation hybrids were shown to differ in their urinary volatile output (Mucignat-Caretta et al. 2010, Chemical Senses, in press). On the other hand, few data are available on the possible differences between females' urine chemical cues.

The aim of this study is to describe the possible chemical variations in the urine volatile profile of female house mice of two different subspecies: Mus musculus musculus and Mus musculus domesticus, in order to support a potential relation of female urine chemical composition with correct mate recognition and choice.

Urine was collected from different groups of adult female mice (Mus musculus domesticus or M. m. musculus, either living in contact zones or originating from segregated areas). Mice were originally caught from different populations in the wild and maintained in the laboratory. Urine volatile chemicals profile was analyzed by sampling the headspace with SPME coupled with GC/MS (full details in: Mucignat-Caretta et al. 2010).

Female mice showed a lower content of volatile urinary molecules compared to males. Moreover quantitative and qualitative variations in the urinary molecules were detected between the two subspecies.

These differences may play a role in the specificity of the odorant pattern that may be involved in recognition and mating segregation.

Pheromone receptors in the noctuid moth *Spodoptera littoralis*

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Insect pheromone communication has been for long a valuable model system for studying fundamental aspects of olfaction since the detection of female released sex pheromone by male moth antennae is remarkably selective and sensitive. However, although sex pheromones have been identified in several thousands of insect species, due to their potential as sex attractant in crop protection strategies, only few insect pheromone receptors (PRs) have been identified and functionally characterized in insects.

Here, we report the identification of the first putative pheromone receptors from the crop pest moth *Spodoptera littoralis.* Through the establishment and bioinformatic analysis of an expressed-sequenced tag library prepared from male antennae, we described 29 sequences encoding candidate olfactory receptors (ORs) in this species. In a phylogenetic analysis, four of the 29 *S. littoralis* ORs fell into the putative lepidopteran pheromone receptor clade and were thus annotated as candidate PRs.

Using qPCR, we showed that expression of the four genes encoding candidate PRs is strictly restricted to antennae. Two are male-specific, consistent with a function in sex pheromone reception, one is largely enriched in male antennae and one appeared to be expressed almost equally in both sexes, consistent with the ability of *S. littoralis* females to perceive their own pheromone. Their developmental expression pattern was reminiscent of that of other pheromone-responsive genes and *in situ* hybridization experiments within the antennae revealed that the receptor-expressing cells were closely associated with the olfactory structures, including pheromone-sensitive ones.

Taken together, our data support the view that we identified new candidate receptors for pheromone components. Functional studies are currently underway to determine the ligand(s) that bind(s) these candidate PRs. The identification of ligand-PR couples will be a keystone for developing further studies on the contribution of PRs to both the selectivity and the sensitivity of the pheromonal system.

Molecular elements of pheromone reception in moths

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Many female moths 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. We have identified candidate pheromone receptors of *Bombyx mori*, *Heliothis virescens* and two *Antheraea* species, which form a relatively conserved group within the olfactory receptor family and are expressed in sensory neurons housed in pheromone-responsive sensilla. Receptor expressing cells were found to be surrounded by cells expressing and secreting pheromone binding proteins (PBPs), which are supposed to mediate the transfer of hydrophobic pheromones through the aqueous sensillum lymph towards the receptors in the dendritic 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 moths pheromone detection system. In addition to receptors and PBPs, a possible role of ''sensory neuron membrane proteins'' (SNMPs) 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. While SNMP2s were expressed in cells co-expressing PBP, apparently the supporting cells, SNMP1s were expressed in only one of the generally two neurons in a single sensillum hair. Our results indicate that SNMP1s may interplay with definite receptors and PBPs in the detection of components of the female sex-pheromone blend.

This work was supported by the Deutsche Forschungsgemeinschaft.

Use trail pheromones in the ant *Camponotus aethiops*: discrimination and generalisation among societies

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Animal societies rely on efficient foraging to optimise their food supply. Many ant species evolved trail pheromone marking to locate food sources and communicate the location to their nestmates. We investigated whether *Camponotus aethiops* ants use trail pheromones and whether these pheromones vary among societies. Freely walking ants were put on a Y-maze differentially marked: one branch previously walked by foragers (either nestmates or non-nestmates) vs. a clean branch; one branch previously walked by nestmate foragers vs. a branch previously walked by non-nestmate foragers. Ants preferred previously used branches (either by nestmates or non-nesmates) to the clean branch, proving *C. aethiops*

using trail pheromones, which were generalised among societies. However, ants preferred that branch marked by nestmates to that by non-nestmates, showing that trail pheromones from different societies could be discriminated. Future work will reveal the actual composition of trail pheromones and how ants may avoid eavesdropping by non-nestmates. Moreover, physiological studies will give light on how these socially active odours are encoded by the ant nervous system.

The smell of disease: Human body odor changes in response to systemic inflammation

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Ability to detect diseases in conspecifics would be advantageous for the individual. In line with this, rodents avoid body odors of infected individuals. To learn whether this is possible by way of human smell, we tested whether body odor would reveal an acute inflammatory response to endotoxin. Eight individuals donated odorous body substances two times by wearing cotton T-shirts following an injection of endotoxin (0.8 ng lipopolysaccharide / kg body weight) at one time and placebo (Saline) at the other. In each case, T-shirts were collected from the donors after 4 hours. The armpit regions of the shirt were cut out, put in plastic bottles, frozen to -35C, and were thawed one hour before presentation. Forty naive panelists smelled all 16 T-shirts from the plastic bottles twice and judged their pleasantness, intensity, and healthiness. Preliminary results were that endotoxin-exposed individuals smelled less pleasant [$F(1,39) = 37.67$, $p < .000$, $\eta_p^2 = .49$], more intense [$F(1,39) = 32.62$, $p < .000$, $\eta_p^2 = .46$], and less healthy [$F(1,39) = 4.22$, $p = .047$, $\eta_p^2 = .10$]. The effect size of the perceived difference between body odors of endotoxin- and placebo-exposed participants was largest for pleasantness and smallest for health. These data support the notion that humans can smell the disease of others. The nature of this perception is discussed and is subject to a follow-up study.

Acknowledgements: This study was supported by grants from the Swedish Research Council (2009) (to MJO), National Institutes of Health – NIDCD (R03DC009869) (to JNL), and Osher Center for Integrative Medicine (to JA).

Postnatal exposure to predator odor induces modification in fear-related behaviors during adulthood without change in corticosterone levels

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Environmental stimuli and adverse experiences in early life may result in behavioral and physiological changes in adulthood. In several animal species, the odors cues are crucial in the setting of adaptive behaviors, especially towards predators. However, little is known about the effects of postnatal exposure to predator odor on the later physiological and behavioral responses to this natural stressor. Thus, the aim of this study was to investigate the effects of a postnatal exposure to synthethic predator odor (TMT) in mice pups on later adult fear-related behaviors and corticosterone levels in response to this specific stimulus. Compared to pups only postnatally exposed to water, results showed that behavioral responses were significantly different in the group of mice exposed to TMT, i.e. a decrease of fear-related behaviors while no differences occurred in the corticosterone levels between both groups.

Expression of genes controlling adult neurogenesis of dopaminergic neurones in the olfactory bulb: a synoptic view

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During neurogenesis nerve cells are generated according to a precise spatio-temporal schedule, so that cohorts of newly-generated neurons settle in defined locations and establish appropriate connections with specific partners. The specification of neural progenitors is subject to complex regulatory mechanisms at precise ontogenetic stages, needed to determine the neuronal types, the required quantities, and the specific location of their implant. In spite of our expanding knowledge of these phenomena, we miss an ensemble view of the process.

To better understand the interplay of the different factors controlling this process we have studied the variation in the expression of more than 40 genes involved in the neuronal differentiation of dopaminergic (DA) neurones during adult neurogenesis in the olfactory bulb at three different stages of maturation. To this end we have exploited a peculiar property of DA neurones in a transgenic line of animals expressing eGFP under the TH promoter, which is that the intensity of the fluorescence reflects their degree of maturation. Using the fluorescence-activated cell sorting after enzymatic dissociation of the olfactory bulb we have separated the fluorescent cells as a function of their level of fluorescence in three groups, corresponding to immature, intermediate and fully mature DA neurones, and performed semiquantitative RT-PCR. The results provide, for the first time, a synoptic view of how a large number of different factors controlling the dopaminergic differentiation vary their expression during the maturation of DA neurones.

We have divided these factors in three groups: 1) Key regulators of the dopaminergic phenotype, 2) Phenotype-independent regulators, and 3) Regulators of OB differentiation through olfactory neuron innervations of the olfactory bulb. The first group includes AP-1, CREB, Dlx1 and 2, Engrailed-1, ER-81, Gsh-2, basic helix-loop-helix transcription factors (Mash1, Id2, and Hes1), Meis-2, Nurr-1, Pax-6, Zic 1 and 3. The second group includes DCX, Ephrin A2, Myst-4Notch-1, Olig-2, Prok-2, PSA- NCAM, genes involved in the reelin cascade (Reelin, APOEr2, VLDLR and Dab1), Shh, Slit 1 and2, Tenascin r, Vax-1. The third group includes ARX, CNG2,Dlx5, FezF1, Big2-Contactin4. For all these genes we provide a description of the variation of expression in three different phases of maturation, description that becomes particularly instructive when the variations are looked synoptically. We believe that this will contribute to a better understanding of the steps required to make dopaminergic neurons in the adult olfactory bulb.

Acknowledgement: this work has been supported by PRIN 2007 and by Programma Ricerca Regione ER-Università 2007/2009, DDG 40/08 and 226/09.

Local modulation of excitatory/inhibitory balance influences oscillatory neural activity in the accessory olfactory bulb of anaesthetised mice

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In mice, mate recognition is proposed to involve a learning-induced increase in feedback inhibition, which is dependent on noradrenaline, and selectively gates the transmission of the learned signal at the level of the accessory olfactory bulb (AOB) [1]. Coupling between excitatory projection neurons and inhibitory interneurons, at reciprocal synapses, generates oscillatory neural activity evident in the local field potential (LFP) recorded in the AOB. In the present study, we have investigated how manipulating the balance of excitatory and inhibitory neurotransmission influences the oscillatory dynamics of AOB activity, with the potential to affect functional coupling with other brain areas[2].

Mice were anaesthetised by intraperitoneal injection of urethane and a bipolar recording electrode with an integral drug delivery cannula was inserted in the mitral/tufted cell layer of the AOB. LFP total power and spectral centroid (weighted mean frequency) in different frequency bands were analysed pre-injection and following a 1ml infusion of either drug or artificial cerebrospinal fluid (CSF) at intervals up to 1 hour post-injection. Infusions of 100pmol of the type II metabotropic glutamatergic receptor agonist DCG IV, 1nmol of the $GABA_A$ receptor agonist isoguvacine and 1 nmol of noradrenaline each led to significant and lasting decreases of around 50% in total power in frequency bands between 4 and 90Hz, compared to CSF. Infusions of DCG-IV and isoguvacine resulted in small, but significant, reductions in spectral centroid in the 12-30Hz frequency band compared to CSF. In contrast, despite having a similar effect on oscillatory power, noradrenaline infusion resulted in a slowly developing increase spectral centroid in the 30-60Hz band compared to pre-injection level.

The results suggest that both power and frequency of oscillatory neural activity depend on the balance between excitatory and inhibitory neurotransmission in the AOB. However, it is doubtful whether such small effects on frequency of network oscillation would be sufficient to decouple neural oscillators in the AOB from neural oscillators in central brain areas, as has previously been hypothesised as a mechanism for memory recall[2].

1. Brennan, PA. (2009) Outstanding issues surrounding vomeronasal mechanisms of pregnancy block and individual recognition in mice. Behavioural Brain Research, 200: 287-249.

2. Taylor JG, Keverne EB. (1991) Accessory olfactory learning. Biological Cybernetics, 64: 301-306.

Acknowledgement: This work was supported by BBSRC grant BB/E020283/1.

Mechanisms underlying olfactory perceptual learning in adult and aging mice: implication of neurogenesis and noradrenaline

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A number of experiments have shown that odor exposure alone, unaccompanied by behavioral training, changes the response patterns of mitral cells in the olfactory bulb. Bulbar granule cells which shape the activity of mitral cells are continuously incorporated in the adult main olfactory bulb under the control of noradrenergic system.

We previously showed that odor enrichment enhances the ability of mice and rats to discriminate between chemically similar odorants and that this improvement, called perceptual learning, is accompanied by an increase of newborn granule cell survival in the olfactory bulb. Olfactory functions are altered by age, including deficits in olfactory perception, olfactory discrimination, and memory. Because olfactory perceptual learning is crucial for basic olfactory functions, we investigated in this study the mechanisms underlying olfactory perceptual learning of adult and aging mice. The experiments reported here first showed that olfactory perceptual learning is impaired with aging and is correlated with a low level of neurogenesis. Second, we showed that stimulation of the noradrenergic system using the dexefaroxan ($\alpha 2$ pre-synaptic receptor antagonist) improves odor discrimination in old mice. Finally, we are also investigating whether perceptual learning differentially affects the synaptic density in the olfactory bulb of young and aged animals.

Decreases in olfactory responses in mouse model for Alzheimer disease

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X11-like (X11L) is a neuronal adaptor protein suppressing the generation of amyloid-proteins. A decrease in the ability of olfactory function is one of typical pothogeneis of Alzheimer disease. X11L proteins have been shown to be expressed in the main olfactory bulb (MOB) and accessory olfactory bulb (AOB). Amiloid-proteins increased with a lack of X11L in mice (Sano et al., 2009). Pheromones contained in urine induce excitation of vomeronasal sensory neurons and neurons in the accessory olfactory bulb (Inamura et al., 1999a, 1999b).

In the present study, we explored Fos-immunoreactive (Fos-ir) structures in the MOB and AOB of X11L-deficient mice after the olfactory and vomeronasal organs were exposed to conspecific urine to explore the effects of amiloid-proteins on the function of the neurons at the MOB and AOB. The density of Fos-ir cells in the MOB after exposure of X11L-deficient male mice to conspecific urine odor was lower than that after exposure of wild males. In immature male of X11L-deficient mice, the density of Fos-ir cells in the AOB after exposure of X11-deficient mice to conspecific urine was similar to that after exposure of wild males. However, the density of Fos-ir cells in the AOB of the X11L-decicient mature males after exposure to conspecific urine was lower than that of wild males.

These results suggest that olfactory dysfunction induced by an increase in amiloid-proteins occurs at the MOB and AOB in mice.

How nasal airflow shapes spatio-temporal representation of odors at the olfactory bulb level

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Olfactory sense and respiration are intimately related: odorant molecules reach receptor cells in the olfactory epithelium in a periodic way, with inspiration. Thus, several studies have demonstrated that olfactory bulb (OB) activity is largely shaped by respiration *(for a review, see Buonviso et al., Chem. Senses, 2006, 31: 145–154)*. When analyzing respiratory modulation of OB activity, it should be keep in mind that anesthetized rat breaths slowly and very regularly whereas behaving rat adapts its sniffing behavior (amplitude, frequency) according to numerous parameters (odor, task, experience···). Recent Ca^{2+} imaging studies has evidenced that nasal airflow parameters could modulated the spatio-temporal representation of the incoming message from neuroreceptors at the glomerular layer level *(Spors et al., J. Neurosci, 2006, 26:1247–1259; Oka et al., J. Neurosci., 2009, 29:12070-12078)*.

The aim of our study was to analyze how nasal airflow shapes the spatio-temporal distribution of odor evoked activity in the rat OB using voltage-sensitive dye imaging (VSDI). VSD signals originate mainly from post-synaptic activity and thus can measure odor-evoked activation of bulbar circuitry *(Spors and Grinvald, Neuron, 2006, 34: 301–315)*. Thus, compared to previous studies based on presynaptic activity measurements, VSD signals take into account the modulation of incoming activity by local interneurons.

Experiments were performed on urethane anesthetized rats. The OB was stained with the blue voltage-sensitive dye RH 1838 and image series of the dorsal OB were acquired with a CCD camera (Optical Imaging Inc.) at 160 Hz. We used a double canulation protocol in order to make nasal airflow sampling independent from animal respiration. Thanks to this technique, we were able to test different parameters of the nasal airflow as the frequency or the strength of inhalation flow. Sniffing frequency varied from 2 Hz to 6 Hz either at low or high flow rate.

Optical signals appeared as increase in fluorescence during odor presentation superimposed with a phasic component appearing after each inspiration. Preliminary results show that glomerular responses remained locked to the nasal respiration cycle even at high frequency sniffing and that the magnitude of this modulation depended on the inspiration strength. Moreover, respiratory modulation is not homogeneous across all activated glomeruli. Data analysis regarding the spatial distribution of respiratory modulation is in progress. These results confirm that intranasal air dynamics plays a critical role in shaping odor representation at the bulbar level.

This work was supported by an ANR grant (#ANR-07-NEURO-030).

Projection pattern of antennal-lobe output neurons in higher olfactory centers of the heliothine moth brain

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Heliothine moths utilize pheromone blends made up of partial identical constituents, however in a species-specific composition. By tracing functionally characterized receptor neurons and antennal-lobe projection neurons, a general chemotopic arrangement of the macroglomerular complex (MGC) has been demonstrated in a number of various species (Christensen et al. 1991; Hansson et al. 1995; Berg et al. 1998; Vickers et al. 1998; Lee et al. 2006). Especially interesting is the fact that the MGC of heliothine species have established two separate pathways — one for pheromone information underlying attraction and sexual behavior, and one for signal information emitted from heterospecifics ensuring reproductive isolation. Among the heliothine species studied so far, the Oriental species, *Helicoverpa assulta*, is unique as concerns composition of the pheromone blend. Whereas the other species rely on *cis*-11-hexadecenal (Z11-16:AL) as the major pheromone component, *H. assulta* uses this substance as the secondary component and *cis*-9-hexadecenal (Z9-16:AL) as the primary one. Consequently, a large number of male-specific receptor neurons tuned to Z9-16:AL and a small number tuned to Z11-16:AL have been identified in *H. assulta* (Berg et al 2005). A third neuron category consistently co?localized with the most numerous pheromone neuron type is tuned to *cis*-9-tetradecenal (Z9?14:AL), a behavioral inhibitor.

Tracing of the male-specific receptor neuron categories and of antennal-lobe projection neurons has demonstrated that each of the three female-produced substances is represented in one of the three MGC units — the major pheromone component and the interspecific signal in the two large units located dorsally, and the secondary pheromone component in the somewhat smaller ventral unit (Zhao and Berg 2010). In the present study, we have reconstructed the various categories of projection neurons and brought the reconstructions into a common reference model for the purpose of visualizing the projection pattern of the neuron categories in protocerebrum. Particularly interesting is to map the representation of insect-produced substances versus plant odours and of pheromones versus the antagonist. For interspecific comparison, we have reconstructed projection neurons tuned to the major pheromone component in the American species *Heliothis virescens*, which correspond physiologically to the category tuned to the secondary component in *H. assulta*.

Berg BG, Almaas TJ, Bjaalie JG, and Mustaparta H. 1998. J Comp Physiol A 183:669-682; Berg BG, Almaas TJ, Bjaalie JG, Mustaparta H. 2005. J Comp Neurol 486:209-220; Christensen TA, Mustaparta H, and Hildebrand JG. 1991. J Comp Physiol A 180:523-536; Hansson BS, Almaas TJ, and Anton S. 1995. J Comp Physiol A 177:535?543; Lee SG, Carlsson MA, Hansson BS, Todd, JT, Baker TC. 2006. J Comp Phys A 192: 351-363; Vickers NJ, Christensen TA, and Hildebrand JG. 1998. J Comp Neurol 400:35-56; Zhao XC, Berg BG. 2010. Chem Sens, doi:10.1093/chemse/bjq043.

Supported by grants from the Norwegian Research Council.

Function of inhibitory Projection neurons for odor coding and their influence on odor-guided behavior in *Drosophila melanogaster*

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The olfactory system of the vinegar fly *Drosophila melanogaster* provides an excellent system to study mechanisms how sensory information is coded and processed in the brain. Excitatory cholinergic olfactory sensory neurons (OSNs), the input neurons of the olfactory system, transfer peripheral information to the antennal lobe (AL), the first order processing center. Within the AL, odors are encoded by specific ensembles of activated glomeruli which constitute its structural and functional units. The peripheral information is initially modulated in the AL via interconnecting excitatory as well as inhibitory local interneurons (LN). Projection neurons (PNs), the output neurons of the Antennal lobe relay the olfactory information to higher brain centers such as the mushroom body calyx (MB) and lateral horn (LH). Three different projection neuron populations can be distinguished: the cholinergic anterodorsal and lateral PN lineage (adPN and lPN), which innervate the MB and LH and the GABAergic ventral PN lineage (vPN), which solely targets the LH. It has already been demonstrated that PNs ramify highly stereotyped in the LH. So far various Ca^{2+}-imaging studies of adPNs and lPNs have been performed since these are covered by the most common enhancer trap line GH146-GAL4.

To functionally characterize inhibitory PNs and to reveal their function for odor guided behavior we performed behavioral assays with intact and silenced vPNs. Additionally we performed two-photon Ca^{2+}-imaging in the AL and LH using an enhancer trap line specifically labeling vPNs, which exhibit a spatial segregation pattern for different odors in the input region, the AL as well as in the output region, the LH.

Identification of a fatty acyl reductase from *Spodoptera littoralis* female gland involved in pheromone production

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The pheromone blend of the Egyptian cotton leafworm, *Spodoptera littoralis,* is a mixture of long chain acetates with differences both in the number of unsaturations and the position of the double bonds. The biosynthetic pathway of all components involves functional transformations of palmitic acid through the action of β-oxidation enzymes, desaturases, reductase and acetyl transferase (Muñoz *et al.* 2008). Among these enzymes, reductase and acetyl transferase have been postulated to be the mainly responsible for the final ratio of the pheromone blend (Jurenka *et al.* 1989).

We present herein our investigations directed to identify the fatty acyl reductase involved in the reduction step of the biosynthesis. Using a degenerate PCR strategy we have amplified a cDNA corresponding to a fatty acyl reductase from *S. littoralis* female pheromone gland. The cDNA sequence showed 40% of aminoacid identity to a fatty acyl reductase previously identified in the pheromone gland of *Bombyx mori* (Moto *et al.* 2003). Subsequently, the transcript codifying for the identified fatty acyl reductase was introduced in a yeast expression vector for further functional analysis. This allowed us to explore the affinity of the enzyme for the different alcohol precursors of the *S. littoralis* pheromone components.

