ABSTRACTS

Thirteenth Congress of the European Chemoreception Research Organisation

Held in Siena, Italy, September 8–13, 1998

The following pages contain the abstracts from the XIII ECRO Congress. The abstracts are organized in alphabetical order by first author's name.

Supported by grant DC01655 from the National Institute on Deafness and Other Communication Disorders.

1. Organizational complexity in lobster olfactory receptor cells

B. W. Ache

Whitney Laboratory and Departments of Zoology and Neuroscience, University of Florida, St. Augustine, FL 32086 USA

The olfactory organ of lobsters, as many other invertebrates, consists of sensilla. Each lobster olfactory sensillum contains ~350 primary bipolar receptor cells, each cell of which gives rise to ~25 outer dendritic segment (cilia). This highly enriched, highly polarized source of olfactory receptor cells facilitates localizing putative intracellular signaling pathways to the ciliary transduction compartment. Recent molecular evidence complements biochemical and electrophysiological evidence identifying the major components of a phosphoinositide signaling pathway in the cilia. Odors activate a PLC through a G-protein coupled receptor that targets an IP_3 receptor in the plasma membrane. The IP_3 receptor is a functional, non-selective cation channel; activation of this pathway excites the cell. Activation appears to be a two-step process that involves secondary activation of a predominantly current-carrying channel, as is being considered for the calcium-activated chloride current in vertebrate olfactory receptor cells. In the lobster, however, secondary activation involves a novel sodium-activated, non-selective cation channel that couples to the second-messenger signaling pathway by an as yet unknown mechanism. The phosphoinositide pathway works not in place of, but rather in addition to, a cyclic nucleotide signaling pathway. In the lobster, odors activate adenylyl cyclase through a different G-protein from the one associated with phosphoinositide signaling. The cyclic nucleotide pathway appears to target a potassium channel that inhibits the cell with the same kinetics as does IP_3-mediated excitation. The ability to modulate excitation through a parallel inhibitory pathway, i.e. to provide bipolar input, provides a functional rationale for having two olfactory second messengers. Complexity in intracellular signaling confers upon lobster olfactory receptor cells the potential to carefully regulate, and most likely integrate, the signal they transmit to the central nervous system.

2. Chemosensory proteins (CSP) of *Schistocerca gregaria*

S. Angeli, F. Ceron, A. Scaloni[1], M. Monti[1], R. Petacchi[2], A. Minnocci[2], G. Monteforti[2] and P. Pelosi

Dip. Chimica e Biotecnologie Agrarie, via S. Michele, 4, I-56124 Pisa, [1]CISESMA—IABBAM, CNR, Napoli and [2]Scuola Superiore 'S. Anna', Pisa, Italy. e-mail: ppelosi@agr.unipi.it

Soluble proteins of chemosensory organs have been described in several orders of insects and include pheromone- and odorant-binding proteins, as well as other polypeptides of similar structure probably involved in gustatory perception. Information of this type is not available for Orthoptera, despite the high economical interest of several species of this order.

In our search for OBP-like proteins in *Schistocerca gregaria*, we have isolated several polypeptides of low molecular weight (~14 kDa) and acidic nature from chemosensory organs, such as antennae, tarsi and upper labrum. N-terminal sequencing revealed that all the isolated proteins belong to the same subfamily, including *Drosophila melanogaster* OS-D and *Cactoblastis cactorum* CLP-1. By using degenerate primers and PCR, we have amplified, cloned and sequenced genes encoding five members of these proteins in tarsi of both sexes. The five gene products contain 109 amino acids, are very similar to each other and present four cysteine residues probably involved in two disulphide bridges. Calculated molecular weights are identical to those measured by mass spectrometry, indicating the absence of other post-translational modifications.

Polyclonal antibodies, raised against the purified protein, have been used in immunocytochemical localization. Both in antennae and tarsi, single-pore gustatory sensilla are labelled, but not olfactory sensilla. Labelling is limited to the sensillar lymph.

The same polyclonal antibodies have indicated in Western blot experiments that the synthesis of these proteins, both in antennae and tarsi, starts at the second instar and reaches high levels of expression at the third instar.