Dichlobenil-induced limited damage of the olfactory system alter exploratory and social behaviour in the mouse

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Damages to the olfactory system may cause behavioural modulation not limited to the sensory sphere. Ablation of olfactory bulbs in rodents induces a behavioural pattern with striking modifications in different behavioural domains, that may be rescued by antidepressant agents. A similar pattern was described also after peripheral destruction of the olfactory epithelium (OE, see Mucignat-Caretta et al. Neurobiol. Dis., 2004, 16, 386).

In the present series of experiments, the intraperitoneal injection of 2,6-Dichlorobenzonitrile induced a selective lesion in the OE of male Swiss mice restricted to the dorsomedial zone (zone I of OE), as revealed by a thinner histological appearance. The lesion was confirmed by immunohistochemistry of the OE and olfactory bulbs, showing modifications in a variety of markers, including cAMP-dependent protein kinases, Olfactory Marker Protein and Tyrosine-hydroxylase in the OE zone I or in its projections to dorsomedial glomeruli in the olfactory bulb. Modifications were also present in the subventricular zone and rostral migratory stream. A week after the injection, mice were tested for a variety of behavioural domains modulated by olfactory system, including exploratory, aggressive and anxious behaviours. Mice were anosmic, as shown by their inability to find a hidden piece of food. However, a reduction in the exploratory behaviour was also demonstrated in the open field test. Concerning social interactions, the olfactory lesioned mice attacked a male adult intruder mouse, similarly to control mice, but also increased the interspecific predatory aggression, similarly to olfactory bulb-lesioned mice. In conclusion, selective damages limited to zone I of OE are sufficient to induce some changes in the olfactory bulb circuitry that ultimately result in behavioural changes, similar to complete OE lesions. These data suggest a selective action of deafferentation on olfactory bulb output to limbic areas involved in behavioural modulation.

Modelling the cellular mechanisms underlying the multiphasic response of moth pheromone-sensitive projection neurons

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In the antennal lobe of the noctuid moth *Agrotis ipsilon,* pheromone-sensitive projection neurons (PNs) exhibit a multiphasic firing pattern when the antenna is stimulated with the pheromonal blend. At low concentrations, the PN response is biphasic and consists of an excitatory phase (E1) followed by an inhibitory phase (I). The duration of E1, but not of I, depends on stimulus concentration and duration. At higher concentrations, the response becomes

triphasic with a long tonic excitatory phase (E2) coming out after the inhibition.

To understand the cellular and synaptic mechanisms underlying these specific discharge patterns E1/I and E1/I/E2, we developed a biophysical model of a PN receiving inputs from olfactory receptor neurons (ORNs). The model is based on patch-clamp recordings from PNs, intra- and extra-cellular recordings from ORNs and PNs, as well as other experimental results on the synapses from ORN to PN and LN (local neuron) to ORN and PN. The PN input is modeled as a population of ORNs firing according to Poisson processes with the same firing rate under pheromone stimulation at various concentrations and durations. The PN model is based on the Hodgkin-Huxley formalism with realistic ionic currents whose parameters were fitted to whole cell patch-clamp data. Simulations revealed that the A current-(transient potassium current) strongly delays the onset of the spike and affects the firing frequency. Simulations showed also that the SK current (calcium gated small conductance potassium current) strongly affects the firing frequency and, more importantly, the hyperpolarization of the inhibitory phase I following the E1 phase. The E2 phase likely results from the long-lasting excitation of ORNs observed at high concentrations.

The interplay of the intrinsic currents affects such characteristics as the onset, duration and frequency of the various phases at different stimulus concentrations and durations. The transient Na^+ and the sustained K^+ currents determine the maximal frequency of the E1 phase. The Ca^{2+} current controls the existence of the I phase and the frequency of the E1 and E2 phases. The I_A current plays a significant role on the ending time of the E1 phase and the onset of the I phase. The SK current controls the duration of the I phase via the time constants of the Ca^{2+} kinetics.

Acknowledgements. This work was supported by French-British grant ANR-BBSRC Sysbio 006 01 ''Pherosys'' and by European FP7-ICT 2007 ''Neurochem''.

An exploration of mammary odor cues in newborn mice

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Among mammals, odor-based communication between females and offspring appears decisive for neonatal survival. It directs neonatal movements to the mammae, and favors subsequent milk intake. Although many species were studied, it is somewhat surprising that the mouse (*Mus musculus*), as a main biological ''workhorse'', has been relatively neglected on that topic. The present study aims to characterize the females' physiological stage and biological substrates that optimally support attraction in neonatal mice.

Six day-old mice were assayed in a series of 2-min tests opposing paired odor stimuli in an age-adapted rectangular choice arena. The tests opposed either anesthetized females lying supine under each half of the choice arena, or biological fluids painted in 2 ipsilateral corners of the arena. Time spent over/against each stimulus in the arena was measured.

When abdominal odours of lactating (LF) and non lactating females (NLF) were simultaneously presented, pups roamed preferentially over the abdomen of the LF. To assess whether nipples were involved, pups were exposed to the abdominal odor of LFs which nipples were either free or covered. It came out that the side with accessible nipple odor was more attractive for pups.

Next, we assessed the attractive power of the blend of exocrine sources that are present on LFs' nipples (a nipple smear was taken), as well as some separate sources presumed to compose it, *viz*. fresh milk, maternal saliva, and pup saliva. Pups spent longer time over all these stimuli when they were paired with a blank (water), indicating that they are detectable and attractive to them. When the nipple smear was paired with fresh milk, maternal saliva, or pup saliva, pups explored the nipple smear as much as its components. Besides, maternal and pup salivas were equally attractive.

Thus, all mammary stimuli assessed here, whether the whole odor mixture or its presumed components, worked as attractants for 6 day-old pups. Such common attractiveness in distinct biological substrates may derive either from overlap in chemical constituents, or from the fact they were all simultaneously reinforced during nursing. Thus, much as in rats, the mammary area of female mice is endowed with multiple, redundant odor cues emitted there or spread there by the self-licking female or by sucking neonate. Six day-old mice pups are able to use them all separately in their orientation responses.

This research is supported by a doctoral grant from CNRS-Region Burgundy, and by continued support from Region Burgundy.

Flavour learning in babies: The role of breastfeeding

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Preferences for certain tastes (e.g. sweet) are present at birth, whereas most flavour preferences are learned as a result of exposure. How flavour preferences develop has already received some attention, but the role of breastfeeding is not fully elucidated. Some studies have shown a better acceptance of weaning foods in breastfed babies than in bottle-fed babies (eg, Sullivan & Birch, 1994; Maier et al., 2008). The effect may well result from flavour-specific learning via mother's milk as suggested by Mennella et al. (2001) or from previous exposure to a variety of flavours (Hausner et al., 2010). To be able to argue for flavour-specific learning via mother's milk it is necessary to demonstrate that a significant amount of dietary aroma compounds transfer into mother's milk. Previous experiments have shown that some flavours transfer to mother's milk, but it has also been suggested that only lipophilic flavour compounds are transferred (Hausner et al., 2008). Based on the existing literature we suggest that at least two learning mechanisms occur; flavour-specific learning and learning not to avoid novel flavour via exposure to a variety of flavours in mother's milk. These possible learning mechanisms will be discussed in relation to the existing literature.

Hausner,H., Bredie,W.L.P., Mølgaard,C., Petersen,M.A. and Møller,P. (2008) Differential transfer of dietary flavour compounds into human breast milk. Physiol Behav, 95, 118-124.

Hausner,H., Nicklaus,S., Issanchou,S., Mølgaard,C. and Møller,P. (2010) Breastfeeding facilitates acceptance of a novel dietary flavour compound. Clinical Nutrition, 29, 141-148.

Maier,A., Chabanet,C., Schaal,B., Leathwood,P. and Issanchou,S. (2008) Breastfeeding and experience with variety early in weaning increase infants' acceptance of new foods for up to two months. Clin Nutr, 27, 849-857.

Mennella,J.A., Jagnow,C.P. and Beauchamp,G.K. (2001) Prenatal and postnatal flavor learning by human infants. Pediatrics, 107, E88.

Sullivan,S.A. and Birch,L.L. (1994) Infant dietary experience and acceptance of solid foods. Pediatrics, 93, 277.

Modulation of facial mimicry by sexual orientation and related social chemosensory context cues

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Facial mimicry has been shown to vary with gender, social context, and with the level of general empathy, while sexual orientation is discussed to affect empathy. Human body odors constitute potent social signals, most probably transmitting information about gender and sexual orientation. Here, we investigate the effect of sexual orientation on facial reactions to facial expressions being presented in the context of gender and sexual orientation related body odors.

Facial electromyographic activity from the corrugator supercilii (indicating frowning) and zygomaticus major (indicating smiling) muscle regions in response to happy and sad faces of 23 (11 gay) men (study 1) and 22 (12 lesbian) women (study 2) was recorded. In addition to the exclusive presentation of the faces, in study 1 gay and heterosexual male and heterosexual female body odors were presented simultaneously with faces of the corresponding gender. In study 2, lesbian and heterosexual female and heterosexual male body odors were presented. The body odor samples were obtained from 11 heterosexual and 11 lesbian women, as well as from 13 gay and 14 heterosexual men. Odor donors' axillary sweat was sampled via cotton pads attached to their armpits over the course of one night. Subsequently, the samples were pooled with respect to gender and sexual orientation. These chemosensory stimuli were delivered using a constant-flow, six channel olfactometer (OM6b, Burghart, Wedel, Germany), and both nostrils were stimulated simultaneously.

Men responded with stronger corrugator activity to sad as compared to happy female faces (500-1000 ms after picture onset). This effect was prolonged in gay men (1000-1500 ms after picture onset). A corresponding differential corrugator response to male faces was observed with gay male context odor only (500-1000 ms after picture onset). In addition, heterosexual men responded with stronger zygomaticus activity when presented with sad as compared to happy male faces, indicating a display of counter-mimicry (1500-2000 ms after picture onset). Heterosexual women displayed stronger corrugator activity in response to sad faces irrespective of the actor's gender (1000-2000 ms after picture onset). In lesbian women, however, a corresponding effect only was evident in response to female faces in the context of heterosexual female body odor (500-1500 ms after picture onset).

Both studies demonstrate variations in facial mimicry with regard to sexual orientation, which may be related to differences in interpersonal empathy. Moreover, the gender of the person modeling the facial expression and social chemosensory context cues modulate facial mimicry. As mimicry shares a bidirectional link with rapport and affiliation, subjectively relevant body odors might prime the motive of affiliation.

Central nervous processing of chemosensory anxiety signals in pregnant women

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Studies in humans indicate that socially relevant emotions like anxiety or stress related experience are

chemosensorily communicated. Additionally, recent EEG studies demonstrated that the processing of chemosensory anxiety signals recruits more neuronal activity than the processing of chemosensory stimuli derived from sport sweat. Moreover, in social anxious individuals, the processing of anxiety related chemosignals is faster than the processing of the control stimuli.Apart from that, it has been demonstrated that physiological responses to stress are diminished during pregnancy.

The current study investigates whether in pregnant and non pregnant women chemical substances taken from individuals experiencing high state anxiety elicit different central nervous processing patterns than chemical substances taken during an equally arousing, but emotionally neutral situation (sport control condition).

Using cotton pads, axillary sweat was collected from a sample of 28 male participants both over the course of one hour before an important oral examination at the university (anxiety condition) as well as during an ergo-meter training (control condition). The EEG of 12 non pregnant women, of 14 women during the first trimester and of 18 women during the third trimester of pregnancy was recorded from 60 scalp locations. Using a constant-flow olfactometer, the chemosensory stimuli were presented during an oddball paradigm and chemosensory event-related potentials (CSERPs) in response to the deviant stimuli were analyzed.

When processing chemosensory anxiety signals non pregnant women showed larger P3-1 and P3-2 amplitudes than when processing control stimuli ($p = 0.01$). In addition, in non pregnant women these components were larger than in both groups of pregnant women (main-effect group: $p = 0.01$).

Regarding the latencies, all groups showed shorter latencies of the P3-2 component in response to anxiety stimuli as compared to control stimuli (main-effect stimulus: $p = 0.01$). In non pregnant women this component exhibited shorter latencies than in pregnant women during the first trimester of pregnancy ($p = 0.05$) and than in pregnant women during the third trimester of pregnancy ($p = 0.001$).

Additionally, in pregnant women during the first trimester of pregnancy, the P3-1 component exhibited a shorter latency in response to anxiety signals than in pregnant women during the third trimester of pregnancy ($p = 0.02$).

Results demonstrate that chemosensory anxiety signals have a higher subjective significance as compared to control stimuli in general (P3 effect). Additionally, with advancing pregnancy delayed and reduced processing of chemosensory anxiety signals is observed. These results suggest that differences in the processing of chemosensory anxiety signals depend on the hormonal state in women, probably based on a stress protection mechanism during pregnancy.

Developmental and activity-dependent regulation of the post-synaptic protein Neurogranin in the olfactory bulb

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Neurogranin (Ng) is a brain specific protein involved in synaptic plasticity, through the regulation of Calmodulin-mediated signalling. In a recent study, we have demonstrated Ng expression in the adult olfactory bulb (OB), where Ng localizes in a large population of GABAergic interneurons in the granule cell layer (GCL) and in a few neurons laying in the deep glomerular layer (GL, Gribaudo et al., J Comp Neurol 2009).

Here we investigate by immunohistochemistry how Ng expression is regulated during OB postnatal ontogenesis in standard conditions and following early sensory deprivation. At postnatal day 0 (P0), Ng identifies a population of granule cells that ramify in the prospective external plexiform layer (EPL). The density of Ng-positive granule cells increases during OB development, acquiring mature features around P20-P30. Starting from P5, besides the GCL, Ng is also expressed at the boundary between the EPL and the GL by a population of cells whose apical processes extensively arborize into the developing glomeruli. The number of cells expressing Ng in this region drastically increases during development reaching a peak at P10 to be than progressively reduced to the adult condition (P30), where only rare Ng-positive elements are identifiable in the deep GL. At the different postnatal ages considered, Ng-positive cells largely co-express markers of tufted neurons (such as Tbx2.1 and CCK), whereas they do not co-localize with juxtaglomerular interneuron markers (CB, CR, TH, GAD67, Gad65). Moreover, analyses on Ng-positive cell body size and position relative to the EPL, indicate these elements represent an heterogeneous population of external/superficial tufted cells that transiently express Ng during development. Interestingly, the time-course of Ng expression in these cells (P5-P20) corresponds to the critical period of glomerular maturation and suggest Ng could play a role in the olfactory nerve-tufted cells synaptic refinements. In order to interfere with these processes we performed early postnatal (P0) naris closure, a procedure of sensory deprivation that causes a decrease in olfactory receptor activity and alters synaptic rearrangements at the glomerular level. Following this procedure at all time points analyzed (P5, P10, P15, P20), the number of Ng-positive cells strongly increases in the EPL/GL area compared to standard conditions, indicating an activity-dependent regulation of Ng expression in tufted cells, that might represents a compensatory mechanism to enhance cell sensitivity in response to decreased incoming stimuli.

ACKNOWLEDGEMENTS: This work was supported by Compagnia di San Paolo (Neurotransplant 2007-0660); PRIN 2007. Marion Denorme is recipient of a exchange Erasmus project from University of Roen.

Sequential Arrival and Graded Secretion of Sema3F by Olfactory Neuron Axons Specify Map Topography at the Bulb

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In the mouse olfactory system, the anatomical locations of olfactory sensory neurons (OSNs) roughly correlate with their axonal projection sites along the dorsal-ventral (D-V) of the olfactory bulb (OB). Here we report that an axon guidance receptor, Neuropilin-2 (Nrp2), and its repulsive ligand, Semaphorin-3F (Sema3F) are expressed by OSNs in a complementary manner that is important for establishing olfactory map topography. Sema3F is secreted by early-arriving axons of OSNs and deposited at the anterodorsal OB to repel Nrp2-positive axons that arrive later. Sequential arrivals of OSN axons, as well as the graded and complementary expression of Nrp2 and Sema3F by OSNs, help to form the topographic order along the D-V axis. The coarse map can be established autonomously, without involving target-derived guidance cues.

Intracellular study of relationships between spiking activity and membrane potential oscillation in freely breathing anesthetized rat

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In the olfactory bulb, an important characteristic is the presence of a respiratory-modulated activity. During odor stimulation, this modulation is distinguishable at the level of global network activity with the presence of a local field potential slow oscillation (1 to 5 Hz) and at the level of spiking discharges by the existence of respiratory spiking patterns. Indeed, several respiratory discharge patterns have been described among which non synchronized patterns (noS) characterized by uniform distribution of action potentials (AP) along the respiratory cycle, excitatory-simple-synchronized patterns (S+), presenting a single increase in firing activity synchronized with the respiratory cycle, and inhibitory-simple-synchronized pattern (S-) presenting a single decrease in firing activity synchronized with the respiratory cycle. In addition, respiratory modulation is observable at the level of intracellular activity with slow oscillations of the membrane potential. Relationships between these three activity levels have been poorly studied despite their importance in olfactory processing. In particular, relationship between respiratory discharge patterns and intracellular slow oscillations has never been described.

In a previous work, we characterized two types of intracellular slow oscillations called positive and negative slow membrane potential oscillations. The percentage of positive type is increased by

hyperpolarization of membrane potential with negative DC current injection. In contrast, the percentage of negative type is increased by depolarization of membrane potential with positive DC current injection and by odor stimulation. In this study, we recorded intracellular activity of Mitral/ Tufted cells in order to examine relationships between these two forms of intracellular slow oscillations and noS, S+ and S- respiration-related spiking patterns. Moreover, we analyzed the fine structure of spiking discharge (single AP in opposition to burst of AP) and their distribution along intracellular slow oscillation cycle.

Surprisingly, despite the fact that respiration shaped both, spiking patterns and intracellular slow oscillations, we found no strong relationship between these two activities. However, our study clearly showed that the cells with noS pattern never exhibited a slow oscillation before or during odor stimulation. Moreover, the proportion of cells presenting S+ or S- respiratory-synchronized pattern together with an intracellular slow oscillation is increased during odor stimulation. For cell exhibiting S+ pattern, the presence of intracellular slow oscillation seemed to influence the respiratory cycle position of single AP as they preferentially occurred just after inspiration/expiration transition. Moreover, the bursts of AP were always localized to a restricted area along the respiratory cycle (more often at proximity of inspiration/expiration transition) whatever the presence or not of an intracellular slow oscillation. This study suggests that respiratory spiking patterns and respiration-related intracellular slow oscillations could participate in different way to olfactory processing.

Activity correlation imaging: visualizing function and structure of neuronal populations in the olfactory bulb

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It is often desirable for the investigation of neuronal networks to simultaneously measure the individual neuron's activities and their morphology. Because of the highly correlated activity in interconnected dendritic trees, it is possible to extract their morphology exclusively from measurements of their temporal activity patterns. Morphological data of different trees is then combined into a high-contrast, multicolor visualization of the neuronal network, having used only a single functional dye. This novel technique, called activity correlation imaging [1], is demonstrated in the Xenopus laevis olfactory bulb, showing both activities of the mitral/tufted cells as well as their projections into the olfactory glomeruli.

We thereby show that mostly distant mitral cells project dendrites into the same glomerulum, that these mitral cells are coupled through gap junctions and that they perform synchronous activity.

[1] Junek S, Chen T-W, Alevra M, Schild D (2009) Activity correlation imaging: visualizing function and structure of neuronal populations. Biophys. J., 96, 3801-3809.

Rat piriform cortex response to odors revealed by BOLD fMRI

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Blood Oxygen Level Dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) has recently been applied to the rodent central nervous system. In the olfactory system, reproducible and relatively specific odor-induced spatial activity patterns have been demonstrated in the olfactory bulb (Martin et al., 2007). These results support previous optical imaging studies showing that odors elicit activity within glomerular layer domains. Despite these promising results, recording of BOLD signal in small animals remains a challenge particularly in deep brain structures. Indeed, fMRI recordings need the use of surface receiving coils to achieve a good signal to noise ratio. Thus, the signal intensity dramatically decreases with increasing depth making difficult to record BOLD signals in ventral structures.

The aim of our study was to record BOLD odor evoked responses in the rat piriform cortex (the main area of olfactory bulb projections), which stretched on the ventro-lateral part of the rat brain. In this study, we took advantage of the use of a new phase-array surface coil. This 4 channel array was made of 4 receivers aligned along the coronal axis. This new coil offered a more homogeneous recording field in the whole rat brain and an increased signal to noise ratio in the brain structures located far from the coil. BOLD responses to different odors were acquired in the piriform cortex of anesthetized rats using a 7 T spectrometer.

When compared with a previous study using a single receiver coil, the probability to record BOLD signal in the piriform cortex was largely increased with the phase-array coil. BOLD responses were mainly recorded in the anterior part of the piriform cortex. According to previous electrophysiological and anatomical studies, PC responses to odors were not spatially organized but extended in a large part of the anterior cortex. These results were the first report of BOLD responses in a deep rat brain structure and demonstrated the advantage of using multi-receivers coils. Data confirmed that BOLD fMRI is suitable for studying spatio-temporal activity associated with odor stimulation in olfactory system, particularly in the olfactory cortex which is less accessible to optical imaging studies.

This work was supported by an ANR grant (#ANR-07-NEURO-030).

Martin et al., fMRI visualization of transient activations in the rat olfactory bulb using short odor stimulations. Neuroimage, 2007, 36: 1288-1293.

Olfaction in Parkinson's disease: Monorhinal olfactory testing in patients with lateralized motor symptoms

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There is little doubt that patients with Parkinson's disease (PD) have important olfactory dysfunctions. Multiple sources of evidence seem to suggest that there is no link between the degeneration in the dopamine nigrostriatal system and the olfactory system, as most studies so far did not show a relationship between the motor and olfactory symptoms in this disease. For example, anti-PD medications do not have effects on the smell deficit, and the olfactory impairment seems to be independent of the disease stage, severity and/or duration. Importantly, a previous study (Doty et al. 1992) showed that there is no association between the side of greater motor involvement and the side of olfactory impairment, and that the olfactory loss is typically bilateral in this population. Yet, these notions have been challenged, as some recent findings suggest a link between the dopaminergic dysfunction and the olfactory decline in patients with PD. The aim of the present study is to reinvestigate the existence of eventual lateralized olfactory deficits in patients with lateralized symptoms of Parkinson's disease.