A role in carbon dioxide sensing has been suggested for the homologous protein of *C. cactorum*. Binding of labelled bicarbonate, however, has been unsuccessful with the proteins of *S. gregaria*, while other prospective ligands are being tested.

3. Pheromone-induced changes in heartbeat frequency in males and females of the moth *Spodoptera littoralis*

Anna Maria Angioy[1], Barbara Pisano[1,2] and Bill S. Hansson[2]

[1]*Department of Experimental Biology (General Physiology), University of Cagliari, I-09126 Cagliari, Italy and* [2]*Department of Ecology, Lund University, S-223 62 Lund, Sweden*

Sensory input has been shown to affect the normal rhythmicity of the heart in a number of insects. We performed electrocardiograms on males and females of the moth *Spodoptera littoralis*, and investigated how stimulations with single female-produced sex pheromone components [(E,Z)-9,11–14:OAc and (E,Z)-9,12:OAc)] and the male-attracting blend of these (99:1) affected the heartbeat frequency in both sexes. Both males and females have been shown to possess antennal olfactory receptor neurons and antennal lobe neurons responding highly specifically and sensitively to the pheromone components and their blend. In the male, the pheromone blend elicits a search behaviour in the wind tunnel. The function of pheromone auto-detection in the female is unknown.

The heartbeat typically followed two phases, a fast and a slow phase. An effective odour stimulus given in the slow phase reverted it into the fast phase but not vice versa. In the male, the response threshold to the major pheromone component was extremely low, 0.1–1 pg deposited on a filter paper in a stimulus pipette. The response to the minor component was, however, totally different. No unambiguous response threshold was evident, even though the number of males responding started to increase at 100—1000 pg stimulus loading. The blend did not lower the response threshold below what was found for the major component. In females, very few responses were registered, and only at a stimulus loading of 1000 pg or higher of the blend. Single components were not tested in the female.

The cardiac response in *S. littoralis* is a highly sensitive method to establish a physiological response to an olfactory stimulation. The male responds to very low amounts of the major pheromone component, concentrations even lower than those active in the wind tunnel. The minor component did, however, exert a much lower effect on the heartbeat, and the components in the complete blend did not synergize to lower the response threshold further. Antennal receptor neurons specifically tuned to each of the two pheromone components display the same sensitivity, and antennal lobe interneurons also respond at similar concentration levels. The differential response to the two pheromone components must thus be elicited at a higher neural level. This level must also be separate from the 'normal' olfactory pheromone pathway, as the wind tunnel response to the blend occurs at a 1000-fold lower concentration in the windtunnel, while no such effect was observed here.

The female heartbeat rate was more or less unaffected by pheromone stimulation. The behavioural significance of pheromone auto-detection is thus probably less for the female than for the male, or the concentration levels used by the female must be very much higher than in the male. Pheromone-specific receptor neurons are fewer in the female than in the male, but express the same sensitivity to the main pheromone component.

4. Conformational analysis by NMR and molecular modelling for designing new floral odorants

C. Anselmi, M. Centini, M. Francioli and A. Sega

Ist. Chimica organica, University of Siena, Italy

The odour of 'white flowers' has been the object of our recent research on odorant molecules. Several aldehydes endowed with this odour are known and widely employed in perfumery and cosmetics. However, there are some problems related to the use of such compounds, owing to their ready oxidatability and to skin irritant properties sometime observed. Therefore, it is of practical interest to obtain molecules with this type of odour and greater chemical stability. As the relationships between odour and chemical structure are still not well defined for this kind of note, we decided to study the conformational parameters of known odorants of this class as a basis for designing new molecules with similar odour and different functional groups. The odorants chosen were hydroxycitronellal and lilial, and a series of THF and THP ethers that best reproduce the typical note of muguet. NMR spectroscopy has been used for a conformations of the molecule in solution, but also on its dynamics.

This study has been performed in two solvents of different polarity, CDCL3 and DMSO, to evaluate the effect of the medium on the preferred conformation of the odorants.

A parallel conformational analysis has been performed using molecular modelling systems: the predictions given by this method were in good agreement with the data obtained from NMR spectroscopy.