So far, we recruited nine non-demented patients with idiopathic Parkinson's disease. Difference of 30 seconds between the two hands on a fine motor dexterity test (Grooved Pegboard) was used as a criterion for classifying patients into bilateral, predominantly left, and predominantly right motor symptom group. Patients were tested monorhinally, i.e. each nostril was tested separately while the other one was sealed with surgical tape. Olfactory function was examined with the Sniffin' Sticks test battery (including threshold, discrimination, and identification). In addition, patients were asked to rate 16 odors for pleasantness and intensity. The testing order of the two nostrils (i.e. ipsilateral and contralateral to the motor symptoms) was counterbalanced across subjects.

Our preliminary results show that while patients with bilateral and predominantly left motor symptoms do not show an asymmetry in their olfactory functioning, those with predominantly right motor symptoms show an olfactory asymmetry by which certain of their olfactory functions are better in the right than in the left nostril. Also, patients with predominantly left motor symptoms appear to show incapacity of evaluating pleasantness of smells with the right nostril. These data suggest that olfactory and motor symptoms of the PD may be related, at least in some patients, and that this question should be pursued further. We continue to collect data and will present results from a larger sample at the conference.

Support: NSERC, RGPIN 335938-08 to JD

Odour recognition memory and odour identification abilities in patients with Mild and Severe Major Depressive Disorder

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Olfactory deficits, in detection, recognition and identification of odorants have been observed in several neurodegenerative (e.g, Alzheimer's, Parkinson's, Multiple System Atrophy, Huntington's) and psychiatric conditions (e.g., Schizophrenia, Anorexia nervosa, Alcoholic/Drug addiction, Major Depression). Among the latter, olfactory abilities in Major Depressive Disorders (MDD), were less investigated and available studies have provided contradictory results. For instance, Olfactory thresholds were found either increased (Lombion-Pouthier et al., 2006), reduced (Postolache et al., 2002) or unchanged (Thomas et al., 2002) compared to healthy controls; odour recognition memory was substantially not investigated, except by Moberg et al. (1986), and by Sreenivasan et al. (1987), who found decreased performance; however, the first on an unspecified odour recognition task, and the second on a matching to sample discrimination task. As to odour identification most studies reported no difference between MDD and controls (e.g., Lombion-Pouthier,et al., 2006; Pentzek et al., 2007; Swiecki et al, 2009), few others decreased odour identification (Serby et al , 1990; Steiner et al. 1993).

These controversial outcomes, could be due to shortcomings, as: small samples size; inclusion of patients having different degrees of pathology in a same MDD group, ages over 65, different number of male and female within and between group.

In the present study we took into account these limitations, and have examined odour identification and odour recognition abilities in two groups of 12 mild MDD patients (*M* age 41.3, range 25-57) and 12 severe MDD patients (*M* age, 41.9, range 23-58) matched for age and gender to 12 Normal controls (*M* age, 39.8,range 23-53). The patients were diagnosed according to DSM-IV (1994) criteria.

Statistical analyses have shown that Severe MDD patients performed worse ($p < .001$) on both tasks than Mild MDD patients and Normal controls (these last not differ significantly from one another).

Results are consistent with previous studies in other domains revealing reliable, although not conclusive, impairments in cognitive function including memory, in patients with MDD (Ilsley et al., 1995; Ravnkilde et al., 2002; Rose & Ebmeier, 2006; Bearden et al, 2006).

The present outcomes stress the relevance to take into account the severity of the disease of MDD patients in the arrangements of the experimental samples, lack of which may contribute to inconsistencies between studies, hindering the appearance of significant effects.

Bearden C. et al. (2006), Patterns of memory impairment in Bipolar disorder and Unipolar Major Depression. Psychiatry Research, 142, 139-150; Lombion-Pouthier S. et al. (2006), Odor perception in patients with mood disorders. Journal of Affective Disorders 90, 187– 191; Rose E. & Ebmeier,K. (2006), Pattern of impaired working memory during Major Depression. Journal of Affective Disorders, 90, 149-161; Serby M. et al. (1990), Olfactory sense in Psychoses. Biological Psychiatry, 28, 829-830; Sreenivasan K. et al. (1987), Righ temporal lobe functions in psychiatric disorders. Indian Journal of Clinical Psychology, 14, 40-42

The influence of distractors on olfactory identification ability in schizophrenia patients and controls: relationships to negative symptomatology

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The olfactory function is impaired in schizophrenia. Indeed, it has been shown that discrimination and memories of odours are affected as well as the estimations of their hedonicity and their familiarity. In addition, an olfactory identification deficit was frequently reported. This deficit is correlated with schizophrenic symptoms, and mainly with its negative side including a flat affect, a social withdraw and a poor personal hygiene. An olfacto-visual tool was created proposing a forced choice through the selection of sources' images to the participants. A score of accuracy and a response time delay are recorded. An error evaluation is performed using distractors which are close to or distant to the target to identify. Based on the olfactory identification scores in schizophrenia patients, the relationships between olfactory identification performance and negative symptomatology were analysed.

Forty seven pleasant and unpleasant olfactory signatures of everyday life objects were proposed to the participants. Preliminary studies have assigned to each odorant a specific image (target) and 4 distractors (2 close to the target and 2 distant) allowing us to create the forced choice. The identification task consists in designating one of the 5 images corresponding with each odour sample. The computerized procedure allows recording responses time delays. A pretest was made with 104 controls. Then, 2 matched groups including 73% men were tested: 30 schizophrenia patients (41.2 ±9.9 years old) and 30 controls (39.0 ±9.4 years old).

The results confirm that the pathological cohort has lower odour identification scores than controls. The analysis reveals that schizophrenia patients chose more responses close to the target than the controls. Taking into account the score of responses close to the target, the clinic group shows 82% of "correct" odour identification (89% for the controls). Interestingly, the incorrectly identified stimuli in both groups are distinct. In addition, we highlight the olfactory identification deficit correlations with negative symptomatology. The results depend on the stimuli hedonic value and on the gender. However, no difference of responses time delays between the 2 groups is shown, without any effect of the hedonic value.

The analyse shows that the patient's choices are rather imprecise. We can consider that they have the ability to designate exactly the odour but some of the studied symptoms could have impaired this accuracy. We suppose that an olfactory training could reduce their difficulties and thus could help olfactory remediation (orthosmia) to attenuate their impact in the everyday life of patients.

This research is funded by the Edmond Roudnitska Foundation.

Delayed decrease of pleasantness ratings in congenital anosmia during the consumption of a simple food

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Food is evaluated for various attributes on the basis of several sensory modalities. One of the key domains of food evaluation is hedonicity. Pleasantness, which has a positive effect on food intake, declines over time as food is consumed. Along with it the reward value of the particular food decreases because of repeated exposure to the stimulus in question and "boredom with taste" prevails, which is referred to as *sensory-specific satiation*

The aim of the present study was to investigate the change of food pleasantness during a meal in individuals who only have a non-olfactory experience with food. Fifteen patients with congenital anosmia and sixteen normosmic controls participated in the study. The participants were presented with ten 10g banana portions which, to prolong the exposure time, were to be consumed in the following manner: visually inspected, smelled and chewed for ten seconds per action. Upon swallowing, the pleasantness of the particular portion was rated on a 21-point scale. For both the anosmic and normosmic group, a decrease in pleasantness ($\chi^2(9) = 31.9$, $p = .0002$; $\chi^2(9) = 64.0$, $p < .001$) across repeated measures was found. Post-hoc tests revealed a significant change in pleasantness ratings between the first and last measure in both the anosmic and normosmic group at $p < .05$ and $p < .005$, respectively. No change in pleasantness ratings between adjacent measures was found in either group but there was a significant decrease in pleasantness ratings over three measures from the fourth measure on (p4-p7, p7-p10) at $p < .005$ in the control group whilst no change was found in the anosmic group.

The results of the present study indicate that the development and/or expression of boredom with flavour of a simple food is less pronounced in the absence of smell.

No brain-response to a human chemosignal in congenital anosmia

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Non-human chemosignaling is mediated by a combination of several chemosensing subsystems. For example, rodent social chemosignaling is mediated in part by the vemoronasal system. The functionality of human chemosensing subsystems beyond the main olfactory system remains poorly understood. To test for such non-olfactory chemosensing, we set out to use brain imaging in order to measure the brain response to a chemosignal in otherwise congenitally anosmic individuals. We hypothesized that if human social chemosensing is mediated by subsystems beyond the main olfactory system, than such anosmic individuals should nevertheless display a brain-reesponse to a chemosignal. We used the sweat-derived compound 4,16-androstadien-3-one (AND), which has been widely considered in the context of human chemosignaling. An olfactometer delivered 6 blocks of 30 seconds of AND followed by 30 seconds of clean air, with a constant sniffing rate of once every 6 seconds (5 sniffs per block). We used a 3-Tesla Siemens Tim-Trio scanner, with acquisition parameters of 30 slices, slice thickness=4 mm, gap=0, TR=1500 msec, TE=23.

Pilot data from two female subjects revealed an AND-induced response in the normosmic, but not anosmic subject ($p < 0.001$). The normosmic response was evident in piriform and orbitofrontal cortex, and in the hypothalamus. These pilot results from congenital anosmia are consistent with those obtained by Savic et al in conductive anosmia, and together suggest that the human brain response to the chemosignal AND is mediated by the main olfactory system.

Early anosmia or hyposmia: MR Imaging and evoked potentials evaluation

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Early anosmia is related to patients reporting a reduced or absence of smell sensation since early childhood. Some forms of early anosmia are associated with genetic disorders and endocrine problems such as in Kallmann's syndrome which is always associated with hypogonadotropic hypogonadism. Other early anosmia, which are not linked to endocrine defects, are considered as "isolated anosmia" (IA). The cause of IA is still unclear but is thought to be associated with alterations in the olfactory pathway, from the olfactory bulbs to the orbitofrontal cortex.

Our purpose was to evaluate patients with IA using MR imaging, olfactory event related potentials and sensory measurements. MR imaging of patients with IA were compared to normosmic subjects. Various regions of interest were examined: olfactory bulbs and olfactory tracts as well as cortical regions (gyrus rectus, olfactory sulcus, medial orbitary gyrus). We noticed various combinations of alterations among our patients. Olfactory event related potentials were recorded at Cz and Fp2 using a paradigm based on the contingent negative variation, including the measurement of hits and false alarm rates. Most recordings were congruent with the declared lack of perception of short odorant stimulations. However in a few cases a small positive event could be recorded at Cz, although not associated with any perception. The contingent negative variation, a signal triggered by the perception of the odorant while the subject is expecting a sound stimulus, was not observed in IA patients. In addition, other sensory measurements using sniff bottles were used to test the olfactory sensitivity and discrimination with longer odorant stimulations and natural sniffing.

Our main conclusion is that IA seems associated with various modifications in olfactory intracranial structures which are considered as essential for a normal olfaction. Short lasting odorant stimulations were not perceived and, in most cases, no OERP was recorded. The origin of the small positive evoked potential observed at Cz in a few patients remains to be determined.

This study was supported by *La Fondation des Gueules Cassées* to AI and DT, and by AP-HP grant project.

Olfactory responses in children with and without autism

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Children with autism often demonstrate difficulties in understanding and reciprocating the emotions of others, but until now it is not clear whether these children suffer from a deficit in feeling emotions, from a deficit in cognitive processing of emotions (including difficulties identifying and describing feelings), or from a deficit in expression of positive and negative emotions.

In this study, we examined the responsiveness of children to odours, since these stimulations are particularly potent to elicit positive and negative emotional reactions. A set of pleasant and unpleasant everyday odours were presented in 8 subjects (8-14 years) well characterized with high-functioning autism and 8 age-matched typically developing controls. Odours were presented by the experimenter in opaque glass jars during 5 seconds. The children were filmed during the whole test and their facial expressions were analyzed by certified experts using the Facial Action Coding System (Ekman and Friesen), an anatomically based instrument in which basic units (named action units) are minimally distinguishable movements of the facial muscles. Heart rate, respiratory rate, and skin conductance were also recorded. Finally, the experimenter asked the subject to report verbally the hedonic connotation of the scented stimulus. Physiological responses to the odorants were extremely similar in autistic and normal subjects, indicating that the chemosensory capacities and the aptitude to feel emotions are not impaired in high-functioning autistic children. Compared to controls, autistic subjects expressed less facial movements, particularly in the lower part of the face. When present, the facial expressions of the autistics appeared less durable and less intense, particularly in the eyes and mouth regions. Finally, a strong correlation between subjective verbal evaluation and facial expressions was observed in control participants, a correlation that was not seen in the subjects of the autistic group. Taken together, these data indicate that autistic children seem to have intact olfactory reception, normal physiological emotions, but clear difficulties to understand and report their own emotions.

These results suggest that autistic children may exhibit specific impairments in the cognitive processing of emotions.

Supported by the Centre National de la Recherche Scientifique, Strasbourg, France.

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.

This work was supported by the Deutsche Forschungsgemeinschaft

OR37-receptors: a unique subfamily of olfactory receptors

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Olfactory sensory neurons (OSNs) which express the same odorant receptor (OR) gene are generally widely dispersed throughout the olfactory epithelium (OE). In contrast, neurons expressing an OR37 receptor are assembled in a small central patch of the nasal neuroepithelium. A detailed molecular characterization of the OR37 receptors revealed that they are not only expressed in cells at a unique topographic region, but display several other special features, as well. Only this group of ORs is characterized by an insertion of six amino acids in the third extracellular loop. The extended loop in the OR37 receptors has a high proportion of charged amino acids which seem to be exposed mainly to one side of a helix. This unique structural feature of the OR37 receptors suggests that they may be tuned to special ligands. Comparative analyses have shown that orthologous receptors exist in diverse mammalian species, e.g. mouse, dog, and primates. Even in humans, this group of receptors exists, with a surprisingly high fraction of potentially functional genes, a scenario that is against the general trend of pseudogenization for OR genes in humans. When compared across all species, the coding sequence of OR37 genes displays an unexpected high degree of conservation; bioinformatic data revealed that these genes apparently are under a negative selective pressure, a feature that is to be quite unusual for OR genes, supporting the idea that these are specially tuned receptors.

The axonal projection pattern of OR37 expressing neurons was analyzed using a gene targeting strategy. The glomeruli from neurons expressing the highly homologous receptors of the mOR37 subfamily are located in immediate vicinity, nevertheless each glomerulus receives input with very high precision from only one, receptor-subtype specific population of neurons. To monitor the unfolding of this precise olfactory map during development, the onset of receptor expression, outgrowth of axons as well as glomerulus formation for two neuron populations expressing different mOR37 subtypes was investigated on transgenic mouse lines. The data indicate a synchronous onset of receptor expression at about embryonic day 10 (E10). From E15, axons of both populations terminate in a common, small area of the presumptive olfactory bulb; the projection into distinct glomeruli is mainly achieved in a short postnatal period until postnatal day 3.

This work was supported by the Deutsche Forschungsgemeinschaft.

Gene switching and odor induced activity shape the OR37-typical patch

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Olfactory sensory neurons which express distinct odorant receptor (OR) genes are spatially organized in the mouse olfactory epithelium (OE). Towards an understanding of the mechanisms which determine these patterns, representative OR genes which are typically expressed in the unique central patch of the OE were investigated. Most OSNs inside the patch that initially selected a representative gene from this group finally expressed another gene from that group, indicating that OSNs inside the patch frequently 'switch' between these genes. If an OSN successively chose genes from the same OR gene cluster, these originated exclusively from one of the two parental chromosomes. A deletion of the cyclic nucleotide gated ion channel in OSNs altered the pattern; distinct OR genes were no longer exclusively expressed in the patch, but additionally in dispersed OSNs.

Together, the results indicate that OSN in the patch initially sample several OR genes for expression; for their correct patterning in the OE, odor induced activity could play a critical role.

This work was supported by the Deutsche Forschungsgemeinschaft.

Interactions between octanal and citronellal at the receptor level

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Most odorants present in the environment are complex mixtures of tens, even hundreds, of components. Psychophysical and behavioral studies have shown that odorant mixtures are perceived as different (synthetic perception) from or like (analytic perception) their components, indicating that the perception to odorant mixtures is not always a simple function of the responses to its individual components. Olfactory perception of mixtures can arise from interactions occurring at the peripheral level, in the olfactory bulb or at the central level. Some studies suggest that interactions at the level of olfactory receptors (ORs) can affect strongly the perception of odorant mixtures. Several types of interactions (such as inhibition, suppression or synergy) have been described in rat olfactory receptor neurons. In addition, using in vitro approaches, it has been shown that some odorants can act both as agonists or antagonists.

In the present study, we investigated the responses of ORs to a binary mixture of octanal and citronellal, a mixture which has been shown to induce a synthetic perception in rat. For this purpose, a heterologous functional expression system (HEK293 cells), in which ORs were transfected, was used. The effects of octanal-citronellal mixture were investigated at different concentration levels and at different ratio. When comparing the responses of ORs to odorants delivered singly or in combination, several types of effects were observed: depending on concentration and OR, the mixture was found to elicit responses lower or higher than, or equivalent to, that induced by single components. These effects can be likened to those observed in electrophysiological and psychophysical studies (inhibition, synergy, suppression and hypo-addition). Our results demonstrate that various types of interactions can occur at the peripheral olfactory stage. Given that several ORs can be

activated by the same mixture, each of them responding to different extent, our observations also highlight the complexity of the processes which may rise at the receptor level.

Acknowledgements: this work was supported by INRA and by the Burgundy council (France).

Novel signaling mechanism of the olfactory receptor hOR51E2 in prostate cancer cells

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Olfactory receptors (ORs) are expressed not only in the sensory neurons of the olfactory epithelium but also in various other tissues. We reported previously that the human OR51E2, which is endogenously overexpressed in prostate cancer cells, can be activated by androstenone derivates as well as the odorant ionone. We could also show that exposure to ionone resulted in an increase of intracellular Ca^{2+} and inhibition of cell proliferation.

Here, we characterized the signal transduction mechanism induced by activation of OR51E2 in LNCaP cells (prostate cancer cell line). Using complementary molecular, biochemical, electrophysiological and live-cell imaging techniques, we found that endogenous Ca^{2+}-selective TRPV6 channels are critically involved in the Ca^{2+} signal induced by OR51E2. Biophysical characterization of the current activated by OR51E2 stimulation revealed characteristic features of the transient receptor potential channel (TRP) V6. The molecular identity of the involved channels was confirmed using RNA interference. TRPV6-mediated Ca^{2+} influx critically depends on Src kinase activity. Src kinase activation occurred independently of G-protein activation, presumably by direct interaction with OR51E2. Thus, Src kinase activation functionally links OR51E2 and TRPV6.

In summary we report the first endogenously expressed OR that signals independently of G-proteins but via direct binding of Src kinase. Ultimately this signaling leads to a Ca^{2+}-influx via TRPV6 channels.

Pyrethroids alter sodium channel gating in honeybee olfactory receptor neurons

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Social structure in a domestic honeybee colony relies on inter-individual chemical communication. Chemical communication is mediated by odors and pheromones detected by olfactory receptor neurons (ORN) localized in antennae. The Colony Collapse Disorder (CCD) has been described in many countries around the world and one of its main symptoms is the desertion of the hive by adult worker bees, leaving the queen with brood and a small number of young bees only. Our hypothesis is that such a desertion could be a consequence of a disruption in the colony cohesion due to a defective peripheral olfactory system. Recent studies have shown that several insecticides can be found inside the hive, including members of the pyrethroid insecticides familly widely used in agriculture (e.g. in *USA*, Mullin et al. 2010 *PLoS One* 5).

To study the consequences of an exposure of isolated ORNs to pyrethroids insecticides, we used electrophysiology. ORNs from honeybee prepupae were isolated and kept in cell culture according a procedure described earlier (Laurent et al. 2002 *Eur J Neurosci* 15). A disposable gravity driven perfusion system or a multicapillary system were used to control extracellular solutions composition and allowed exposure of ORNs to various insecticides and neurotoxins. In appropriate ionic conditions, we characterized under voltage-clamp the sensitivity of the voltage-dependent sodium current (INa) to the fish toxin tetrodotoxin (TTX), and to type I and type II pyrethroids.

Our results show that pyrethroids induce a TTX-sensitive tail current upon repolarization by slowing down the sodium channel closing. On average, after a single 3 ms stimulation, 10 μM permethrin or tetramethrin modify 20 ± 4 and 22 ± 7% of sodium channels respectively. Evidence for a higher percentage of modified channels at these concentrations are given by repetitive stimulations, since more than 80% channels happen to be modified by a train of 10 pulses at 35 Hz. Similar percentages were obtained in the presence of some invertebrate toxins. Whereas the sea anemone toxin ATX-II slowed inactivation by a factor 150, the scorpion toxin AaHII had no effect at a concentration of 20 nM. Interestingly, ATX-II increased the maximal current peak amplitude by a factor 1.5, which suggests that a large fraction of channels inactivate without opening at Vmax. ATX-II has no effect on current deactivation. In the presence ATX-II, a single 3 ms pulse give a maximal tail current amplitude and a fraction of modified channels close to 80%, a result that challenges the hypothesis of a use-dependent effect. The pyrethroid-induced modification of sodium channels involved in action potential could be responsible for abnormal information processing in antennal olfactory receptor neurons and would lead to defective detection of odors and pheromones if pyrethroids reach sufficient concentrations *in vivo*.

Acknowledgements: authors thank INRA-Santé des Plantes et Environnement and Région PACA (PhD fellowship to A.K.).

Ca^{2+}-activated Cl^- channels of the ClCa family are present in rat olfactory cilia and may participate in odor transduction

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The Ca^{2+}-activated Cl^- channel (Cl_{Ca}) is a key constituent of odor transduction. Odor binding to a specific receptor in the cilia of olfactory sensory neurons (OSNs) triggers a cAMP cascade which mediates the opening of a cationic cyclic nucleotide-gated channel (CNG), allowing Ca^{2+} influx. Ca^{2+} ions activate Cl^- channels, generating a prominent Cl^- efflux, which substantially contributes to the receptor potential. In spite of its fundamental role of in chemotransduction, the molecular identity of the Ca^{2+} activated Cl^- channel remains controversial. We explored the possibility that any of the members of the ClCa family of Ca^{2+}-activated Cl^- channels expressed in the rat nervous system, ClCa4l and ClCa2, may

correspond to the odor transduction channel. We addressed this question by performing nested RT-PCR of whole epithelia and single OSNs, establishing that both channels express in these neurons.