The information obtained with these commercial products have provided the theoretical basis for the design and synthesis of new classes of compounds with floral odour.

5. Odor properties of cyclo-aliphatic hydrocarbons

C. Anselmi, M. Centini, M.I. Paoli and A. Sega

Scuola dì Spec. in Scienza e Tecnologìa Cosmetiche and Ist. Chimica Organica, University of Siena, Italy. e-mail: anselmic@unisi.it

Hydrocarbon molecules with characteristic and pleasant odor are common in nature in several essences and fruits. In the literature no systematic work has been ever published on odor-molecular structure relationships in the class of hydrocarbons whose odor is in general defined as 'grey', being devoid of any interesting note. In the present paper some structures are examined which are similar to the megastigmatrienes, natural compounds present in the passionflower juice (*Passiflora edulis sims*, *P. flavicarpa*) to which they confer the fragrance. The *P. edulis sims* and the *P. flavicarpa* differ not only in the colour of their skin but also in their shape, dimension and fragrance. The purple fruit's fragrance has been defined as fruity–floral, the yellow fruit's fragrance as exotic with a pungent sulfur note. A small amount of megastigmatrienes are present in their juice, but they contribute decisively to its fragrance.

Since hydrocarbons generally show weak odorous characteristics, analogues of megastigmatrienes were examined and to this purpose we synthesized cyclopentane, cycloheptane and cyclohexane derivatives containing an alkenylic or alkenylidenic side chain, which exhibit a mainly fruity and intense fragrance, mostly when containing a double bond side chain.

The odor threshold of limonene, which is one of the strongest and most typical hydrocarbon odorants, proved intermediate

among the threshold values measured for two butenilydene derivatives.

The influence of an alkylic substituent on the cycloalkane ring was also studied. Data show that steric hindrance near a double bond reduces the intensity of the odor. It appears that the size of rings having the same side chains deeply affects the odor.

Open chain compounds and polycyclic structures containing a dienic conjugated system were synthesized in order to highlight the effect on odor of the molecule features (rigidity or flexibility).

6. Convergence of the subfamily signatures in the honeybee colony

Gérard Arnold, Brigitte Quenet[1] and Claudine Masson

Centre Européen des Sciences du Goût, CNRS, 21000 Dijon, et Neurobiologie Expérimentale et Théorie des Systèmes complexes, CNRS UPR 9081, INA-PG, F-75231 Paris Cedex 05 and [1]Neurobiologie Expérimentale et Théorie des Systèmes complexes, CNRS UPR 9081, ESPCI, F-75005 Paris, France

In honeybee colony, workers with an ability to discriminate between super-sisters (coefficient of relationship, $r = 0.75$) and half-sisters ($r = 0.25$) should have a large fitness advantage by nepotistic behavior. But certainly this ability would form the basis for strong conflicts between the workers of the various subfamilies, and should be detrimental to the overall colony. Therefore the colony may benefit by reducing or eliminating information about subfamily discrimination. Keller (1997, TREE 12: 99) suggested that, in social insects, kin discrimination might be selectively disfavoured by scrambling recognition labels. The aim of our work was to test the hypothesis that the subfamily hydrocarbon profiles of the worker bees tend to be homogenized in the hive conditions. We have compared the hydrocarbon profiles of workers ($n = 117$) which were reared in their normal environment (parental hive) and workers ($n = 117$) which were isolated since their emergence.

The workers were assigned to their respective subfamily using two highly variable microsatellites loci (Arnold *et al.*, 1996, Nature, 379: 498). Pentane extracts of these workers were individually analyzed for cuticular hydrocarbons by GC/MS. A correspondence factorial analysis applied to the data matrix (234 individuals × 22 hydrocarbon concentrations) shows a clear separation of the two types of rearing conditions and points out the most characteristic compounds. There is greater variability in the concentration of the hydrocarbons in the isolated bees than in the hive bees, which proves an homogenization of the subfamily signature in the hive bees. In order to determine whether this homogenization affects uniformly all the subfamilies, we have compared the Mahalanobis distances of the mean hydrocarbon profiles of the subfamilies. We demonstrate that the profiles of most subfamilies converge. This convergence might be due to an active or a passive transfer of the hydrocarbons between the workers. This mechanism can be an inherent limitation of the intra-colonial discrimination system of the workers, which would probably reduce the number of recognizable subfamilies in the colony and finally reduce the number of intra-colonial conflicts. Conversely, the subfamilies bearing distinct profiles should have a better discriminatory ability, which perhaps induces nepotistic behavior. If there is only a small number of such subfamilies, this could explain why nepotistic tendencies are so weakly expressed in the bee colony.