Full length sequencing of amplified ClCa expressed in rat olfactory epithelium indicated that ClCa4l is the most abundant of both channels. Immunoblotting with an antibody generic for both channels revealed expression in the ciliary membrane. This result was confirmed by inmmunofluorescence in cryosections of the olfactory epithelium. In addition, by means of RT-PCR we detected TMEM16A and B, but not Best-2, in the rat olfactory epithelium.

Altogether, the evidence raises the possibility that ClCa4l and perhaps also ClCa2 underlie the transduction Ca^{2+}-activated Cl^- conductance in rat olfactory cilia.

Supported by MIDEPLAN ICM-P05-001, FONDECYT 1080653, TW007920 Fogarty Int Center NIH.

Calcium-activated chloride currents in olfactory sensory neurons and in HEK293 cells heterologously expressing TMEM16B

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Olfactory transduction takes place in the cilia of olfactory sensory neurons. Odorant molecules bind to odorant receptors on the ciliary membrane, activating a second messenger enzymatic cascade that produces a cAMP concentration increase. Cyclic nucleotide-gated channels allow Na^+ and Ca^{2+} influx and Ca^{2+} ions open Cl^- channels. Olfactory sensory neurons maintain an elevated Cl^- concentration in the cilia; therefore Cl^- ions will exit, amplifying the odorant response.

The Cl^- component accounts for most of the odorant-induced depolarizing current and thus is critical for olfactory sensation; nevertheless the molecular identity of the olfactory calcium-activated chloride channel is not definitely established.

TMEM16B, a member of the new transmembrane protein TMEM16/Anoctamin family, has been shown to produce Ca^{2+}-activated Cl^- currents when heterologously expressed in HEK293 cells.

Moreover different groups recently showed that the TMEM16B gene is selectively transcribed in mature olfactory sensory neurons, and protein expression is limited to the sensory cilia. These findings support the hypothesis that TMEM16B is a promising candidate to be part of the ciliary Cl^- channel and may contribute to the excitatory Cl^- current during odor detection.

Despite the emerging role of TMEM16B in the cilia of olfactory sensory neurons, a side-by-side comparison between its electrophysiological properties and those of the native channel involved in olfactory transduction is lacking.

In this study we used the whole-cell voltage-clamp technique to record currents elicited by selective Ca2+ photorelease in the cilia of isolated olfactory neurons from mouse and in HEK293 cells transfected with the olfactory splice variant of TMEM16B.

We determined the anionic permeability of both channels by substituting external Cl with other anions. To study the pharmacology we applied the following chloride current blockers at the external side of the membrane: Niflumic Acid, DIDS, SITS, NPPB and 9AC.

Our data show several similarities between the properties of the two currents despite some differences remain to be explained.

The comparison between the chloride current in the cilia of olfactory sensory neurons with that induced by TMEM16B expressed in HEK293 cells will be a further point to establish the molecular identity of the Cl^- component in the olfactory transduction.

Calcium-dependence of the TMEM16B-chloride channel: a candidate calcium-activated chloride channel in olfactory transduction

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Olfactory transduction in vertebrates involves a depolarizing chloride current activated by intracellular calcium. Indeed, the binding of odorants to receptors in the cilia of olfactory sensory neurons activates a transduction cascade via a cAMP-signaling, which results in the opening of cyclic nucleotide-gated channels (CNG). The consequent Ca^{2+} influx through CNG channels produces an increased Ca^{2+} concentration in the cilia that activates Ca^{2+}-activated Cl^- channels (CaCCs). Since olfactory sensory neurons maintain an unusually high intracellular Cl^- concentration, CaCCs produce an efflux of Cl^-. The Cl^- current contributes to the amplification of the primary depolarization due to CNG current. Despite the fact that Ca^{2+}-activated Cl^- current constitutes up to 90% of the total transduction current, the molecular identity of the Ca^{2+}-activated Cl^- channel (CaCC) is still elusive.

TMEM16B (also called ANO2) has been recently proposed to be the CaCC in olfactory sensory neurons and its functional properties closely resemble those of the native olfactory CaCC measured in inside-out excised patches. Channel gating is both Ca^{2+}- and voltage-dependent, but the mechanisms underlying the activation are still unknown. TMEM16B does not have any apparent canonical Ca^{2+}-binding site, but the first putative intracellular loop contains five consecutive glutamic acid residues. To test the hypothesis that this region is involved in channel activation, mutants of the glutamic acid residues were obtained and the functional properties of wild-type and mutant channels were measured and compared.

After expression of wild type and mutants of the mouse TMEM16B in a heterologous system (HEK293T), electrophysiological recordings with the patch-clamp technique in the inside-out configuration were performed. The exposure of the intracellular side of the channel to various Ca^{2+} concentrations rapidly activated a current both in wild-type and in mutant channels, indicating a direct activation by Ca^{2+}. Currents showed a time-dependent decrease (inactivation) at -50 mV in the presence of a constant high Ca^{2+} concentration and an irreversible rundown. The rectification properties of current–voltage relations were Ca^{2+}-dependent. In wild-type channels the current–voltage relation was outwardly rectifying at 1.5 μM Ca^{2+}, linear at 3.8 μM Ca^{2+}, and inwardly rectifying at 13 and 100 μM Ca^{2+}. The dose-response relationship of wild-type currents was obtained and fitted with the Hill equation. The

Ca^{2+} concentration for half-maximal current activation was 4.5 μM at -50 mV and 2.6 μM at +50 mV, while the Hill coefficient was >1. For some mutants the same analysis revealed differences in Ca^{2+}- and/or voltage-dependence.

These preliminary results indicate that the region rich in glutamic acid residues located the first putative intracellular loop could be involved in Ca^{2+}- and voltage-dependence of the TMEM16B channel.

Bioluminescence Resonance Energy Transfer studies demonstrate Constitutive and Specific Homo-oligomerization of the Human Olfactory Receptor OR1740, a Rhodopsin-like GPCR

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Olfaction confers mammals the ability to perceive a myriad of odorants. Olfactory clues represent an essential survival tool for food searching, avoidance of danger and reproduction. The olfactory receptors (ORs) belong to the large super-family of G protein coupled receptors (GPCRs). GPCRs have been documented to form oligomers thought to play important roles in receptor trafficking to the cell membrane, ligand binding, G protein coupling, and intracellular signaling. To date, no study has thoroughly elucidated mammalian ORs dimerization, even though heterodimerization of ORs was reported in rodents and insects (Hague et al, 2004; Neuhaus et al, 2005; Bush et al, 2007). The relationship between a GPCR activation and its association/conformation state remains unknown.

In a previous paper (Vidic et al, 2008), the bell-shaped dose-response curve observed for receptor interaction with odorants in the absence of OBPs (odorant binding proteins) prompted us to propose a model for OR-OBP-odorant ligand interaction. Our model is based on two hypotheses, the association of ORs into homodimers, and the competitive binding of odorant and OBP to the receptor. On the one hand, we elucidated the specific role of OBP for maintaining OR activity at high odorant concentration (Vidic et al, 2008). On the other hand, investigation of the association state of ORs was crucial to fully validate the adequacy of our whole mechanistic model.

Therefore, the human OR1740 receptor, heterologously expressed in *S. cerevisiae,* was again used to address whether mammalian ORs exist as homo-oligomers and whether this phenomenon may be involved in receptor signaling upon odorant ligand activation. Co-immunoprecipitation and bioluminescence resonance energy transfer (BRET) approaches were performed to demonstrate that the OR1740 receptor constitutively homo-dimerizes. Using membrane subfractions preparations, this constitutive homo-dimerization was shown to occur early in the biosynthesis pathway, in the endoplasmic reticulum (ER). Furthermore, agonist binding induced a modulation in BRET levels, suggesting a conformational rearrangement of the receptor related to its activation/signaling state. The bell-shaped curve observed for the BRET variation as a function of odorant ligand concentration corroborates our previous studies (Minic et al, 2005; Sanz et al, 2005; Vidic et al, 2008).

For the first time, our results unambiguously show that mammalian ORs can self-associate into dimers early in the ER, and are constitutively expressed at the plasma membrane. This study also demonstrates that ligand-mediated conformational change can promote an inactive state of the OR dimers at high ligand concentration. In conclusion, the existence of the OR homo-dimers supports and validates our model based on the tripartite OBP-odorant-OR partnership (Vidic et al, 2008).

The authors thank Dr Ralf Jockers and Dr Jean-Louis Baneres for fruitful discussions.

Odorant dependent secretion on the antennal surface in the cockroach, *Periplaneta americana*

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Temporal resolution is an important feature of olfactory perception allowing orientation towards an odor source. It substantially depends on stimulus inactivation rate. Until now, only the mechanisms of enzymatic degradation of pheromones were discovered and their role in source localization and mate finding was suggested.

Present study describes the odorant dependent liquid secretion onto antennal surface in response to plant-derived odors in American cockroach. Optical registration of odor stimulated antennae discovered the layer of clear liquid which was absent in non-stimulated or ambient air stimulated antennae. Long alcohol-sensitive sensilla which lack pores on the basal parts of their shafts did not change their responses over a 100 min of periodic stimulation with an odorant. Medium sized sex pheromone sensitive sensilla decreased their firing slightly. Decrease in action potential rates of short alcohol-sensitive sensilla in response to an appropriate odorant with time corresponded to the liquid accumulation. The mechanical blockage of pores at the basal parts of sensilla with this secretion is likely to be responsible for the effect.

The main questions arose are 1) what is the chemical composition of the secretion, and 2) whether brain is involved into mediating the signal of odorant presence. Freely behaving insects enhance antennal grooming when exposed to the olfactory stimuli, supposedly cleaning out the surplus of secretion with odorant dissolved in it. It is interesting to note that frequency of the leg grooming did not change under exposition to odor stimuli. Thus the displacement behavior as a reaction to odor is the less plausible explanation. The mechanism of odorant elimination described here seems to be non-specific and does not depend on biological significance of the stimulus.

The study was supported by RFBR grant #10-04-01042a

Processing of odour mixture complexity in newborn rabbits

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Humans have limited abilities to elementally process odour mixtures. In a mixture including less than 16 odorants we do not recognize more than 4 components, and in a mixture including more than 16 odorants we do not recognize any of them. At a certain level of complexity, mixtures are then configurally processed in humans[1].

Here, we asked for the level of complexity required to induce a configural processing of odour mixture in another species, the European rabbit, at an important period of development, the neonatal period. We used a 6 components mixture which forms Red Cordial (RC) blending in humans[2]. We have previously shown that rabbit pups efficiently extract all the elements from this complex mixture after learning RC mixture. However, they do not respond to the RC mixture after learning one component, which suggests that they do not recognize the learned component within the mixture[3]. One may hypothesized that perceptual blending occurs in this mixture and led, inr rabbits as in humans, to the emergence of an odour quality different from the quality carried by each component. Alternatively, one may also suggest that a 6 components mixture, whatever the mixture, contains too much un-learned information to allow the extraction of previously learned fractional information. To evaluate such possibilities we conditioned[4,5], in a first experiment, 55 one-day-old rabbits to one component of the RC mixture. We tested, 24h later, their oral response[4,5] to the mixture deleted of one component (5 possible quinary mixtures). Between 83 and 94% of the rabbits responded to the quinary mixtures. Moreover, in a second experiment, we began to evaluate the responsiveness of pups to the RC mixture but including various ratios of 2 of the 6 components or all of the 6 components. Final results will validate one or the other hypothesis and shed some light on whether the high number of information or the intrinsic blending properties of a mixture lead to a configural processing.

Supported by grants from the Burgundy Region, the EU-ERDF and IFR 92 to GC and TTD and by a grant from the French MESR to CS.

[1]*Jinks, A., and Laing, D.G. (1999). Perception 28, 395-404.*

[2]*Le Berre, E., Thomas-Danguin, T., Beno, N., Coureaud, G., Etievant, P., and Prescott, J. (2008). Chemical Senses 83, 193-199.*

[3]*Sinding, C., Thomas-Danguin, T., Schaal, B., and Coureaud, G. (2010). XXXII AChemS Annual Meeting, St Pete Beach, Florida,USA, April 20-25.*

[4]*Coureaud, G., Thomas-Danguin, T., Le Berre, E., and Schaal, B. (2008). Physiology & Behavior 95, 194-199.*

[5]*Coureaud, G., Hamdani, Y., Schaal, B. and Thomas-Danguin, T. (2009). The Journal of Experimental Biology, 212, 2525-2531.*

Banana odor blend processing in the olfactory circuitry of *drosophila melanogaster* – Linking gas-chromatography with optical imaging techniques

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Natural odors occur in complex blends consisting of many different components. Such a blend might be characterized by a single key compound (a single dominant compound) or by several compounds. The central question of this study was if a blend including several key compounds is characterized by the mere sum of these compounds or by non-linear compound interactions.

We addressed this question by investigating physiological responses to single odor compounds and the complete odor blend emitted from a banana. These fruits are one of the most favored breeding places and a highly attractive food source for the fruit fly, *Drosophila melanogaster*. In these flies olfactory sensory neurons (OSN) convey odor information from the antenna and the maxillary palps to the antennal lobes (ALs). Neurons expressing the same type of receptor proteins converge into spherical AL sub-structures called olfactory glomeruli. Here, OSNs synapse onto projection neurons (PN) carrying odor information to higher brain structures and onto local interneurons (LN) that synapse onto neurons of other glomeruli providing a network with modulatory capacity. The concert of active glomeruli in the AL is odor compound and odor blend specific. How the network processes simultaneous input regarding several different odor compounds to form a specific activity pattern is poorly understood.

Using coupled gas-chromatography / calcium imaging techniques active banana odor compounds were characterized and their activity patterns in the olfactory system of the fruit fly were described. Comparison between calcium dynamics among OSN input and PN output channels revealed significant differences due to network processing. Comparisons between single compound and whole banana blend stimulation responses revealed blend interactions only in few glomeruli.

We could observe multiple processing effects revealing that the processing of a natural banana odor blend is not linear. Network processes altered component representations and changed their similarity to the blend. The center of AL activity, glomerulus DM2, was most strongly activated by most blend components, both in excitatory and inhibitory interactions. The GC-imaging method thus allows detailed investigations of the olfactory network and will be a highly useful tool in future investigation of insect-insect and insect-plant interactions.

Candidate chemosensory ionotropic receptors in a lepidoptera

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Recently, a new family of candidate chemosensory receptors was discovered in *Drosophila melanogaster* (Benton et al, 2009). These receptors are related to ionotropic glutamate receptors (iGluRs) and thus were called ionotropic receptors (IRs) but, phylogenetically, they do not belong to the iGluR family.

Through Blast analyses of an expressed sequenced tag library prepared from male antennae of the noctuid moth *Spodoptera littoralis*, we identified twelve unigenes encoding proteins related to *D. melanogaster* and *Bombyx mori* IRs (V. Croset and R. Benton, personal communication). Their full length sequences were obtained and their expression patterns were determined though RT-PCR in different tissues and according to the developmental stages. Eight

putative IR transcripts appeared to be exclusively expressed in the chemosensory organs, antennae and proboscis, and their expression was restricted to late pupal and adult stages. The others, although expressed in various tissues, were all enriched in adult antennae, two of them being also expressed in larval antennae.

Analyses of the deduced protein sequences revealed considerable variations in the predicted ligand-binding domains and, like most *D. melanogaster* IRs, none retained the three glutamate-interacting residues found in iGluRs, suggesting different binding specificities. Phylogenetic analysis showed that these sequences were more closely related to *B. mori* and *D. melanogaster* IRs than to iGluRs.

Taken together, our data suggest that we identified in *S. littoralis* new members of the insect IR chemosensory receptor family.

*Benton,R., Vannice,K.S., Gomez-Diaz,C., & Vosshall,L.B. (2009) Variant ionotropic glutamate receptors as chemosensory receptors in Drosophila. Cell **136**, 149-162.*

Acknowledgements: We thank R. Benton and V. Crozet for providing unpublished *B. mori* IR sequences.

Conserved response to food compounds in olfactory receptor neurons of two scarab beetles, *pachnoda interrupta* and *P. marginata*

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Few studies have systematically addressed evolutionary changes in olfactory sensory neuron (OSN) assemblies, either by genetic drift or as an adaptation to specific odor environments. We have studied the sense of olfaction in two related scarab beetles, *Pachnoda interrupta* Olivier and *P. marginata* Drury (Coleoptera: Scarabaeidae: Cetoniinae), which are both opportunistic polyphages, feeding mainly on fruit and flowers. The two species occur in dissimilar habitats: *P. interrupta* is found in dry savannah, and *P. marginata* in tropical parts of equatorial Africa.

To study how these species may have adapted their sense of olfaction to their odor environments, we utilized single unit electrophysiology on olfactory sensilla with a wide selection of food-related compounds. Despite the differences in habitat, we found that the species shared most of the physiological types of OSNs encountered, although their proportions frequently varied between the species. The high degree of conservation in olfaction between the species implies that a similar sensory strategy is efficient for food search in both habitats. However, the presence of shifts in proportions of receptor neuron classes, and slight shifts in response profiles and/or presence of some OSN classes unique to either species, may reflect adaptation to a different set of hosts.

Dose-response recordings reveal a high degree of specificity of selected olfactory sensory neurons to single compounds typically emitted by plants, or known as defensive or pheromome compounds in other scarab beetles, i.e., chemically similar compounds elicit responses only when presented at doses increased by several orders of magnitude.

Expression of odorant binding proteins and olfactory receptors on the antenna of the mosquito *Anopheles gambiae*

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The afrotropical mosquito *Anopheles gambiae* is the major vector for transmission of malaria, causally linked to the requirement of a blood meal by female mosquitoes to complete their gonotrophic cycle. Female *A. gambiae* rely on their sense of smell to find a blood host, sugar sources and oviposition sites, while nectar feeding male mosquitoes mainly locate hosts plant odours. Both sexes of *A. gambiae* detect and discriminate volatile odorants by means of olfactory receptor neurons (ORN) located in sensory structures, named sensilla. The majority of sensilla populate the antennae, whereas much lower numbers are found on the maxillary palps and the probosces. After entering a sensillum through pores in the cuticle odorant molecules are supposed to be captured by odorant binding proteins, which solubilize the odorant molecules in the sensillum lymph and transfer them towards olfactory receptors in the dendritic membrane of the ORNs. Large and diverse gene families encoding 57 candidate odorant binding proteins (AgOBPs) and 79 putative odorant receptors (AgORs) have been annotated from bioinformatic screens of the *A. gambiae* genome. However, the number and topographic distribution of cells expressing the different AgOBPs or AgORs is largely unknown.

We have adapted a whole mount *in situ* hybridization method based on fluorescent riboprobes to analyse the antennae of *A. gambiae* mosquitoes. This allowed us to visualize in the female and male antenna the cells, which express subtypes of AgOBPs and AgORs. Specifically, using labeled probes for the odorant binding protein AgOBP1 and the olfactory receptor types AgOR1 and AgOR2 the topographic distribution of cells was determined, which express olfactory proteins that have been indicated to play an important role in the detection of human host odors.

This work is part of the ENAROMATIC project supported by a grant from the European Communitýs Seventh Framework programme (FP7/2007-2013) under grant agreement FP7-222927.

In vitro characterization of two antennal Odorant-Degrading Enzymes in the noctuid moth *Spodoptera littoralis*

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The olfactory system has evolved mechanisms for inactivating odors to minimize signal saturation. Various enzymes have been shown to degrade pheromones in insect antennae, suggesting that these Odorant-Degrading Enzymes (ODEs) could participate in odorant inactivation after interaction with receptors and play a role in signal termination. However, few functional data are available on purified ODEs and their precise function in the dynamic of olfactory responses is not yet clearly established. Moreover, if the diversity of Odorant-Binding Proteins and Olfactory Receptors has been well investigated among various insect species, such data on ODEs are still lacking.

By bioinformatic screening of a male antennal EST library from the moth *Spodoptera littoralis*, we have identified a high diversity of putative Odorant-Degrading Enzymes belonging to various families (cytochrome P450, esterases, UGT, GST). In particular, we have isolated 19 sequences of putative antennal carboxylesterases. This family is of great interest because *S. littoralis* uses a mix of esters as sex pheromone. Phylogenetic analysis showed that these genes were distributed into the various clades of carboxylesterases previously defined, in agreement with presumptively different cellular localisation and substrate preference. Expression patterns of the corresponding genes were studied between tissues and toward development by RT-PCR and qPCR, leading to the identification of several adult and antennal-specific or antennal-enriched genes, putatively involved in odorant catabolism (Durand *et al.* 2010). Two candidate enzymes, SlCXE7 and SlCXE10, were produced *in vitro* using the baculovirus expression system in order to test their catalytic properties. SlCXE7 was able to efficiently degrade the major component of the sex pheromone blend, the (Z,E)-9,11-tetradecadienyl acetate, contrary to SlCXE10, which efficiently degrades a green leaf volatile, the (Z)-3-hexenyl acetate.

In conclusion, we have for the first time revealed the occurrence of a high diversity of esterase genes expressed in the olfactory organ of an insect species. This diversity could reflect the fact that esters are widespread among semiochemical signals in insects. Moreover, our results suggest that this diversity could be associated with a functional specialization of the various enzymes toward different volatile acetate substrates.

Durand, N., G. Carot-Sans, T. Chertemps, N. Montagne, E. Jacquin-Joly, S. Debernard & M. Maibeche-Coisne (2010) A diversity of putative carboxylesterases are expressed in the antennae of the noctuid moth Spodoptera littoralis. Insect Mol Biol, ***19,****87.*

Molecular mode of action of insect repellents

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Broad spectrum of activity and a variety of effects are the hallmark of insect repellents suggesting these compounds target multiple molecular components. The study of DEET in particular exemplifies the seemingly contradictory molecular modes of actions these compounds exert on the olfactory system. Using the two-electrode voltage clamp of Xenopus oocytes expressing two highly divergent *Aedes aegypti* ORs, we show that insect repellents exhibit both activating and inhibitory activities on ORs and provide a parsimonious explanation for the mode of action of insect repellents on mosquito ORs.

This work was supported in part by a grant to J.C.D. from the Deployed War Fighter Protection (DWFP) Research Program, funded by the US Department of Defense through the Armed Forces Pest Management Board (AFPMB).

Octenol receptor of the yellow fever mosquito, aedes aegypti: comparison of activity of agonists and antagonists in heterologous expression and in vivo studies

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Mosquitoes are attracted to their hosts by a variety of odorants including CO2, lactic acid, ammonia and 1-octen-3-ol. Our recent expression of the octenol receptor, OR8, along with its obligate co-receptor, OR7, in Xenopus oocytes allowed for structure activity studies which revealed the specificity of the receptor for (R)-(-)-1-octen-3-ol and the responsiveness of the receptor to various analogs. Differential effects of known insect repellents on the receptor assemblage were also recently revealed.

Here we report results of electrophysiological recordings of neural activity from the "C" neuron within the capitate basiconic sensilla on the maxillary palps expressing OR8 in response to octenol analogs and insect repellents used in the heterologous expression studies. Our data demonstrate the specificity of the octenol receptor neuron to be primarily a product of the OR while other aspects of neuronal responses likely reside with other components of the sensillum.

This work was supported in part by a grant to J.C.D. from the Deployed War Fighter Protection (DWFP) Research Program, funded by the US Department of Defense through the Armed Forces Pest Management Board (AFPMB).

Morphology and electrophysiology of olfactory sensilla on the antennae of female cotton leafworm, *Spodoptera littoralis*

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We studied morphology and physiology of olfactory sensilla on the antennae of female cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Using Scanning Electron Microscopy (SEM) six morphological types (long trichodea, short trichodea, basciconic, coeloconic, auricilic and groovedpeg) of olfactory sensilla were identified.

Electrophysiological responses from 450 olfactory receptor neurons (ORNs) housing in the olfactory sensilla from two specific antennal segments (15^{th} segment from both, base and tip of the antenna) were recorded to unravel the mechanisms underlying host and oviposition site selection in female. Odor stimuli comprised of flower odours, green leaf volatiles (GLVs), induced plant odours, oviposition deterrents, general host plant odours and female own

sex pheromone components elicited strong, selective and specific responses in 91 and 82 ORNs from the 15th proximal and distal flagellomeres respectively. Altogether 29 plant origin and 5 pheromone stimuli were used.

Responding ORNs from both segments were classified into fifty different ORN classes according to their response spectra. Fifteen ORNs classes were found commonly on both flagellomeres and the remaining were found either on dorsal or on proximal flagellomeres. The number of ORNs characterized was found very similar to the number of glomeruli characterized previously from the antennal lobe of female. The majority of the ORNs responded to only one or a few of the tested compounds. In general, only one of the two receptor neurons in a sensillum responded to any of the compounds tested except few cases where both receptor neurons housed in the same sensillum responded but to different stimuli. The majority of the receptor neurons displayed a high sensitivity to GLVs and floral compounds. Two morphological types were found physiologically less-responsive to tested stimuli. May this study will help to understand the olfactory system and host selection mechanism in lepidopteron species.

Anderson, P., Hansson, B.S. & Löfqvist, J. (1995) Plant-odour-specific receptor neurones on the antennae of female and male Spodoptera littoralis. Physiological Entomology, ***20****, 189-198.*

Anton, S. & Hansson, B. (1994) Central processing of sex pheromone, host odour, and oviposition deterrent information by interneurons in the antennal lobe of female Spodoptera littoralis (Lepidoptera: Noctuidae). Journal of Comparative Neurology, ***350****, 199-214.*

Couton, L., Minoli, S., Kiêu, K., Anton, S. & Rospars, J.-P. (2009) Constancy and variability of identified glomeruli in antennal lobes: computational approach in Spodoptera littoralis. Cell and Tissue Research, ***337****, 491-511.*

Hansson, B. & Anton, S. (2000) Function and morphology of the antennal lobe: new developments. Annual Review of Entomology, ***45****, 203-231.*

The ICE[3] project is funded by Swedish research council (FORMAS) and PhD scholarship is awarded by HEC, Pakistan.

Olfactory responses to neuro-transmitters in goldfish

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Olfactory signal processing in the fish olfactory bulb is mediated by several neurotransmitters, including glutamate, gamma amino butyric acid (GABA), acetylcholine and serotonin. Of these, only glutamate is a known food-related odorant with specific receptor proteins identified in the olfactory epithelium of Atlantic salmon (*Salmo salar*) (Pang et al, 1994) and channel catfish (*Ictalurus punctatus*) (Ivanova et al, 1993). Evidence of olfactory responses to other neurotransmitters in fish is limited only to the study of catecholamines and their metabolites in goldfish (*Carassius auratus*) (Hubbard et al, 2003). In present work we demonstrate the presence of membrane receptors to above mentioned neurotransmitters in the goldfish olfactory epithelium transcriptome and show that several are able to elicit olfactory responses, as measured by the electro-olfactogram (EOG).

Goldfish possesses a strong olfactory sensitivity to serotonin, glutamate and adrenaline but it is not responsive to GABA or acetylcholine. In the olfactory epithelium we could isolate mRNA for the neurotransmitter G-protein coupled receptors GABA (*gababr1*), glutamate (*mGluR1*), acetylcholine (*chrm5*), adrenaline (*adrb3*) and serotonin (*5-HT-1F*) together with subunits of receptor channels for glutamate (gria2), GABA (gabrg2) and serotonin (htr3a).

Absence of responses to GABA and GABA (B) receptor specific agonist baclofen, as well as to acetylcholine indicates that those neurotransmitters are not odorant stimuli in goldfish and rule out the possibility of their olfactory epithelium putative receptors being chemosensory and implying rather a regulatory role in this part of olfactory system.

Cross-adaptation with the glutamate type 1 receptor specific agonist quisqualic acid and with the serotonin type 1 receptor agonist 1-naphthyl-piperazine hydrochloride (1NPH) demonstrated that olfactory sensitivity to glutamate and serotonin relies on the identified receptors mGluR1 and 5-HT-1F, respectively. Also, responses to the agonists were specific and did not overlap with other types of odorants, such as amino acids or bile acids, or with each other.

In conclusion, we demonstrate for the first time specific olfactory responses to serotonin and provide evidence that classical neurotransmitter receptors can serve as chemosensory receptor. While the biological significance of olfactory sensitivity to this and other biogenic amines remains unclear, it is a useful tool to study olfactory transduction in fish. Since the signaling pathways for both glutamate and serotonin receptors are characterized and specific agonists identified, it can be used to evaluate the efficiency of adenylate cyclase or phospholipase modifying agents, therefore providing a direct link between any particular odorant and the signaling pathway it is activating.

Acknowledgements: While carrying out this work NK received doctoral fellowship SFRH/BD/12736/2003 from Fundação para a Ciência e Tecnologia, Portugal.

Olfactory sensitivity to Ca^{2+} and Na^{+} in the European eel (*Anguilla anguilla*)

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The European eel lives most of its life in fresh water but reproduces in sea water. Thus, we hypothesize that eel uses olfaction to sense environmental salinity and is consequently involved in physiological processes such as osmoregulation and migration. For this reason we expect that olfactory responses to the main cations present in the aquatic environment (Ca^{2+} and Na^{+}) will be mediated by olfactory receptors, and such responses will change according to the physiological status of individuals.

To test our hypothesis, we first assessed olfactory sensitivity to Ca^{2+} and Na^{+} by extra-cellular recording from the olfactory nerve of sea- or freshwater adapted eels. Also, we tested whether olfactory responses to Ca^{2+} may be mediated by the Ca2+-sensing receptor (Ca^{2+}-SR) by assessing olfactory sensitivity to two known agonists of the mammalian Ca^{2+}-SR; neomycin and spermine. The effect of the ion-channel blocker, tetracaine, was also assessed. Finally we studied the distribution of Ca^{2+}-SR in the olfactory epithelium by immunocytochemistry.

Freshwater or seawater adapted eels responded to increases in external [Na^{+}].

Conversely, freshwater eels respond to increases in external [Ca^{2+}] whereas seawater-adapted fish responded to reductions of external [Ca^{2+}] in a concentration-dependent manner. Together, these

results suggest that olfactory sensitivity in the eel is modulated according to the aquatic environment. Eels had high olfactory sensitivity to neomycin with an EC_{50} of 0.04 mM, similar to that of Ca^{2+} (0.11 mM). Furthermore, the I_{max} (1 mM) of the response was similar to that of Ca^{2+} (0.9 mM). Sensitivity to spermine (EC_{50}; 0.43 mM) was significantly less than that to Ca^{2+} but the I_{max} of the response was similar. Tetracaine inhibited the olfactory responses to both cations (less than 0.1 mM stimulus). The presence of Ca^{2+}-SR in olfactory epithelia was revealed by immunocytochemistry using antibodies raised against seabream Ca^{2+}-SR. These receptors were distributed throughout the olfactory lamellae in freshwater eels but confined to the interlamelae region in seawater adapted eels. Taken together, these results suggest that a Ca^{2+}-SR may be responsible for the olfactory sensitivity to calcium.

Olfactory sensitivity to inorganic cations has already observed in goldfish and seabream [1,2]. This sensitivity may aid osmoregulatory mechanisms and/or avoidance of potentially lethal salinities [1,2]. In the eel, sensitivity to changes in environmental [Ca^{2+}] and [Na^{+}] could play a role in migration to the spawning site.

1. Hubbard, P.C., Barata, E.N. and Canario, A.V.M. (2000). Olfactory sensitivity to changes in environmental [Ca^{2+}] in the marine teleost Sparus aurata. J. Exp. Biol. 203, 3821-3829.

2. Hubbard, P.C., Ingleton, P.M., Bendell, L.A., Barata, E.N. and Canario, A.V.M. (2002). Olfactory sensitivity to changes in environmental [Ca^{2+}] in the freshwater teleost Carassius auratus: an olfactory role for the Ca^{2+}-sensing receptor? J. Exp. Biol. 205, 2755-2764.

Funding: FCT grants SFRH/BPD/26339/2006 and POCI/BIA-BMC/55467/2004.

Oviposition in *Manduca sexta*: Behavioural responses, chemical stimuli, and their sensory perception

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In the field, *Manduca sexta* hawkmoths prefer to oviposit on plants from the Solanaceae and Martyniaceae families. Gravid females avoid previously feeding-damaged plants since the latter recruit parasitoids and predators through the release of volatile secondary metabolites. However, a systematic analysis of female egg deposition behaviour and the underlying neuronal processing of volatile stimuli mediating attraction to non-flowering plants is lacking.

We report on preferences of ovipositing females with respect to (a) plant species, (b) caterpillar feeding-damage, (c) chemically induced plant defence, and (d) larval faeces present on host plants. By combining gas chromatographic electrophysiological techniques and statistical methods we describe blends of components that are both perceived and characteristic for preferred and non-preferred plants at the species and the plant state level.

Single sensillum recordings reveal that headspace volatile collections from different host plant species elicit responses in different, i.e. plant species-specific arrays of sensilla. However, olfactory sensory neurons from the same array of sensilla respond whether headspace volatiles are collected from feeding-damaged or undamaged individuals of the same plant species.

Results from behavioural tests indicate that female avoidance of feeding-damaged plants compared to intact host plants is not mediated by plant-derived odorants alone but by the combined scent from the plant and larval faeces. Fatty acids are the predominant compounds emanating from the faeces. We demonstrate their physiological activity, which has hitherto been overseen.

Sensory organs on larval antenno-maxillary complex of *Melolontha melolontha* (Coleoptera: Scarabaeidae)

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Little knowledge is available on the chemosensory system of rhizophagous *Melolontha melolontha* larvae, which are known as major pest insects in European agriculture and horticulture (*1*). *M. melolontha* larvae are known to orient along a CO_2 gradient, but CO_2 receptive structures have not been localized and specified yet (*2*). In lepidopteran larvae, candidate structures for CO_2 reception are the digitiform organ and placoid structures on maxillary palpi (*3*). We identified similar structures on *M. melolontha* larval antennae and mouthparts. Further potential chemoreceptive areas of *M. melolontha* larvae are the apical termini of antennae, labial and maxillary palpi, each bearing various blunt sensilla of different shapes and possibly different functions.

We will present an overview of the chemosensory structures present on *M. melolontha* larval antennae and palpi, based on scanning electron microscopy and electrophysiological data.

1. R. Hauss, F. Schütte, Z Angew Entomol 82, 275 (1977).

2. T. Hasler, PhD thesis, ETH Zürich (1986).

3.T. A. Keil, Tissue Cell 28, 703 (1996).

Egg deposition elicits Brachiaria plants to produce defence signals that attract natural enemies antagonistic to a major cereal pest

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Plants subjected to insect attack usually increase volatile emission which attracts natural enemies and repels further herbivore colonisation. Less is known about the capacity of herbivores to suppress volatiles and the multitrophic consequences thereof. In our study the African forage grass, Brachiaria brizantha, was exposed to ovipositing spotted stemborer, Chilo partellus, moths. A marked reduction in emission of the main volatile, (Z)-3-hexenyl acetate (Z3HA), occurred following oviposition but the ratio of certain other minor component volatiles to Z3HA was increased. While further herbivore colonisation was reduced on plants after oviposition, the new volatile profile caused increased attraction of an adapted parasitoid, Cotesia sesamiae. Our results show that insect

responses are dependent on the quality of volatile emission rather than merely the quantity in this multitrophic interaction.

Macroanatomy and untrastructural studies on olfactory sensory epithelial components of *Pseudapocryptes Lanceolatus* (Bloch and Schneider)

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Olfaction is important modality of chemoreception, which mediated through olfactory apparatus and analyzes the chemical nature of the environment. The present study deals with the macroanatomy and cytological details on sensory components of olfactory apparatus of *Pseudapocryptes lanceolatus* (Bloch and Schneider) in relation to their role in olfaction. To study the olfactory sensory components of *P. lanceolatus*, the olfactory apparatus was dissected out, fixed (aqueous Bouin's fluid) and examined under light microscope (LM) and the tissue was further fixed in 2.5% glutaraldehyde in 0.1(M) phosphate buffer (pH. 7.2 – 7.4) for 1hour at 4° C and examined under transmission electron microscope (TEM: MORGAGNI- 268D) operated at 40kV respectively. The olfactory apparatus of *P. lanceolatus* is a unilamellar structure and associated with ethmoidal sac and lachrymal sac. The olfactory apparatus is externally lined by olfactory epithelium. It is a pseudostratified structure and comprises of different type of cells *viz.*, sensory receptor cells, supporting cells, basal cells, *etc.*. These cells are morphologically different. Among the epithelial cells, sensory receptor cells are the bipolar neuron and possess dendron, perikaryon and axon. There are two type of sensory receptor cells present within the olfactory epithelium *i.e.,* ciliated sensory receptor cells, microvillous sensory receptor cells. In both the sensory receptor cells, dendron moved towards the nasal cavity but the length of the dendron differs among the sensory receptor cells. The tip of the dendron protrudes to form the olfactory knob. In ciliated sensory receptor cells, the olfactory knob shows cilia but in microvillous sensory receptor cells, the olfactory knob possesses numerous microvilli. The basal body of the cilia is associated with well developed centriole. Numerous neurotubules are linearly arranged within the cytoplasm of dendron and passes through the perikaryon towards the end of axon. This structure is present only in both types of sensory receptor cells. The perikaryon shows spherical nucleus with greater euchromatin materials. Rough endoplasmic reticulum (rER) is present at the peripheral region of the nucleus. Ribosomes are closely associated with nuclear membrane. It is also evident that free ribosomes and polyribosomes are also present in between the lamellae of rough endoplasmic reticulum. It is assume that polysomal structures are the active area of protein synthesis in sensory receptor cells. Golgi complex is also noted within the cytoplasm. The distribution of the mitochondria at dendron, perikaryon and axon region is clearly marked with adequate density. The end of the axon shows large accumulation of synaptic vesicles. Our observation indicates that these structures of sensory receptor cells are the notable area for olfactory signal propagation as well as neural transmission.

Smell of death elicit evasive behavior in zebrafish

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Trace amine-associated receptors (TAARs) have gained substantial interest as olfactory receptors for trace amines and their metabolites. In a recent study, we have delineated the taar gene family in jawless, cartilaginous and bony fish (zero, two, and more than hundred genes, respectively). We conclude that taar genes are evolutionary much younger than the related OR and ORA/V1R olfactory receptor families. Phylogenetic analysis of taar genes from 14 species show that they fall into 3 classes. Two classes of teleost taar genes, but not the third, have retained the aminergic ligand-binding motif, a characteristic motif in mammalian TAARs. Within the third class, the dn/ds analysis suggests both minimal global negative selection and an unparalleled degree of local positive selection. Taar genes from all three classes are expressed in subsets of zebrafish olfactory receptor neurons, supporting their function as olfactory receptors. The highly conserved taar1 gene is not expressed in the olfactory epithelium and may constitute the sole remnant of a primordial, non-olfactory function of this family. Expression of most TAAR genes in larval zebrafish is restricted to olfactory epithelium.

For some mammalian TAARs amine ligands have been identified, although their biological function is yet to be understood. Species-specific rapid evolution of taar gene repertoires suggests an independent evolution of teleost taar ligands. We have identified diamines as specific ligands for a taar receptor. Diamines occur naturally (putrescine, cadaverine) as bacterial breakdown products of basic amino acids and as such indicate decaying organisms. These diamines activate a sparse subset of olfactory sensory neurons as indicated by c-fos expression in olfactory epithelium. Zebrafish exposed to even low concentration of diamines show strong aversion, while food and water controls show the expected behavior (attraction and no response, respectively). Ligand binding and behavioral responses are saturable with increasing concentrations of diamines. The ligand spectrum of the TAAR receptor closely parallels the behavioral effectiveness of these diamines. Our results are consistent with the existence of a defined neuronal microcircuit in vertebrates that elicits a characteristic behavior upon activation of a single olfactory receptor, a novum in the vertebrate sense of smell.

An edibility/toxicity sensor in the nose

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The mammalian nose is equipped with several structurally and functionally distinct chemosensing subsystems. One such subsystem is the septal organ of Masera (SOM). The SOM is a distinct patch of ~10,000 olfactory receptor neurons situated in front of (ventral to) the main olfactory epithelium. Most SOM receptor neurons express one receptor type, half of them expressing a

receptor named SR1, and the remaining half predominantly expressing one of eight different receptor types. In other words, the SR1 response profile dominates the SOM response profile. Despite nearly 70 years of research, the functional role of the SOM remains unknown.

In a critical recent effort, Grosmaitre et al. (2009) measured the response of mouse SOM receptor neurons to many different odorants. They found strong responses to a variety of compounds. Because the responses were not restricted to any known chemical group, the authors concluded that the SR1 receptor has an unusually broad response profile.

Here we tested the hypothesis that the SOM may serve as a mechanism for initial characterization of odors. One of the critical initial decisions an animal must make regarding an odor is whether it is edible or toxic. To ask whether the SOM is tuned to odorant edibility/toxicity, we obtained the lethal-dose 50 (LD50) values for the odorants tested by Grosmaitre et al. Strikingly, SOM neural responses were significantly correlated with LD50, whereby increased response reflected higher edibility and lower toxicity ($r = 0.6$, $F = 18$, $p < 0.0002$). In other words, whereas the SOM was broadly tuned in terms of chemical structure, it was in fact narrowly tuned in terms of ecological significance. It was tuned to an edibility/toxicity axis.

To ask whether SOM edibility/toxicity-tuning is reflected in behavior, we investigated whether toxicity influenced preferences between 32 odorant pairs in five C57BL/6 mice per odorant comparison. We found that LD50 values predicted mice preferences for both scented drinking water ($T(31) = 4.8$, $p < 0.0001$) and a scented environment ($T(9) = 2.9$, $p < 0.05$). We are now testing these preferences in SR1-deleted mice.

The Grueneberg ganglion – a dual sensory organ?

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The Grueneberg ganglion (GG) is a cluster of neuronal cells in the anterior nasal region which project axonal processes to the olfactory bulb and express the olfactory marker protein (OMP). Moreover, GG neurons are endowed with distinct olfactory receptors, indicating that the GG is not a homogenous population of neurons and that it may operate as a chemosensory subsystem. In search of stimuli activating the GG, we have found that a large subset of murine GG neurons is activated by cool ambient temperatures. These coolness-induced responses were particularly intense in neonates and restricted to a subset of GG neurons.

Since a line of evidences suggests that the GG serves a chemosensory function, it was assessed for responsiveness to an array of odorants. These approaches revealed that GG neurons were activated by a limited number of chemically related odorous substances. Responses induced by these odorants were dose-dependent and confined to subsets of GG neurons. The odor-evoked responses were detectable in early postnatal stages but not in the GG of juvenile or adult mice. However, responsiveness to GG-activating odorous substances was not restricted to the GG but also occurred in the main olfactory epithelium.

The observation that GG neurons are activated by cool temperatures and given odorants indicates that these cells might function as dual sensors. In fact, cool temperatures were found to enhance odor-evoked responses of GG neurons. These findings support the concept that the GG of neonatal mice operates as a dual sensory organ which is activated by both odorous compounds and cool ambient temperatures.

This work was supported by the Deutsche Forschungsgemeinschaft.

Investigations of the multisensory modalities of the mouse Grueneberg ganglion

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Four distinct olfactory subsystems compose the mouse olfactory system, the main olfactory epithelium (MOE), the septal organ of Masera (SO), the vomeronasal organ (VNO) and the Grueneberg ganglion (GG). They are implicated in the sensory modalities of the animal and they evolved to analyse and discriminate molecules carrying chemical messages, such as odorants and pheromones.

We first investigated the morphology of the most recently described olfactory subsystem, the Grueneberg ganglion. We found that, under a water-permeant keratinized epithelium, glial cells and ciliated neurons compose this olfactory ganglion. These particular morphological aspects are similar to the olfactory AWC neurons of *C. elegans* which were used for further functional comparisons. Investigations of the signalling elements present in GG neurons revealed that, as for AWC neurons, or pGC-D expressing neurons of the MOE, proteins participating in a cGMP cascade were found, such as the particulate guanylyl cyclase pGC-G and cyclic nucleotide-gated channel subunit CNGA3. We found pGC-G expression to be restricted to neuronal primary cilia, while CNGA3 was found not only in cilia but also in axons and neuronal somata. Calcium imaging experiments, performed with a membrane-permeant analogue of cGMP, the 8-Br-cGMP on acute mouse Grueneberg ganglion slice preparations, confirmed that cGMP could be a second messenger implicated in the transduction machinery of these neurons. Interestingly sensory neurons such as olfactory neurons of the MOE, of the SO and AWC neurons respond to both chemical and physical stimuli. Similarly, we found GG neurons to detect highly volatile chemical molecules and temperature changes. Indeed, the presence of alarm pheromones (APs) secreted by stressed mice, elicited strong neuronal stimulation. The transient response to APs, recorded in almost all tested GG neurons, was dependant from calcium from both extra and intracellular origin. The linear temperature sensitivity was observed in 72% of GG neurons and it was exclusively dependant of extracellular Ca^{2+} entry. The presence of parallel transduction pathways might explain the dual sensing properties of GG neurons. We investigated the physiological relevance of these different sensing modalities for the mouse. The isolated nature of the GG at the tip of the nose makes it suitable for lesion experiments, allowing axonal projection bundles of GG cells to be axotomized. Thermotaxis and huddling behaviours were evaluated with a thermal camera and demonstrated that GG axotomy did not influence the pup performances in these early life mouse

behaviours linked to functional temperature sensing. On the other hand, axotomy has, as a consequence, a total abolishment of the stereotypic freezing behaviour elicited by the presence of APs.