7. Establishing a spatial map of odor quality

Richard Axel

Departments of Biochemistry and Molecular Biophysics, Pathology, and Howard Hughes Medical Institute, College of Physicians and Surgeons, Columbia University, New York, NY 10032, USA

Mammals possess an olfactory system of inordinate discriminatory power. How is the diversity and specificity of olfactory perception accomplished? Analysis of the spatial patterns of expression of odorant receptor genes suggest a logic for olfactory perception in which neurons expressing a given receptor, and therefore responsive to a given odor, project to precisely defined loci in the olfactory bulb, creating a topographic map of receptor activation. The identity of an odorant stimulus will therefore be encoded in distinct spatial patterns of activity in the brain. Support for this model derives from recent genetic experiments that permit the visualization of the individual axons from sensory neurons expressing a given receptor as they course through the olfactory epithelium into the olfactory bulb. These experiments reveal that neurons expressing a given receptor project to only two topographically fixed loci in the mouse olfactory bulb such that the pattern of convergence is absolute and invariant. The bulb, therefore, provides a spatial map that identifies which of the numerous receptors have been activated such that the quality of an olfactory stimulus would be encoded by specific combinations of glomeruli activated by a given odorant. How do neurons expressing a given receptor know which target to project to in the olfactory bulb? In recent experiments, we have used gene targeting to introduce either deletions or substitutions into specific odorant receptor genes, and have visualized the effect of these mutations on the precision of axon targeting. These data support a model in which the olfactory receptor plays an instructive role in axon targeting as one of a complement of guidance receptors involved in the generation of a precise topographic map.

8. Analysis of the piriform cortex response to direct stimulations

T. Ballain, J.L. Martiel and M. Cattarelli[1]

TIMC, UMR CNRS 5525, Faculty of Medicine, F-38706 La Tronche and [1]CESG-CNRS, 15 rue Hugues Picardet, F-21000 Dijon Cedex, France

The pyramidal cells of the piriform cortex are connected through either very short-range connections or two association fiber systems: one rostro-caudal and the second caudo-rostral. The role of this cortex in the olfactory information processing would be better understood if a precise distribution of synaptic contacts between pyramidal cells was available. However, data are lacking concerning the density of the connections between pyramidal cells and the synapse number. We present here an attempt for having direct information on the intrinsic associative fiber systems of the piriform cortex by mapping of the piriform activity in response to a punctual stimulation of the cortex itself.

Experiments were carried out on anaesthetized and curarized rats. Stimulations were delivered on the cortex (0.1–2 mA, 200 μs, $n = 5$, $f = 8$ Hz) via a bipolar electrode (125 μm between the tips). According to the deepness of the electrode tip, we stimulate either the afferent projections of the olfactory bulb or the intrinsic association fiber systems or directly the pyramidal cells. The cortical activity was mapped on a 144 photodiode array with a voltage-sensitive dye (RH 795).

We set up a mathematical method based on the detection of slope discontinuity on the signal to identify the cortical responses automatically. We derive a series of information matrixes as well as amplitude and time-delay histograms of cortical responses. These data allow us to determine the connections between remote part of the piriform cortex. Then, we look at the presence of wave I and wave II corresponding to the activation by afferent inputs or via the association fiber systems. The distribution of wave II amplitudes and latencies yields the first elements necessary to the mathematical mapping of association fiber and synapse densities. We propose a representation of the intrinsic association fiber and synapse densities. Since the response characteristics are different from one stimulation to the other, we may derive a mechanism for the response changes in terms of synaptic conductance modifications.