In summary, the multisensory modalities of the mouse Grueneberg ganglion is described and is related to a high degree of complexity required for the animal to sense its environment.

Subsystem-specific odorant-processing in the main olfactory system of larval Xenopus laevis

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The main olfactory epithelium (MOE) of larval *Xenopus laevis* contains at least two subsets of olfactory receptor neurons (ORNs). One subset lacks the canonical cAMP transduction pathway and responds to amino acid odorants, the second subset responds to pharmacological agents activating the cAMP cascade. The ORN axons of these subsystems project to distinct glomerular clusters in the main olfactory bulb (MOB). The 'amino acid-sensitive' cluster is situated in the lateral MOB, the 'cAMP-specific' cluster in the medial MOB. Furthermore these two clusters have been show to have an unequal composition of presynaptic proteins. Together, this shows that the main olfactory system of this species is made up of at least two different and substantially diverse subsystems.

Here we set out to allocate groups of waterborne odorants (bile acids, amines, alcohols, amino acids, nucleotides) to the abovementioned subsystems using functional Ca^{2+} imaging in acute slices of the olfactory system. We recorded odorant-induced responses of ORNs in the MOE and glomeruli of the MOB. We also tested whether ORNs responsive to specific groups of odorants are coupled to the cAMP-dependent transduction pathway. Also, by retrograde labeling of ORNs via electroporation of biocytin into the lateral and medial part of the glomerular layer, we were able to show that the two subsets of ORNs have a spatially distinct distribution in the MOE.

The outcome of the present work is a further step in an effort to understand the functional significance of the different subsystems that coexist in the main olfactory system of larval *Xenopus laevis*.

Supported by grants from the DFG Schwerpunktprogramm 1392 (project MA 4113/2-1) to I.M., and the DFG CMPB (Project B1/9) to I.M. and D.S.

Sensory aspects of offensive odours: "*musty*" is not always perceived the same

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In literature, the odour character of several compounds e.g. 2,4,6-trichloroanisole, 2,4,6-tribromoanisole and geosmin are described as musty (Curtis, 1774; Buttery, 1976, Mottram, 1998).

Interestingly, a musty compound can either be accepted as characteristic flavour in foods, but also rated as off-flavour compound. For example, geosmin is one of the main flavour impact of beetroot (Mottram, 1998) but has also be identified as the reason for off-flavours in drinking water industry (Suffet, 1999).

Also, direct orthonasal or retronasal comparison of "musty" compounds results in a clear perceptual differentiation. E.g. when asking a trained panel to name more specific odour character attributes, the annotations obtained can vary to a major extent.

For example, in the case of 2,4,6-trichloroanisole, geosmin and 2,3-dimethyl-5-ethylpyrazine, the description "musty" is elaborated by adding the attribute "earthy" for geosmin and 2,3-diethyl-5-methylpyrazine, while the descriptor "cork-like" is clearly related to 2,4,6-trichloroanisole. Still, when asking an untrained panel, "musty" is obviously used as the universal term for a broad range of substances.

Regarding to that, the aim of the present study was to test if untrained panelists are able to differentiate and name the sensory differences between"musty" compounds.

Within a consumer survey, 200 untrained panellists were asked to describe the odour of the respective substances. In a first session the panellists were asked to describe the odours using their own words. In a second session, four attributes were provided based on previous sensory evaluations by a trained panel. These were most specifically: corky, leatherlike, earthy and musty. The panellists were asked to choose among these attributes according to their orthonasal sensory impressions. In addition, hedonic rating of panelists was recorded.

The data obtained from the untrained panel in comparison to the trained panel will be presented, and will be discussed in relation to the complexity of the descriptor *musty*.

The authors acknowledge the support of Fraunhofer IVV in conducting these investigations.

Buttery, R. G., Guadagni, D. G., & Ling, L. C. (1976). Geosmin, a musty off-flavor of dry beans. Journal of Agricultural and Food Chemistry, 24(2), 419-420

Curtis, F., Dennis, C., Gee, J. M., Gee, M. G., Griffiths, N. M., Land, D. G., et al. (1974). Chloroanisoles as a cause of musty taint in chickens and their microbiological formation from chlorophenols in broiler house litters. J Sci Food Agric, 25(7), 811-828.

Mottram, D. S. (1998). Chemical tainting of foods. Journal of Food Science and Technology, 33, 19-29.

Suffet, I. H., Khiari, D., & Bruchet, A. (1999). The drinking water taste and odor wheel for the millennium. Water Science and Technology, 40(6), 1-13.

Determinants of the pleasantness of odor mixtures

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Objective: To investigate if pleasantness of odor mixtures is determined by (linear) combinations of the pleasantness of the components and whether odor pleasantness can be deduced from the chemical nature of the odor.

Methods: Thirty seven Ss participated in the experiment. Stimuli consisted of 19 *natural* (food) odors and 52 different mixtures of the 171 possible binary mixtures. The (component) odors varied in chemical complexity, consisting of between 40 and 800 different

molecules each. Ss sniffed and rated the following properties of each of the 71 stimuli contained in small vials and presented in randomized sequences: pleasantness, intensity, perceived complexity and novelty. Ratings were repeated 2-4 times and were divided over two sessions on separate days.

Results: Partial least squares analysis demonstrates that pleasantness is inversely correlated with perceived complexity and novelty. Pleasantness of odor mixtures is *not* solely determined by a linear combination of pleasantness of components as indicated by many examples where pleasantness

1) of a mixture falls below pleasantness of either of the components

2) of mixture (A,B) is different from pleasantness of mixture(A,C) even though odors B and C have similar pleasantness

3) of mixtures (A,B), A,C), (A,D) and (A,E) is similar despite very different pleasantness of B, C, D and E.

Perceived complexity is *not* correlated with chemical complexity.

Conclusions: A linear combination of pleasantness of component odors clearly cannot account for our data obtained with natural odors. Furthermore, since perceived complexity and novelty of a particular odor change with exposures to it and since pleasantness is strongly inversely correlated with these quantities, odor pleasantness cannot be deduced from the (unchanging) chemical nature of an odor.

Modulation of odor pleasantness with age

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The development of odor hedonic perception across the life span is still unclear. Is odor pleasure maintained during aging or is it impaired, like other aspects of olfaction? To answer this question we performed two experiments. In a first psychophysical study (experiment 1), we measured hedonic responses to odors in young and older adults and found that seniors rated pleasant odors as less pleasant than did young adults ($p<.005$). However unpleasant odors were rated identically by young and old adults. In a second study (experiment 2), we replicated the above findings using a physiological measure, namely olfactory evoked potentials. In line with the results from Experiment 1, we showed that the latency of the N1 wave of the olfactory evoked potentials was longer for old adults than young adults only for pleasant odors ($p<.04$). Taken as a whole, the present results suggest that olfactory pleasure is dulled with age while odor unpleasantness is maintained. They also strengthen the notion that aging is accompanied by ''olfactory anhedonia'', a trouble that may have serious effect on quality of life.

The effect of congruent and incongruent ambient odors on the evaluation of public goods

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A long series of studies showed that the ambient odors influence the high-level cognitive processes, including the economic decision making. For example, Hirsh (1995) showed that ambient odors modulate decisions in a risky gambling game. In particular, people are willing to bet higher amount of money in a room with a pleasant ambient odor than in an odorless one. The choice of the ambient fragrance is crucial. For example, the congruence between the odor and a product increases its perceived quality and tend to increase the sells (Bone & Jantrania, 1992).

Our paper focuses on the public goods (*e.g.* a forest ecosystem), which do not have a market price. In order to estimate their economic value was created the Contingent Evaluation procedure. In particular, we ask to the participants if they are willing to give an amount of money to save a forest ecosystem from a parasite infestation.

The aim of the study is to show the influence of the ambient odor on the evaluation of a public good.

Our hypothesis is that a pleasant and congruent odor (semantically related to the public good) will lead to an increase of the WTC (willingness to contribute).

Two fragrances were chosen with a pilot study. Vanilla and pine essential oil fragrances were similar in the intensity, familiarity, pleasantness and unpleasantness evaluation scores. The pine fragrance is semantic related to the forest ecosystem, while the vanilla fragrance is not related.

The experiment has a between subject design with 3 conditions of ambient odor stimulation (pine fragrance, vanilla fragrance and no odor condition). The fragrance was diffused in a previously aired room and the participants (N=270, 51,5% female) were not aware of the presence of the ambient odor.

The results show both a general effect of olfactory stimulation and a congruency effect on the WTC. In particular, in presence of an ambient olfactory stimulation, more participants are willing to contribute to the public good than in a room without fragrance.

Regarding the congruence effect, the results show three significantly different level of WTC. The conditions with a pleasant odor report a significantly higher level of WTC in comparison to the control condition (no odor condition/forest ecosystem). In addition, with a pleasant and congruent stimulus (pine/forest ecosystem) the WTC is significantly higher than the WTC in the condition with a pleasant but non congruent ambient fragrance (vanilla/forest ecosystem).

In conclusion, our findings support the hypothesis that ambient fragrances influence an economic decision like the willingness to support public goods.

Bone P.F., Jantrania S. (1992). Olfaction as a cue for product quality. Marketing Letters, vol.3, pp.289-296.

Hirsch A.R. (1995). Effects of ambient odors on slot-machine usage in a Las Vegas casino. Psychology & Marketing, vol.12, pp.585-594

Individual differences in the awareness of odours

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In the present study, we investigated whether individual differences in the subjectively reported level of odours awareness in the environment might be explained by demographic aspects or other individual parameters and to odour recognition abilities.

Seventy-six adults (29 women), aged between 20 and 56 years, took part in this study. Fifty-six were Fondazione Edmund Mach's employees while the remainders were students of the Viticulture and Oenology degree course of the University of Trento. The study was organised in six testing sessions: On every session, participants were presented with 12 familiar odours and were asked to recognize them. Olfactory stimulation consisted of 36 odours taken from the ''Nez du Vin'' masterkit [1] (sessions 1-3) applied on cotton wool placed inside glass vials with screw caps and of 36 compounds (sessions 4-6) dissolved in hydro-alcoholic solution and poured (20 ml) in plastic glasses with lids. On each trial, the participants had to smell the vial/glass content and to identify the substance by either clicking on one of the 90 names presented on the screen or by writing down a name by using a virtual keyboard. Before testing, participants were asked to fill in a questionnaire devised to collect demographic data and general information about them (e.g., age, smoking habits, etc.) and to complete a computerised version of the Odour Awareness Scale (OAS) [2]. Additionally on the first and last testing sessions, participants had to evaluate how good they believed their olfactory abilities were by using a 9-points rating scale. Data analysis was conducted by performing a series of one-way ANOVAs on OAS scores averaged for sub-groups of participants segmented by the factors under study. The results revealed that women were more aware of odours than men and that smokers appeared to give less relevance to odours than no-smokers. Age had a significant effect on OAS scores, with younger people (<30 years) being less aware of odours than older people. Additionally, people with food allergies were significantly more aware of odours than other people. As for performance on odour identification tests, there was no significant effect of the scores on the OAS test on the participants' accuracy. Moreover, the effect of self-evaluation of olfactory abilities was significant only at the beginning of the study, with people with high evaluation of their sense of smell being more aware of odours than people giving medium evaluations.

These results suggest that the level of awareness of odours is modulated by both individual (e.g., gender) and cognitive (e.g., self-evaluation) aspects whilst no relation between odour identification and OAS scores was found.

1) McMahon & Scadding (1996), Food Qual. Prefer., 20, 83-91.
2) Smeets et al. (2008), Chem. Senses, 33, 725-734.

Structure and variability of odor-related affective feelings

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In response to the need for a comprehensive approach to the wide range of affective feelings elicited by everyday odors, we developed the Geneva Emotion and Odor Scale (GEOS, Chrea et al. Chem. Senses 34: 49–62, 2009). The procedure used to construct this self-report scale was a 3-step reduction of 480 affective terms taken from the literature, rated by more than five hundred French-speaking participants for their relevance in describing affective feelings experienced when smelling a large variety of odorants. Similar scales were then developed in England (LEOS, Liverpool, N = 540 participants) and in Singapore (SEOS, N = 354 participants) using the same procedure (Ferdenzi et al., in preparation). Familiarity, intensity, pleasantness and identification scores were also collected in the last step of the procedure involving 56 odorants. The scales developed in the three cultures are constituted by 6 or 7 dimensions. Among them, four were interpreted the same way in the three cultures, although the terms comprised in these dimensions slightly differed: a) Sensuality Desire, b) Disgust, c) Energy and d) Happiness Well-being. Culture-specific dimensions also emerged, such as Spirituality in Singapore that was associated with the odors of incense and its ingredients and reflects the widespread religious use of incense in the Chinese community of Singapore (79% of our sample). Significant cultural differences were found on the four common affective dimensions, and on familiarity, intensity, pleasantness and identification for several odors such as durian, cinnamon and wood (repeated-measures ANOVAs, corrected for multiple testing). Sex and age were investigated as factors underlying inter-individual variation on these variables (2-way ANOVAs). Consistent with the literature, odors were better identified by women (2/3 of the participants), who also rated them as more familiar than men did. On the contrary, average pleasantness and affective ratings of men and women did not differ. Participants were divided into five age groups, from 16 to more than 55 years old. Although no age difference was found for pleasantness scores, there was a linear decrease with age for the negative but not for the positive affects (only the ratings of Well-being slightly decreased with age). Intensity, familiarity and identification did not vary with age and no significant gender by age interactions were found.

To conclude, the affective scales developed in three different cultures showed that odors trigger affective feelings that varied as a function of culture (partly due to exposure) and age (possibly due to more general changes in emotional responses), but not as a function of gender.

Odors eliciting Fear: a Conditioning Approach to Idiopathic Environmental Intolerance

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Individuals with Idiopathic Environmental Intolerance (IEI) seem to fear odorous chemicals. To determine whether IEI can be conceived of as an ''odor phobia'', we tested whether odors can elicit

stress responses (1); and whether odors, after association with fear compared to before, are evaluated as less pleasant (2)and avoided more (3).

Method: Using differential classical conditioning paradigm, an odor (either rotten egg as CS+ [CS: Conditioned Stimulus] and peach as CS-, or vice versa), were conditioned to an electrical shock as Unconditioned Stimulus (US). The acquisition phase consisted of 6 presentations of the CS+ followed by electrical shock and 6 presentations of the CS- alone. The extinction phase consisted of 6 CS+ and 6 CS- trials, with no US. Stress reponse was assessed via Skin Conductance Response (SCR); pleasantness via rating scales; avoidance via sniffing.

Results: SCR increased significantly to both odors, but stress to rotten egg did not extinguish (1); a significant (p =.03) change in pleasantness during acquisition was encountered, with the CS+ being liked less after conditioning (2); sniffing volume associated with the CS+ decreased immediately after the first trial(s), but reduction in volume was not significant over 6 trials (3).

Discussion: The finding that odors can be conditioned to stress responses, leading to reduced liking for these odors, support a fear conditioning conception of IEI.

However, the absence of extinction and avoidance of the CS+ are problematic in the light of the theory and additional research is needed, especially since extinction would be the treatment of choice in view of the theory.

Acknowledgements: NWO Vidi 452-03-334

Olfactory identification capacities in novice and wine experts

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In a number of olfactory complaints, qualitative deficits are reported. However, most of the available clinical tests focus on the olfactory sensitivity evaluation. We have designed a procedure to test the ability of different categories of patients to identify odorant sources. In short, the participants have to identify a series of artificial odour samples representing familiar odorant sources. Our test showed its utility in pathological situations. Now, we wanted to enlarge its use to non clinical purposes. We choose to test its applicability in the context of the wine expertise.

Comparing the olfactory sensitivities of wine experts and novices, several papers have concluded to the absence of difference of sensitivity between wine experts and novices. However, the quality of a wine is defined by experts who evaluate its organoleptic characteristics. Their odour identification capacities are decisive. Moreover, experts have developed semantic abilities to describe more precisely wines than novices. We explored the odour identification as a function of expertise using an original procedure: visual images and forced choice to facilitate identification. In this study, we compared olfactory identification abilities of 39 wine experts (46.5 ± 9.8 years old; 18 males), and 33 novices (43.8 ± 10.6 years old; 14 males). Smelling successively 47 odorous materials from vials, participants have to designate the corresponding image choosing between 5 photographs. The series of images included the target, two adjacent objects and 2 clearly not related. The presentation of the odorant stimulus and the response recording was assisted by a computer, allowing a regular timing of the test and recording of the response time delay.

The performances were significantly different ($F(1,70)=5.4$, $p=0.023$). Mean scores were higher in wine experts (75.3% ± 9.7) than in novices (69.9% ± 9.8). 50-63 years old showed an impairment of capacity of olfactory identification.

In order to reduce the difficulty of the test, it is possible to consider the "nearby" targets as good targets . In that condition, the performances of the both experimental groups are not significantly different, the novices reaching the level of the experts (i.e. about 90%). This sign of imprecision could suggest that the odorant representations of smelling objects proposed in the test are so not good or that the analysis of the odour perception is really not similar in experts and novices (Experts were more precise than novices in their responses). One interpretation of this difference is related to the fact that experts answer less faster than novices. Indeed, their response time delays were significantly longer ($F(1,70)=3.99$; $p=0.049$). Interestingly, the incorrectly identified stimuli in both groups are distinct.

Adaptation of UPSIT for RF population

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Olfactory system disorders received much clinical and experimental attention within last two decades as several severe neurological conditions have been associated with peripheral or central deficits of the olfactory system. Despite its importance no test assessing olfactory function is developed so far in Russian Federation. Our study aims to adopt one of existing olfactory –assessing tests for further use in population of Central Russia. The University of Pennsylvania Smell Identification Test (UPSIT) is a scratch and sniff test used in USA since 1984. The UPSIT is potentially applicable for population of other countries but obviously requires cross-cultural adaptation. One of the important steps of adaptation is detection of unidentifiable and unfamiliar odors within testing samples. In our preliminary study we tested the identifiably of odor samples present in the UPSIT 40-item test. All subjects were healthy adults of 19-35 years of age with no apparent olfactory problems, Moscow residents as well as people from rural area (n=140). As a control group we used individuals with known medical history; lack of allergic inflammation was confirmed by NO level measurement in the

nasal cavity. Average score of correct answers out of 40 odor samples was 34.95±2.34 which is comparable with scores reported for North America, Italy and Greece (Doty et al., 1984; Parola & Liberini, 1999; Economou, 2003). 50% of odors were identified correctly by 95% tested subjects. Though 3 odorants yielded alternative responses more frequently than correct ones: ''lime'' (89% error, most frequent alternate ''turpentine'' in 76.5% of cases), ''lilac'' (68% error, most frequent alternate ''whiskey'' in 77% of cases) and ''fruit punch'' (68% error, most frequent alternate ''soap'' in 90% of cases). More than 90% out of respondents reported a mismatch of their conception of ''lilac'' and ''grass'' odors and samples in the UPSIT. ''Fruit punch'' was named as unfamiliar odor by 63% of subjects. Among other text clues present in the UPSIT ''skunk'','' root beer'', ''pumpkin pie'' , ''musk'' and ''cheddar'' were named unfamiliar by more than 50% of respondents. Data obtained confirms potential applicability of the UPSIT for the assessment of olfactory function in Central Russia population. Further cultural adaption is required though.

Supported by RAS Programme ''Basic Science – for Medicine''.

Insulin, a survival factor for olfactory epithelium

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In mammalians, olfactory epithelium (OE) is continually renewed throughout adulthood. This epithelium is stratified, composed of progenitor cells at its basal region that give rise to olfactory neurons (ORNs) and to sustentacular cells that surround neurons. Due to their position in the nasal cavity, ORNs are exposed to multiple toxic factors and high levels of odorants, that induce their cell death. Therefore, the regenerative capacity of the OE is essential for the maintenance of olfactory function and requires tight controls of cell death and proliferation.

Our team is working on the influence of the nutritional status of the animal on its odors perception. OE, irrigated by the peripheral circulation, is under metabolic hormones influences. In this context, we have shown that OE is an insulin target. Most of OE cells displays high level of insulin receptors and we demonstrated that this level is up-regulated when circulating insulin level is low, following a 48 h diet. Furthermore, this hormone is able to reduce ORNs response to odorants*.

Some aspect of OE apoptosis has been described, showing caspase-3 and -9 involvement in this process**, however survival factors for OE remain to be characterized. In the present study, we examined the effect of insulin on primary OE cell cultures and on olfactive ODORA cell line. First, we showed that serum depletion in primary OE cells induced a mitochondrial-dependent apoptosis process, as assessed by flow cytometry after $DiOC_6(3)$/IP, that involved caspases activation, as shown by the inhibitory effect of zVAD-fmk. This apoptosis process can be accelerated in presence of etoposide, a DNA damage agent that induces p53 activity. Etoposide treatment of primary OE or ODORA cells in serum free medium induced the cleavage of both caspase-3 and caspase-9, as well as caspase-3 activity in primary cells. The addition of insulin in the culture medium partially protected OE cells from p53-dependent apoptosis. Specific cell death analysis by immnucytochemistry suggested that ORNs are very sensitive to p53-dependent apoptosis and can be partially rescued by insulin treatment.

These data were confirmed *in vivo*. Apoptosis was induced in OE by bulbectomy. The number of apoptotic cells was significantly decreased in animals receiving intra nasal insulin delivery. Our data present evidences that circulating or local insulin could be involved in OE renewing process.

**Lacroix MC et al. 2008, J Neuroendocrinol., 20:1176.*

***Cowan C, 2201, J Neurosci., 21:7099.*

The endocannabinoid 2-AG controls odor sensitivity in larvae of Xenopus laevis

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The search for food as well as the subsequent food intake is known to be guided among others by the sense of smell, and it has been suggested that the feeding state modulates the olfactory sensitivity. However, the underlying mechanisms responsible for the functional interaction between olfaction and food intake are as yet poorly understood. It is well-documented that the endocannabinoid system is important for energy homeostasis and nutrition at central stages. The endocannabinoid system may therefore functionally link the feeding state and the olfactory sensitivity of an animal.

In the present study we identified 2-AG as an endocannabinoid acting in the olfactory epithelium of larval Xenopus laevis. Suppression of 2-AG production delayed and decreased odorant-induced responses. Two differential synthesizing pathways exist in the olfactory epithelium: First, 2-AG is produced by diacylglycerol lipase β in olfactory receptor neurons and acts as an autocrine modulator at CB1 receptors on dendrites, second, 2-AG is produced by diacylglycerol lipase α in sustentacular supporting cells and acts as an paracrine modulator at CB1 receptors on dendrites. Synthesis of 2-AG in sustentacular supporting cells depends on hunger. Full animals exhibit low 2-AG concentrations in their olfactory epithelia. Vice versa, food-deprived animals have increased concentrations of 2-AG, which renders olfactory receptor neurons more sensitive. As a consequence odorant detection thresholds are decreased in these animals.

Taken together, the activity of the endocannabinoid system in the olfactory epithelium modulates olfactory sensitivity in a hunger-dependent manner and may thus control food intake.

Evidence for Multidrug resistance transporter activity in the olfactory epithelium of rodents

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We observed that intact main olfactory epithelia from mice and rat had only slow and impaired accumulation of fluorescent calcium indicators like Fura-2, fluo -3 and fluo-4. Fluorescent probes are well characterized substrates for multidrug resistance (MDR) transporters and limited dye uptake or extrusion of dye is often associated with MDR transporters which are ATP-driven export pumps.

To investigate the presence and the functional activity of MDR transporters in the olfactory epithelium we used the fluorimetric calcein–acetoxymethyl ester extrusion assay on olfactory epithelial slices of rodents. We applied verapamil or cyclosporin to block P-glycoprotein (MDR 1) transporters, and probenecid or MK-571, respectively, which are known inhibitors of multidrug resistance-associated proteins (MRP). Calcein uptake measured as an increase in fluorescence intensity was accelerated 1.3 - 2.5 fold in the presence of the four different MDR modulators in rat, and 1.7 - 2.3 fold in mice. This suggests that calcein was prevented from exiting the cells via mdr-mediated transport in the presence of these inhibitors.

To test, if MDR transporters may be involved in the olfactory response we examined odorant evoked field potentials using electro-olfactogram recordings (EOG), extracellularly recorded from a population of olfactory sensory neurons in response to odorants. We monitored EOGs responses from different locations of the main olfactory epithelium in response to two single and a mixture of odorants. In the presence of the inhibitors of probenecid or verapamil peak amplitudes of EOG responses from rat and mice in the different recording sites were significant smaller for all odorants tested. This suggests that P-glycoprotein- like and MRP-like transporters are functional in the main olfactory epithelium of rodents and exert a physiological effect on the olfactory response.

A.M is supported by a grant from the Fondation Edmond Roudnitska

Psychobiological effects of perinatal exposure to odorants in mice. Part I: Morphological changes in the olfactory epithelium

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Centre des Sciences du Goût et de l'Alimentation, UMR 6265 CNRS-Université de Bourgogne-INRA 15 rue Picardet 21000 Dijon, France. It is now well established that ciliated neurones embedded in the olfactory epithelium are the sites for odorant molecules detection through a cAMP transduction pathway. It is also known that odorant-evoked activity contributes to olfactory epithelium organisation and axon targeting to the olfactory bulb. However the influence of external factors such as long term odorant exposures on the olfactory epithelium remain unknown.

In order to answer this crucial question we have taken the following experimental approach. We exposed MOR23-IRES-tauGFP and M71-IRES-tauGFP mice to lyral or acetophenone daily for 21 days starting at birth. The odorant was placed as 20 µl of pure odorant on a small cotton pad placed in the cage. Animals were then weaned at P20/P21, killed and dorsal olfactory epithelia were dissected out from the septum. Tissue samples were fixed in PBS containing 4% Paraformaldehyde and 0.2% Glutaraldehyde, rinsed in PBS and flatmounted between slide and coverslip. Fluorescence images were taken using a 10 times objectives and the whole septal olfactory epithelium was reconstructed using the Image J software. GFP containing neurones were counted and plotted against the total septal olfactory epithelium surface. In control mice (without odorant exposure) MOR23 GFP containing neurones could be seen scattered across the whole dorsal olfactory epithelium without any clear distribution pattern. Their density was 81.75 +/- 5.63 neurones/mm2 (n=12) and no significant difference was found between the left (85.37 +/- 16.26 neurones/mm2, n=5) and the right side (83.18 +/- 5.92, n=5). Postnatal exposition to lyral dramatically reduced the density of MOR23-GFP containing neurones to 29.39 +/- 4.16 (n=10, $p<0.001$ compared to the control). In order to verify whether lyral only affect MOR23-GFP or has some unspecific effect we exposed postnatally M71-GFP mice to this odorant. In these conditions no reduction of M71-GFP containing neurones could be seen. Meanwhile, the postnatal exposition to acetophenone does not reduce the density of M71 neurones in M71-GFP mice. Further behavioural experiments were carried out and the resulting observations are presented in Oostindjer et al., (partII).

As a conclusion we can state that long term exposition to odorant does remodel the olfactory epithelium during development.

This work was funded by CNRS and Conseil Régional de Bourgogne.

Psychobiological effects of perinatal exposure to odorants in mice. Part II: Behavioural alterations

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Early exposure to odorants pre-, post- or transnatally is known to result in a decreased avoidance or increased preference for these odorants in mammals. There are also indications that olfactory sensitivity for these odorants can be affected. We exposed C57-Bl/6 mice of Mor23-GFP and M71-GFP lines to lyral (LY) or acetophenone (AC), respectively, either transnatally through the water of the mother ($1*10^{-5}$ M) or postnatally in air for 1 hr per day (20 µl pure).

Animals were weaned on postnatal day 20 (P20), and 3 tests were performed:

1) A 3-min exploration test on P20 in a rectangular arena presenting LY and AC under a wire mesh to measure the time spent in each half of the arena.

2) A 2-bottle choice test on P21, whereby LY and AC-flavoured water ($1*10^{-5}$ M) was provided to assess intake.

3) An olfactory sensitivity test based on an habituation procedure on P21 using two 2-min habituation trials with water and 6 dishabituation trials whereby either LY (in concentrations ranging from $9.77*10^{-9}$ to $1.0*10^{-5}$ M) or AC ($9.54*10^{-7}$ to $1.0*10^{-5}$ M) was presented to assess time spent exploring and number of visits to the stimuli, assuming increased exploration when the odorant is perceived.

Control animals of both lines displayed a preference for LY compared to AC in the exploration test (LY: 99 vs. AC: 81 s, Mor23: p=0.04, M71: p=0.03), while transnatal AC exposure resulted in an absence of preference for either odorant (91 vs. 89 s) and postnatal exposure in a higher preference for AC (78 vs. 108 s, p=0.02). In contrast, LY exposure decreased preference for LY, both trans- and

postnatally exposed animals showing no preference for either odour (transnatal: 89 vs. 91; postnatal: 86 vs. 94 s).

We furthermore show results of 2-bottle choice test, and the effects of LY and AC exposure on behavioural olfactory sensitivity. These results are linked with density of Mor23- and M71-receptor-expressing olfactory sensory neurons which show high affinity to LY and AC, respectively, presented in the poster by Tazir et al (Part I).

This research was supported by a doctoral grant from CNRS-Region Burgundy, and by continued support from Region Burgundy.

Influence of nutritional and metabolic status on different olfactory-driven behaviours tests: a way to regulate food intake?

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In humans, it is an every-day experience that nutritional status conditions both olfactory detection thresholds and detection, at least for food-related odours, so these modulations are part of the control of food intake (Albrecht et al, Rhinology, 2009). However, less information is available concerning possible modulations of odour detection in pathologies such as obesity and diabetes.

In rats, locomotor and sniffing activities induced by food odour and sensitivity to a neutral odour are increased by fasting (Prud'homme et al, Neuroscience, 2009; Aime et al, Behav Brain Res, 2007). We wondered if long-term modifications of nutritional or metabolic status were able to modify various olfactory-driven behaviours. Two different olfactory-based behavioural tests were performed, in rats either fed or after an acute period of fasting: i) food odour presented in tea-balls on top of cages (recording of locomotor activity and sniffing behaviour of the tea-ball) ii) hidden chocolate cookie test (latency to find the cookie under a clean bedding). Long-term modifications of nutritional status were induced in Wistar rats fed 2 hrs per day for 2 weeks, leading to 75% of control body weight. Restricted animals spent more time active and sniffing the tea-ball with food odour, and were also slightly more efficient in finding a hidden cookie than the control animals fed 8 hrs per day. We then decided to perform similar experiments on strains of rats with "genetic" metabolic modifications: obese Zucker rats lacking functional leptin receptors, and genetically lean Lou/C rats. At 2 months, Zucker fat rats were significantly more efficient in finding a hidden cookie than their lean controls, and than Lou/C and Wistar rats. At 4 months, both Zucker fat, Zucker lean and Wistar rats were more efficient than Lou/C rats, suggesting an inverse relationship between olfactory performance and body weight (Zucker fat: 500 ± 8 g, Wistar: 435 ± 6 g, Zucker lean: 372 ± 9 g, Lou/C: 247 ± 9 g). This inverse relationship was confirmed by the tea-ball test performed at this age: Zucker fat and Wistar rats are significantly more interested in food odour than their respective counterparts Zucker lean and Lou/C rats. Interestingly, after a 48 hrs-fasting period, the time spent sniffing the tea-ball was increased only in lean Zucker and Lou/C rats, leading to the conclusion that obese rats probably lost a feed-back mechanism linked to negative alliesthesia of food odour when satieted.

Since insulin and leptin receptors are present in the olfactory system (Baly et al, Brain Res 2007; Lacroix et al, J Neuroendocr, 2008), the role of metabolic hormones insulin and leptin in these behavioural modulations will be discussed.

Taste responses in the nucleus of the solitary tract of the actively licking rat

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There is increasing evidence that the active acquisition of a sensory stimulus (e.g., licking, sniffing, whisking, or moving the eyes) influences neural responses at multiple levels of the nervous system. Moreover, the effects of this acquisition process are often apparent at the first synapse at which sensory signals enter the brain. For example, in the gustatory system, mechanosensory, somatosensory and gustatory inputs generated by volitional licking of taste stimuli can interact in the nucleus of the solitary tract (NTS), the first relay in the central gustatory neuraxis. While much is known about NTS responses in the anesthetized animal, little is known about taste-related activity in the NTS during active consumption. To address this, bundles of eight microwires were chronically implanted into the NTS of male Sprague-Dawley rats. Following recovery, animals were water deprived and placed into a testing chamber with free access to water and tastant solutions. Taste stimuli were NaCl (0.1M), citric acid (0.01M), sucrose (0.05M), quinine HCl (0.0001M), monosodium glutamate (0.1M) and water, presented in randomized order for five consecutive licks followed by five water licks presented on a VR5 schedule. As in anesthetized rats, the majority of taste-responsive cells responded to multiple stimuli. In some cells, taste responses were apparent within 100ms after a stimulus was delivered, and often began within 20-40 ms. In other cells, taste responses took several hundred ms to begin. In still other cells, taste-related activity did not begin until after the taste stimulus presentation was terminated. Many cells showed lick-evoked activity in addition to taste-related firing. These data suggest that taste stimuli generate a spatiotemporal pattern of responsiveness in the NTS where different groups of cells respond at different times with respect to the first lick of a taste stimulus. Because it has been shown that rats can identify a taste stimulus within ~200 ms, we focused our analyses on this early response interval. The contribution of firing rate and temporal coding was assessed with a family of metrics that quantify the similarity of spike trains in terms of spike count and spike timing. These analyses showed that spike timing conveyed a significant amount of information about taste quality in a subset of cells in the earliest portion of the response but total information contributed by spike count and spike timing was relatively small (< 1 bit) during this response interval. Further analyses of simultaneously-recorded NTS cells suggests that

synergistic activity across groups of cells may underlie rapid taste identification. However, further elaboration of the taste response continues for several hundreds of ms, and may contribute to hedonic evaluation of taste stimuli and/or orofacial reactivity.

Supported by NIH grant RO1-DC006914 to P. Di Lorenzo.

The number of taste bud cells that belongs to each cell type is in equilibrium in fungiform and soft palate taste buds of mice

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Taste buds are functional units for sensing taste substances and for transmitting taste information to the taste nerves. Each taste bud consists of several cell types with different chemosensitivities. In addition, taste bud cells (TBCs) are short lived of ~10 days and turnover of TBCs continuously occurs in taste buds. Numerical studies on the expression of taste transduction-related proteins within a single taste bud are essential on understanding this complicated unit. We investigated the ratio of these cell types in number per taste bud in fungiform papillae and soft palates, classifying the cell type immunohistochemically with antibodies against PLCβ2 (a phospholipase), IP_3R3 (an IP_3 receptor subtype), gustducin (a G-protein α subunit), Gγ13 (a G-protein γ subunit), and SNAP-25 (a SNARE protein).

Here we show that the ratio is similar among taste buds in each locus, though the rate is different between the loci. The number of total TBCs and that of each immunohistochemical cell type linearly increase as a function of the area of taste buds in both loci. The slope of the regression lines for the number of respective cell types and total cells is steeper in soft palates. The order of slopes for each cell type is TBCs immunoreactive to IP3R3/PLCb2, Gγ13/gustducin, and SNAP-25 in fungiform papillae. That in soft palates is the same except the slope for TBCs immunoreactive to IP3R3/PLCb2 is close to that for Gγ13/gustducin. Few TBCs are immunoreactive to both SNAP-25 and others.

These results show that the number of each cell type depends on the area of taste buds in both loci, though TBCs are more tightly packed in soft palate taste buds than in fungiform ones, and suggest that the ratio of each cell type in number is in equilibrium in soft palate and, probably, fungiform taste buds. We discuss the mechanism that maintains the equilibrium.

This work was supported in part by funds Narishige Neuroscience Research Foundation and a Grant-in-Aid for Young Scientists (#20770124) by MEXT of Japan.

4-aminopyridine differentially affects spike generator and receptor potential in the labellar "salt" receptor of the blowfly *Protophormia terraenovae*

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In the blowfly the taste information is carried to the central nervous system by a spike activity the frequency of which is directly correlated to the receptor potential amplitude (Morita, 1992; Masala et al., 2009).

The present study investigates, in the labellar chemosensilla of the blowfly *Protophormia terraenovae*, if the spike frequency can be modulated independently from the receptor potential amplitude. To this end, the voltage-gated K^+ channels inhibitor, 4-aminopyridine (4-AP; Kim & Mistretta, 1993), was used.

By means of the extracellular side-wall technique (Morita, 1992), the receptor potential and the spike activity were simultaneously recorded following stimulation with 50, 150 or 500 mM NaCl, before, 5 and 10 min after the administration of 1, 10 or 20 mM 4-AP, dissolved in saline.

The results show that the receptor potential evoked by NaCl stimulations is not affected by 4-AP applications, while the spike activity is significantly decreased by all 4-AP concentrations from 5 min after the inhibitor administration. At the highest dose of 4-AP and at the longest interval from application (10 min), receptor potential amplitude is also decreased, and this may be explained as by a delayed effect of the inhibitor probe on the transduction mechanism or by a toxic concentration effect.

In conclusion, our data suggest that in the blowfly *P. terraenovae* the spike activity can be modulated without modifying the receptor potential amplitude.

Kim M, Mistretta CM. (1993). Brain Res. 612:96-103.

Masala C, Solari P, Sollai G, Crnjar R, Liscia A. (2009). J. Comp. Physiol. A 195:1141-1151.

Morita H. (1992). Zool. Science 9:1-16.

An attempt to establishment *in vitro* models for analyses of taste buds with clonal cell lines derived from murine tongue

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Taste bud is a small organ consisting of 50-100 cells, and the cells are immunohistochemically and morphologically categorized into 4 types. Details of cell differentiation and function of the gustatory organ remain unanswered.

Establishment of *in vitro* systems would stimulate analysis of taste buds. The strain of *p53*-deficient mouse has been shown to be a useful source to establish clonal cell lines from various tissues. We collected taste buds from a tongue of the mouse and established clonal cell lines (TBD cell lines). In this study, we characterized TBD cell lines and attempted to develop culture methods for the cell lines. Expression patterns of some taste receptors in four TBD cell lines, TBD-a1, TBD-a5, TBD-a7 and TBD-c1 were examined with RT-PCR. All of the lines expressed T1R3 for sweet and umami taste, and T2R8 for bitter taste. A candidate salty receptor alpha-ENaC was also expressed in the cell lines. While, a candidate sour receptor HCN4 was expressed in TBD-a1 and TBD-a7 cell lines, and another candidate receptor PKD2L1 was slightly expressed in TBD-a1 and TBD-c1 cell lines. Hence, to examine whether TBD cell lines can

response to taste stimuli, we performed calcium-imaging analyses. Some of TBD cell lines responded to citric acid, and a transient elevation of intracellular Ca^{2+} was elicited. The result suggests that these TBD cell lines can respond to sour taste stimuli, and could be useful models *in vitro* of taste receptor cells. Next, we attempted to develop a 3-dimensinal culture method for the cell lines. TBD cell lines cultured in collagen gels formed cell clusters that had an internal cavity. Deposition of laminin, one of marker proteins for basal membrane, was immunohistochemically detected at the surface of the clusters. In addition, electron microscopic observation revealed microvillus-like structures at the internal surface of the cavity and tight junction-like intercellular structures in TBD-a5 cell clusters.

These results suggest that TBD cells develop 3-dimensinal architectures with a basal membrane-like structure and apical-basal polarity. In conclusion, TBD cell lines are useful models for analyses of gustatory function and taste cell differentiation.

This work was supported by the Bio-imaging Center and the Open Research Center of Japan Women's University.

Intermittent delivery of salt or sugar distorts perception of saltiness or sweetness

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A gustometer was developed to deliver a fluid stream, with computer control over the concentration and timing of delivery of up to four tastants. An open-loop mouthpiece, with rapid waste removal, enabled rapid changes to stimuli. A method was developed to collect time-intensity data from a trained taste panel of ten human assessors.

It was hypothesised that a taste stimulus could be intermittently discontinued, but perceived intensity would be maintained. To test this hypothesis, a block design experiment, with two salt concentrations (0·8%, 0·4% w/v NaCl) each delivered over a range of six cyclical stimulus 'on' by six stimulus 'off' times (2 x 6 x 6 = 72 samples), was carried out each with a total sample duration of minimum 20s.

There was a strong positive correlation between the proportion of time that the stimulus was on and overall perceived intensity of saltiness; however the loss of perceived intensity of saltiness did not scale with the reduction in salt. The most extreme proportional reduction in salt content was perceived as equal in saltiness intensity to a continuous delivery of half the concentration. This is equivalent to a 70% reduction in salt content while maintaining perceived saltiness. This result held true for both salt concentrations tested.

The same hypothesis and design were used to test the perception of sweetness intensity with cyclical delivery of sugar solutions (15%, 6.7% w/v sucrose). As for salt, a comparison of continuous delivery with intermittent delivery demonstrated up to a 70% reduction in sugar delivered for an equal overall perceived intensity of sweetness at both concentrations.

Assessors' time-intensity curves did not follow the cyclical changes in salty or sweet stimuli, however some assessors reported changes in quality of the stimuli (from tastant to water or vice versa). To test assessors ability to detect the loss of tastant stimulus, assessors were tested with two concentrations of each of salt and sugar, and a set of timing strategies in which the stimulus was turned off once during the course of delivery. Assessors observed changes to the stimulus quality in all but the shortest 'off' times.

This work suggests a fundamental perceptual mechanism by which taste perception may be distorted by intermittent delivery of a tastant. If this mechanism can be exploited in the production of processed foods, then it may play a role in the reduction of salt and sugar intake, and consequent effects on public health.

EAG responses of the medfly *Ceratitis capitata* to fruit and foliage headspace of host-plants: a comparison between wild and lab reared insect populations

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The Mediterranean fruit fly, *Ceratitis capitata* Wied., is a worldwide pest for horticulture because of its high biological potential, the difficulty of control and the broad polyphagy, mainly addressed, in Southern Europe, to pomaceous (apples and pears) and citrus (oranges) cultures. Information in the literature on the olfactory sensitivity in the medfly is still incomplete and only a few data are reported on the responses to alcohols, aldehydes, esters and acids, but with no reference to the physiological state (Light et al., 1988).

Aim of the present work was to obtain further knowledge on the typology and topology of olfactory chemosensilla on both - medial and lateral - antennal surfaces in males and females. In addition, their electrophysiological activity has been evaluated in response to extracts of fruits and foliage from various host-plants and their primary compounds in two different - wild and lab reared - medfly populations, also in relation to the sex and to the physiological (virgin vs. mated) state of the insects.

HRSEM morphological analysis has highlighted the presence of 4 different olfactory chemosillum types on both the medial and lateral antennal surface which may be classified as basiconic, clavate, trichoid and grooved sensilla. No difference in number and distribution has been observed between sexes, except for the female lateral surface, where a statistically higher number of clavate sensilla was detected.

The EAG responses show that 1) the male olfactory sensitivity of lab reared medflies to headspace collected from fruits of lemon, orange, clementine, prickly pear, apple and their relative foliage was higher than that of females, while an opposite situation was found for wild insects; 2) same results were obtained when virgin and mated insects were considered; 3) virgin males and females displayed a higher sensitivity than the mated ones in the wild and lab populations, respectively; 4) within each population, the olfactory sensitivity was higher in mated males than mated females in lab population, while the opposite was found for wild insects.

The olfactory sensitivity of the medfly to host-plant fruits and foliage in relation to physiological state and rearing conditions is discussed.

Light DM, Jang EB, Dickens JC. (1988). J. Chem. Ecol. 14:159-180.

Morpho-functional characterization of taste labellar apparatus in the medfly *Ceratitis capitata* in response to host-plants

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The Mediterranean fruit fly (*Ceratitis capitata* Wied.) is a widespread polyphagous pest for horticulture, mainly targeting, in Southern Europe, pomaceous (apples and pears) and citrus (oranges) cultures. Despite this, information available in the literature concerning taste receptors in the medfly is largely lacking and only a few data on the responses of tarsal and labellar chemosensilla to salts and sugars are reported (Gothilf et al., 1971; Angioy et al., 1978).

Aim of the present work is to provide a morpho-functional map of taste labellar chemosensory apparatus, by investigating the relative distribution of different sensillum types and evaluating their electrophysiological activity following stimulation with a few compounds from the fruits of various host-plants, also in relation to the sex.