9. Olfactory behaviors in *C. elegans*

C.I. Bargmann, M. de Bono, P. Sengupta and E.R. Troemel

Howard Hughes Medical Institute and The University of California, San Francisco, CA, USA

An animal's ability to discriminate among different stimuli allows it to generate diverse behavioral responses to its environment. We are studying olfactory recognition and discrimination in the nematode *C. elegans*. Different chemicals can elicit distinct behaviors, and molecules that are very similar in structure can be distinguished by the animal. To ask how this specificity arises, we are studying olfactory behaviors at the cellular and molecular levels.

We used genetic methods an olfactory receptor-ligand interaction *in vivo*. In genetic screens with the odorant diacetyl, we found mutations in the *odr-10* gene, then cloned it and showed that it encodes a predicted G protein-coupled receptor. Based on its genetic and molecular properties, ODR-10 is likely to be a specific receptor for diacetyl. In collaboration with Kai Zinn's group, we have reconstituted the diacetyl-ODR-10 interaction in cultured mammalian cells. We also found that *C. elegans* may have as many as 1000 olfactory receptor genes in five different gene families, explaining its ability to recognize many odorants.

The odorant diacetyl is always attractive to wild-type animals. This behavioral specificity could reflect a property of *odr-10*, the diacetyl receptor, or a property of the AWA olfactory neurons that express *odr-10*. To distinguish between these models, we expressed *odr-10* in the AWB olfactory neurons, which detect repellents. When *odr-10* is expressed in AWB, the resulting transgenic animals avoid diacetyl. These results indicate that the receptor is not intrinsically coupled to attractive behaviors. Rather, the AWA neurons recognize attractants and the AWB neurons recognize repellents: each neuron interprets the odorants that it detects in a stereotypic fashion.

Natural variation in sensory behaviors is observed in many animals. We have discovered a polymorphism in wild *C. elegans* populations that leads to distinct solitary or social behavior patterns. The gene responsible for this behavioral variation encodes a neuropeptide receptor that interacts with the olfactory signalling pathways.

10. Taste buds in ectoderm are induced by contact with endoderm: implications for mechanisms governing taste bud development

Linda A. Barlow

Department of Biological Sciences, University of Denver, Denver, CO, USA

In both mammals and amphibians, taste buds arise from either endodermal or ectodermal epithelia. In amphibians, only the most anteriorly located taste buds are ectodermal, while the intermediate and posterior taste buds are derived from endoderm. This dichotomy in embryonic origin poses an interesting developmental question: are the cellular mechanisms used to generate taste buds comparable for endoderm and ectoderm? Until now, taste buds were believed to arise as a result of induction by gustatory nerves, and thus a distinction based on taste bud origin seemed irrelevant. However, recent results indicate that taste bud development is independent of nerve contact. In fact, in amphibians, taste bud formation has been shown to be an intrinsic feature of oropharyngeal endoderm. This finding, as well as the widely disparate embryonic histories of endoderm and ectoderm, leave open the question of how taste buds form in ectoderm.

Given that taste buds form autonomously in endoderm, I hypothesized that ectoderm is induced to make taste buds by contact with endoderm. To test this hypothesis, explants of presumptive oropharyngeal endoderm from albino embryos were paired with presumptive oral ectoderm from pigmented embryos, allowed to heal and placed in culture until taste buds normally develop. In these explants, taste buds formed in both endodermal and ectodermal epithelia. Interestingly, ectodermal taste buds were found primarily at junctures between endoderm and ectoderm. Ectodermal explants alone failed to make taste buds, indicating that contact with endoderm is necessary for the genesis of ectodermal taste buds. However, the ability of endoderm to induce taste buds did not extend to ectoderm from other regions of the embryo, indicating that only oral ectoderm is competent to respond to signals from oropharyngeal endoderm.

These results indicate that transmission of signals responsible for patterning of taste buds spread from endoderm to ectoderm, and will be discussed in the context of our new model (Barlow and Northcutt, 1998, NY Acad. Sci., in press; Northcutt and Barlow, 1998, Trends Neurosci., 21: 38–42).

11. Inhibitory effects of taurine on relay neurons of the rat olfactory bulb *in vitro*

Ottorino Belluzzi, Michelino Puopolo, Alessio Colombari and Igor Kratskin[1]

Dip. Scienze Biomediche e Terapie Avanzate, Sez. Fisiologia, Università di Ferrara, Italy and [1]Smell and Taste Ctr., University of Pennsylvania School of Medicine, Philadelphia, PA, USA. e-mail: o.belluzzi@dns.unife.it; kratskin@mail.med.upenn.edu

In the mammalian brain, taurine (TAUR, 2-aminoethanesulfonic acid) is a ubiquitous free amino acid that is involved in a number of fundamental processes, which include osmoregulation, regulation of Ca^{2+} fluxes, neurotransmitter release and neuronal inhibition. Brain TAUR has heterogeneous regional distribution, being particularly concentrated within the olfactory bulb (OB) where TAUR is abundantly present in primary olfactory afferents and different type neurons. Despite this fact, the effects of TAUR

on olfactory structures have not been investigated yet. Patch-clamp whole-cell recordings in slices of the rat OB showed that application of 5 mM TAUR produces a potent and reversible inhibition of relay neurons, mitral and tufted cells. Under current-clamp conditions, a shift of the membrane potential towards the chloride equilibrium potential, and a 75% reduction in the membrane resistance were observed. Under voltage-clamp conditions, TAUR induced a current reversing at the chloride equilibrium potential. Bicuculline (10 μM), but not strychnine (3 μM), antagonized these TAUR effects. Actions of TAUR were completely maintained under the blockage of synaptic transmission, thus indicating that they were not mediated by the secondary release of GABA from bulbar neurons. These results showed that TAUR directly and effectively increases Cl^- conductance in the somatic membrane of the OB relay neurons. In voltage-clamp experiments, TAUR reversibly reduced by 55% synaptic currents evoked in mitral cells by olfactory nerve stimulation. There is evidence that this action of TAUR is due to presynaptic inhibition of transmitter release. The study provides the first demonstration of TAUR actions in the olfactory system, and suggests that TAUR may modulate, via two distinct mechanisms, synaptic transmission from olfactory axons to relay neurons of the OB.

12. Low-frequency oscillations in the rat olfactory bulb *in vitro*

Ottorino Belluzzi, Andrea Urbani and Michelino Puopolo

Dipartimento di Scienze Biomediche e Terapie Avanzate, Sezione di Fisiologia, via Fossato di Mortara 17/19, 44100 Ferrara, Italy. e-mail: o.belluzzi@dns.unife.it

Highly synchronized, low-frequency neuronal oscillations (LFO) were found in the rat olfactory bulb (OB) under the blockage of $GABA_A$-mediated inhibition and in the absence of Mg^{2+} ions. In this study, characteristics and possible mechanisms of LFO were analysed using patch-clamp whole-cell recordings in rat OB slices. LFO were present throughout the OB, in both relay (mitral and tufted cells) and intrinsic (periglomerular and granule cells) neurons. LFO consisted of large (~20 mV) depolarizations, occurring with extreme regularity every ~18 s, and lasting ~3 s. In relay neurons, LFO triggered high frequency repetitive firing, whereas only single action potential occasionally accompanied LFO in intrinsic neurons. Paired-cell recordings from mitral cells showed that, within the mitral cell layer, these oscillations were in phase. Under voltage-clamp conditions, LFO-underlying currents reversed at 0 mV. The LFO pace was reset by olfactory nerve stimulation, while intracellular injections of a depolarizing current did not disrupt LFO pacing. When part of the glomerular layer was ablated, LFO persisted in relay neurons located on the intact side of the slice, whereas on the lesioned side the oscillations disappeared. LFO were reversibly abolished by Mg^{2+} ions, clearance of $GABA_A$ blockers, substitution of Ca^{2+} ions with Ba^{2+}, by blockers of the Ca^{2+} channels (100 μM Cd^{2+}), by blockers of the NMDA channels (5 mM D-AP5) and by 3 μM TTX, but not by blockers of non-NMDA receptor-channels (10 μM NBQX). Several lines of evidence suggest that LFO are the result of intrabulbar network interactions rather than of a pace-maker current mechanism: (i) oscillations are present simultaneously in different neurons; (ii) they are prevented by the blockage of synaptic transmission; (iii) hyperpolarization of a single cells did not change the oscillation frequency in that cell; and (iv) LFO are interrupted following lesioning of intrabulbar connections in the external plexiform layer.