HRSEM analyses have given evidence of the spatial distribution of the taste chemosensilla on the labellar surface. In particular, 6 pairs of "largest" trichoid chemosensilla are found, each containing 4 different neurons, as also confirmed by TEM imaging. The electrophysiological analysis, focused on the "largest" sensilla by way of the extracellular single sensillum recordings (Hodgson et al., 1955), confirmed the activity of 4 different neurons, on the basis of their amplitude and shape, in both males and females. In details, as assessed by the dose-response relationship curves, a best-responding cell in response to sucrose and fructose was identified in both sexes, with a peak spike frequency at 50 mM for both sugars. Similarly, NaCl elicited a response from a "salt" unit in a dose-response manner. Malic acid, a component of apple pulp, and citric acid, a component of citrus fruit, elicited an inverse dose-response-relationship of all chemoreceptors in both sexes, probably due to pH-dependent effect on the response.

Finally, in both sexes the largest sensilla display high sensitivity, with polyneural responses, to cold-pressed extracts from the skin and the pulp of orange, apple, prickly pear and lemon, while they do not following stimulation with the water-soluble fractions of same fruits.

The comprehension of medfly taste responses to component of host-plants may be critical for population control programmes.

Angioy AM, Liscia A, Pietra P, Crnjar R. (1978). Boll. Soc. Ital. Biol. Sper. 21:2105-2107.

Gothilf S, Galun R, Bar-Zeef M. (1971). J. Insect Physiol. 17:1371-1384.

Hodgson ES, Lettvin JY, Roeder KD. (1955). Science 122:417-418.

Olfactory and gustatory processing in a premotoric area; the laterale protocerebrum in a model insect

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We are studying chemosensory coding and learning in heliothine moths, a subfamily of numerous species distributed on all continents, including some of the most devastating insects in agriculture. In previous studies, we have functionally characterised olfactory receptor neurons (ORNs) for plant odorants according to primary and secondary odorants, using electrophysiological recordings from single ORNs linked to gas chromatography and mass spectrometry. So far, biologically relevant odorants for 20 ORN types have been identified. The gustatory receptor neurons (GRNs) have been classified by screening putative tastants, resulting in classification of four major GRN types. In present studies we focus on how biologically relevant plant odours- and taste information is processed and integrated in input as well as output neurons in the premotoric area, the laterale protocerebrum (LP) of the moth *Heliothis virescens*. By intracellular recordings combined with fluorescent staining, confocal microscopy and 3D reconstructions, neurons are physiologically and morphologically characterised. Transformation of the neurons into the standard brain atlas allow identification of the projection pattern both in the Calyces of the Mushroom Bodies (second order olfactory area, important in learning and memory formation), the LP and the suboesophageal ganglion/tritocerebrum (SOG; primary gustatory areas) as well as identification of innervated glomeruli by antennal lobe (the primary olfactory centre) neurons (Kvello et al. 2009, Løfaldli et al. 2010). Whereas most central gustatory neurons are located in the SOG/tritocerebrum, some of them project in several areas of the brain, including the LP, anterioventral of the olfactory area. Different types of central olfactory neurons have been characterised, and some, including output neurons, respond exclusively to one particular mixture when tested for single components in the mixture and different blends of the same mixture. The central gustatory neurons integrate information about several taste modalities from GRNs on all appendages (Kvello et al. 2010). Neurons situated in the LP might also participate in a possible integration of olfactory and gustatory signals in the brain.

Is taste perception driven by change-detection rather than level-detection?

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In classical psychophysics, the perceived intensity of a tastant solution is defined by its concentration. Apart from long-term time effects like the reduction of perceived intensity by sensory adaptation, taste intensity is generally conceived of as a the result of a level detection process: The proximal stimulus at a taste receptor is invariable, and so is the behavioral response.

Recent investigations in our laboratory contradict such a level-detection interpretation of taste transduction. Inspired by earlier observations of taste enhancement by alternating tastant concentrations over time [1], variable stimuli were presented to study effects on perceived taste intensity. Taste enhancement was observed for stimuli that produce synchronous alternating proximal stimuli (i.e. sucrose solutions delivered by a gustometer) and for stimuli that produce asynchronous alternating proximal stimuli (i.e. bread crumbs with spatially varying salt concentrations and gels with spatially varying sucrose concentrations) compared to constant stimuli of the same average concentration.

These observations suggest that perceived taste intensity is a product of transient receptor responses, integrated over space and time. Perception of constant stimuli can then still be understood considering that tastant solutions produce variable proximal stimuli upon oral processing with concentration gradients proportional to the tastant concentration. Hence, a change-detection hypothesis of taste perception predicts that taste intensity depends on the magnitude and quantity of positive time-concentration gradients, as proximal stimuli.

This hypothesis was tested by delivering tastant solutions of alternating concentrations to fixed portions of the human tongue. To achieve this, two closed chambers covering 240 mm2 on the anterior tongue were flushed with fixed tastant solutions or sequences the same tastant solutions (2s) alternated with water (2s). Although the total amount of tastant presented in the continuous condition was twice the amount presented in the alternating condition, the change-detection hypothesis predicts higher taste intensities and lower taste threshold for the alternating condition. Observed detection thresholds were in line with this for NaCl and citric acid solutions but not for sucrose solutions. Observations provide further support for change detection as the primary process underlying taste perception.

The effects of franones flavors on the intakes of umami solution in short time one-bottle test in non-deprived mice

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Maillard reaction participates not only in the formation of color but also in aroma generation during cooking and thermal processing in the food industry[1)]. The volatiles from the Maillard reaction contributes to the sweet and bakery aroma of savory foods and beverages, such as cooked chicken, roasted beef, coffee, beer and so on. But why these aromatic compounds attract us is not certain.

We conducted behavioral experiments to explore possible interactions of flavor preference between Maillard reaction products (MRP) and umami substances in non-deprived mice. In the present study, we investigated the short time one-bottle intakes of the oxygen containing Maillard reaction flavor, furaneol (2,5-dimethyl-4-hyfroxy 2(3H) furanone) and sotolon (3-hydroxy-4,5-dimethyl 2(3H) furanone) and its combination with umami taste solution. The intakes of 0.1 mg/kg furaneol solution was consumed more than 10 mg/kg solution in the short time one-bottle test. We tested three concentrations of soloton (0.01, 1, 100 mg/kg) and there was no difference in intakes of these solutions. 0.1 and 10 mg/kg furaneol flavored umami solution containing 10 mM monosodium glutamate (MSG) was consumed more than same concentration of MSG solution in the short time one-bottle test. 100 mg/kg sotolon flavored umami solution tended to be consumed more than umami solution. These results suggest that particular concentrations of these furanones flavors enhance short time intakes of umami solutions rather than glutamate only.

We continue to investigate the other flavors, such as nitrogen containing MRP of pyrazines and sulfur containing MRP of thiols.

1) Cerny, C. The aroma side of the Maillard reaction. Ann. N.Y. Acad. Sci. 1126:66:71 (2008)

This work was supported by Society for Research on Umami Taste.

Sweet taste intensity is enhanced by temporal fluctuation of odor and taste, and depends on phase shift

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Pulsatile stimulation enhances taste intensity compared to continuous stimulation with stimuli of the same net tastant concentration. In the present work, we studied the effects of pulsatile delivery of odors and tastants on their combined contribution to taste intensity.

Effects on taste perception were evaluated for odor and taste pulsation and the aroma pulse-taste pulse phase shift. High-concentration sucrose pulses were alternated with water rinses every 2.5s. Four different odor (isoamyl acetate) versions were presented: (1) no odor, (2) continuous odor (3) odor pulses in-phase and (4) odor-pulses out-of-phase with taste pulses. Odor-taste combinations were evaluated for sweetness intensity by a 15-member trained panel using time-intensity analysis.

Sweetness intensity was enhanced by pulsatile stimulation of sucrose or isoamyl acetate. In addition, taste enhancement by odor and tastant pulses was additive if both were presented out-of-phase which resulted a sweetness intensity enhancement by more than 35% compared to a continuous sucrose reference of the same net sucrose concentration. Odor induced sweetness enhancement can be explained by cross-modal aroma-taste integration. Amplification of odor-taste integration by pulsatile stimulation may be attributed to a potentiated afferent input of odor and taste information prior to odor-taste integration. Alternative mechanisms include the importance of swallowing on odor-taste integration.

Oolong tea is suitable beverage for fatty foods

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Fat is known to enhance the flavor and taste of food. Though fatty food stuff is delicious, we are easy to be lost interest in because of its greasy. Oolong tea is usually served for Chinese cuisine. There is something about oolong tea that we instinctively choose for Chinese cuisine. We focus that the interaction between oiliness and property of oil.

Human sensory evaluation was carried out for estimation of oiliness after cleansing with various tea solutions. Healthy adults subjects (n=84) were recruited. They relished whip cream followed to rinse with either mineral water or oolong tea, and scored the oiliness

in VAS chart. The result showed that the oolong tea deduced the after-oiliness significantly. Next, dispersibility was observed after 1, 5 and 10 min. after mixing. Interfacial tension between oil and aqueous solution was also measured by the Face surface tensiometer CBVP-A3 which is based on the method of Wilhelmy methods. Corn or soybean oil was used for oil, and mineral water, green tea, barley tea or oolong tea was used for aqueous solution. The results showed that oolong tea significantly reduced the interfacial tension compared with other solutions. And oil dispersibility was poor in mineral water.

These results suggest that oolong tea has affinity with oil so that remaining oil in tongue is washed out with it, although the effective component of oolong tea is not clear yet.

Olfactory learning during sleep

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Sleep is a state of relatively suspended sensory and motor activity. Sleep is distinguished from quiet wakefulness in part by decreased reactions to external stimuli. However, evidence suggests that external stimuli, mainly auditory, are nevertheless processed during sleep. Olfaction may offer unique insight into sensory processing during sleep, in that it entails an implicit sensorymotor measure of stimulus content, namely sniffing. During wake, sniffs are truncated in response to either intense or unpleasant odorants. Previous work suggested that odorants presented during sleep don't wake, but do modify respiratory patterns.

Here we used measures of sniffing during sleep to ask three independent questions:

1. Is odorant pleasantness processed in sleep?
2. Can odors presented during sleep condition a response generated during sleep?
3. Can odors presented during sleep condition a response generated in later wake?

We recorded the following physiological measurements from 5 subjects: electroencephalogram, electrooculogram, electromyogram, electrocardiogram, blood oxygenation, and nasal respiration. Subjects wore a small nasal mask where we could deliver odorants at a flow rate of 3 LPM in a controlled fashion, with no non-olfactory cues as to odorant onset and offset.

During sleep, partial conditioning between odor and sound was generated at a ratio of 2:1. On each Conditioning trial (two thirds of all trials), an auditory stimulus was triggered by an inhalation, and was followed by an olfactory stimulus. We used two auditory stimuli (pure tones of 1200 Hz or 400 Hz, duration = 1 sec), each paired with one of two olfactory stimuli (the pleasant odor of deodorant or the unpleasant odor of rotten fish (duration = 3 sec). About half an hour after the subjects woke up they were tested for a conditioned response: three auditory stimuli were presented, 1200Hz, 400Hz and a new tone of 800Hz that was not present during the night (8 repetitions each), while nasal respiration was recorded.

As to the first question, we found that odor pleasantness influenced respiratory patterns in sleep in a manner similar to wake: unpleasant odorants were followed by truncated respirations. As to the second question, we found that sounds paired with pleasant or unpleasant odorants later induced dissociable respiratory responses during sleep, even in the absence of odor. This suggests learning during sleep. Finally, regarding the third question, our preliminary data suggests that odor-sound pairings learned during sleep are then retained during wake. We stress, however, that these findings rest on a pilot effort of 5 subjects, and therefore depend on confirmation in a larger sample.

AMPA receptors in the basolateral amygdala are involved in the memory processes underlying acquisition of olfactory trace conditioning in the Rat

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Conditioned odor aversion (COA) is a trace conditioning that results from the association between a novel odor (conditioned stimulus, CS) and a delayed visceral illness (unconditioned stimulus, US). Previous experiments of ours have clearly implicated the basolateral amygdala (BLA) in the processes that control the association between the memory trace of the CS and the delayed US. In particular, pre-CS but not post-CS infusion of the NMDA antagonist d-APV has resulted in a deficit of COA thus suggesting that NMDA receptors in the BLA are important during the early phase of the CS memory trace formation. Regarding the different role of the NMDA and AMPA receptors in synaptic plasticity phenomenon, the present study aimed at characterizing the role of the AMPA receptors during memory processes that underlie COA acquisition.

Thirty Long-Evans rats bilaterally implanted with cannulae were microinjected with DNQX (0.5 mg) or artificial CSF either 5 min before the CS presentation (pre-CS), 5 min after the CS presentation (post-CS) or 20 min after the US administration (post CS-US). The results showed that only pre-CS injection of DNQX impaired COA learning. Taken together with our previous data, these results show that AMPA, as well as NMDA receptors activation is essential during the CS presentation for the olfactory memory trace to be developed. In reference to the role of the AMPA and NMDA receptors in LTP, it is suggested that olfactory memory trace of the CS is subjected to short term memory processes that may imply the participation of the NMDA but also the AMPA receptors in the BLA. Since AMPA receptor trafficking has been implicated in the changes in synaptic strength at cortico-amygdala glutamatergic synapses associated with fear conditioning memory, a second study investigated the involvement of AMPA receptor subunit glutamate receptor GluR1 and GluR3 during the process of olfactory memory trace acquisition. To this aim, Long-Evans rats were subjected to an olfactory CS presentation during acquisition of COA and synaptic expression of GluR1 and GluR3 in the BLA region was studied in order to test the effect of olfactory stimulation on a possible an enhancement of AMPA subunits expression.

Results are discussed in terms of contribution of BLA in synaptic plasticity that could underlie memory processes of olfactory trace formation during COA learning.

Amygdala PKMζ role in the formation of taste aversive memories in rats

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The protein kinase Mzeta (PKMζ) activity is necessary and sufficient for long-term potentiation (LTP) maintenance (Sacktor et al., 1993; Ling et al., 2002, 2006; Serrano et al., 2005; Sacktor, 2008;). Inhibition of PKMζ activity by intracerebral injection of zeta inhibitory peptide (ZIP) has shown a clear breakdown of different memories retention, including spatial, conditioned fear, objects recognition and inhibitory avoidance learned responses (Hardt et al., 2009; Serrano et al., 2008). Taste aversion memory has showed a great sensitivity to the blockade of PKMζ when applied to the insular cortex (IC) but only in consolidated memory (Shema et al., 2007; 2009)

In the present experiments we used adult male Wistar rats in order to investigate the effect of bilateral ZIP injections in the basolateral amygdala (BLA) on the acquisition and the short-term memory of the conditioned taste aversion (CTA). ZIP microinjection (10nmol/μl) in the BLA applied either during the CS-US interval or 30 min after training. Tests showed a weaker aversion in the group receiving ZIP injection in the interval CS-US.

These findings support a role of amygdala PKMζ in the formation of taste aversive memories. The results add to a bulk of data which point to a general critical role of PKMζ in the plastic changes involved in different types of memories independent of the neural circuit involved.

Hardt O, Migues PV, Hastings M, Wong J, Nader K: PKMzeta maintains 1-day- and 6-day-old long-term object location but not object identity memory in dorsal hippocampus. Hippocampus 2009 Oct 5. [Epub ahead of print]

Ling DS, Benardo LS, Sacktor TC: Protein kinase Mzeta enhances excitatory synaptic transmission by increasing the number of active postsynaptic AMPA receptors. Hippocampus 2006, 16(5): 443-452.

Ling DS, Benardo LS, Serrano PA, Blace N, Nelly MT, Crary JF, Sacktor TC: Protein kinase Mzeta is necessary and sufficient for LTP maintenance. Nat. Neuroscience 2002, 5(4): 295-296.

Sacktor TC: PKM ζ, LTP maintenance, and the dynamic molecular biology of memory storage. Prog Brain Res 2008, 169: 27-40.

Sacktor TC, Osten P, Valsamis H, Jiang X, Naik MU, Sublette E: Persistent activation of the ζ isoform of protein kinase C in the maintenance of long-term potentiation. Proc Natl Acad Sci U.S.A. 1993, 90: 8342-8346.

Serrano P, Yao Y, Sacktor TC: Persistent phosphorylation by protein kinase Mzeta maintains late-phase long-term potentation. J Neurosci 2005, 25(8): 1979-84.

Shema R, Sacktor TC, Dudai Y: Rapid Erasure of Long-Term Memory Associations in the Cortex by an Inhibitor of PKMζ. Science 2007, 317: 951-953.

This research was supported by the grants PSI2008-03933 (MICINN. Spain) and HUM 02763 (Junta de Andalucía. Spain).

Dorsal hippocampal and perirhinal neurotoxic lesions and taste intake suppression induced by context aversion learning in rats

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The hippocampal system may play various roles in learning involving context processing. These hippocampal functions may include from perceiving complex configurations to establishing associations either with the CS, US, or second order US-CS modulation (Holland and Bouton, 1999). Thus, an intact hippocampus seems to be required for associative learning involving both appetitive and aversive stimuli Context aversions develop after several context-visceral illness pairings and it is demonstrated by drinking supporession when a new taste solution is available in the aversive context. To our knowledge there is only one study demonstrating that dorsal electrolitical lesions impair the illnes-induced context aversion (Aguado et al., 1998). However, since electrolitical lesions disrupt both the local hippocampal tissue and connecting fibres in the area, it is needed to investigate the effect of neurotoxic lesions in a a similar behavioral procedure. In addition to bilateral dorsal hippocampal lesionss the effects of bilateral perirhinal lesions were also explored, since this area has been involved in other memory tasks.

In the first experiment, male adult Wistar rats were acclimated to two different contexts being one of them safe and the other aversive. They were subjected to a behavioral procedure including three training sessions in which i.p. injections of lithium chloride (LiCl 0,15M; 1% b.w.) or sodium chloride (NaCl 0,15M; 1% b.w.) were applied before the animals being confined to a distinctive spatial context where they remained for 45 minutes. Drinking suppression of a taste solution (NaCl 1%) at the aversive context was used as an index of context aversion. Unexpectedly, the results indicated an increased saline solution intake during the context aversion test, instead of a taste solution drinking suppression, thus poninting to a potential context preference. However, spatial context aversion learning was evident in a latter test using the conditioned blocking effect.

In the second experiment, the same procedure was applied to those groups receiving neurotoxic lesions (NMDA ; 0.06M) either of the dorsal hippocampus (AP: -3,3 ; ML: ± 1,8 DV: -3) or the perirhinal cortex (AP: -3,3 ; ML: ± 6,0 DV: -7,5). No effect of the lesions was evident since similar results were obtained in lesioned and sham-operated groups, thus pointing to the fact that these areas might not be required for some context-dependent types of learning.

Aguado L, Hall G, Harrington N, Symonds M. Illness-induced context aversion learning in rats with lesions of the dorsal hippocampus. Behav Neurosci. 1998 Oct;112(5):1142-51.

Holland PC, Bouton ME. Hippocampus and context in classical conditioning. Curr Opin Neurobiol. 1999 Apr;9(2):195-202.

Grants PSI2008-03933 (MICINN. Spain) and HUM 02763 (Junta de Andalucía. Spain) both supported by FEDER funding.

The participation of testosterone in the retention of the extinction memory of conditioned taste aversion learning in male mice

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Mice acquire the conditioned taste aversion (CTA) memory by application of novel taste, sodium saccharin (Sac), as a conditioned stimulus (CS) and followed by an i.p.injection of LiCl as an unconditioned stimulus (US). Mice are to drink Sac again by repeated presentation of CS without US, which is called the extinction of CTA. Recent studies have demonstrated that the extinction is a process of relearning, where mice acquire the extinction memory of CTA. Male mice (C57BL/6) sexually mature between 5 weeks old (5wk) and 7wk. Male mice at 5wk or 7wk (5wk- or 7wk- extinction group) underwent the training and conditioning periods followed by the extinction period. The retrieval test of the extinction memory of CTA at 12wk showed that mice of 7wk-extinction group represented significantly higher retention of the extinction memory than 5wk-extinction group. In other words, the retention of the extinction memory is dependent on the degree of the sexual maturation. This result suggests the effect of hormone on the retention of the extinction memory during maturation period. Therefore, we examined the effect of an androgenic hormone, testosterone, on the retention of the extinction memory. We chronically implanted pellets containing several doses of testosterone (0-2.0 mg) into castrated male mice at 5wk. After acquisition of CTA memory at 7wk, mice underwent the extinction period for 7 days. The retrieval test of extinction memory of CTA memory at 12wk showed that mice implanted with 0.5 mg testosterone, which is close to physiological appropriate value, represented significantly higher retention of the extinction memory than mice implanted with other doses.

These results suggest that testosterone enhances the retention of the extinction memory in male mice.

This work was supported by Bio-Imaging Center and Open Research Center, Japan Women's University.

An Olfactory Mirror: A Method for Assessing Self-Recognition in Macrosmatic Mammals

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The current benchmark test for animal self-recognition is the mirror-based mark-test, where an animal in front of a mirror directs behavior at a mark on its own body that can only be seen with the help of the mirror. Despite some controversy, it is largely accepted that primarily great apes pass the mark-test, and therefore only these animals have true self-recognition and possibly its implied self-awareness. Here we suggest that the failure of other mammals at the mark-test may be a reflection of the sensory modality in which it was conducted. Whereas the primary sense of most great apes is vision, the primary sense of most other mammals is olfaction.

Thus, we set out to test the hypothesis that macrosmatic mammals have olfactory rather than visual self-recognition. To this end, we developed an olfactory mirror. In the olfactory mirror an animal receives through a sniff-port its own body smell that was sampled directly prior to entering the device.

The animal model we chose is the pet dog (canis familiaris). An individual dog's smell can stem from different sources, including the anal glands and interdigital scent glands. In order to accustom the dog to the mirror, in daily sessions we used the olfactory mirror to reflect either the anal or interdigital smell within a two-alternative discrimination task. Using an electronic nose (Cyranose, Smith Detection), we verified that these two odorants were indeed different Surprisingly, a 4 year-old female Labrador is slow to acquire the discrimination. Currently, at 10 days of training, performance is still at chance levels. Once discrimination is learned, we intend to "taint" one of the odorants, either anal or interdigital, while monitoring the spontaneous behavior of the dog. If the dog then moves to examine the now tainted body part, this will imply self-recognition and arguably awareness, consistent with that revealed in the mirror test.