13. The Scandinavian odor-identification test

Bende Mats, Nordin Steven, Bramerson Annika and Liden Edvard

Department of Otorhinolaryngology, Central Hospital, Skovde, Sweden

The Scandinavian odor-identification test (SOIT) was developed to address the need for a culturally valid odor-identification test for clinical use on the Scandinavian population. Sixteen odorous test stimuli were selected that were relatively identifiable, familiar, strong in intensity and pleasant according to healthy participants. Four response alternatives were then selected for each test stimulus based on a confusion matrix of identification rates obtained from healthy participants, in a manner that controlled for task difficulty. Results on the SOIT from healthy persons and hyposmic patients showed satisfactory test–retest relaibility, split-half reliability and validity. Cut-off scores for olfactory diagnosis (normosmia, hyposmia, anosmia) based on normative data obtained from 171 healthy persons showed a satisfactory sensitivity and specificity of the SOIT. Assessment of 22 anosmic patients revealed that three of the test stimuli were significant trigeminal stimulants.

14. Odor effect on a decision task

M. Bensafi, C. Rouby, V. Farget, M. Vigouroux and A. Holley

Laboratoire de Physiologie Neurosensorielle, Neuroscience & Systèmes Sensoriels, CNRS ERS 5020, Université Claude Bernard Lyon1, 43, bd. du 11 Novembre 1918, F-69622 Villeurbanne Cedex, France. email: bensafi@olfac.univ-lyon1.fr

We studied the possible influence of odors and their hedonic tone on a behavior—decision making—which is supposed to be exclusively controlled by cognitive processes. Neuropsychologists (Bechara *et al.*) explored decision making through a game of chance in which patients with frontal lesions differed from controls concerning their intentional strategies: they seemed insensitive to the risk of loosing everything.

We realized a computer version of this 'poker game' in which subjects could press four different buttons to win or lose money according to their choice. To test whether their strategy could be influenced by an olfactory stimulation, we diffused a pleasant odor during 4 s after a win. This odor 'announced' that the renewal of the same choice would also be followed by a win, but the significance of the odor diffusion was not given to the subject.

The results show that the odor of vanilla actually favoured the repetition of a winning choice. This conservative strategy was observed during the beginning phase of the game, where the odor seemed to reduce the subjects' spontaneous tendency to check all buttons instead of renewing the previous choice. About 50% of the subjects became conscious of the presence of an odor during the test, but no one used it as a conscious clue to reinforce a previous choice. However, this influence of odor cannot be considered as unconscious: only the subjects who detected it showed an increment in their repeated choices compared with controls.

15. Comparative study of the macroglomerular complex in the antennal lobe of heliothine moths

Bente G. Berg, Tor Jørgen Almaas, Jan Bjaalie[1] and Hanna Mustaparta

Department of Zoology, Norwegian University of Science and Technology, N-7034 Trondheim, Norway and [1]Department of Anatomy, University of Oslo, Norway

The macroglomerular complexes (MGC) of the antennal lobes in five heliothine species have been compared with regard to structure and functional organization. Projections of specific receptor neuron types in the MGC were studied by electrophysiological recordings combined with application of cobalt-lysine which resulted in single labelled axon terminals in different compartments. Histological investigations included camera-lucida drawings and 3-D computer reconstructions of the MGC. Some intracellular recordings from antennal lobe projection neurons combined with neurobiotin stainings have also contributed to the results. In all five heliothine species investigated, the MGC consisted of three or four compartments, of which one large unit (the cumulus) was located at the entrance of the antennal nerve. Input to the cumulus by receptor neurons tuned to the major pheromone component was shown in *Heliothis virescens, Helicoverpa zea* (American species) and *Heliothis armigera* (Eurasian species). In *H. virescens* another large dorso-medial compartment received input from a small population of receptor neurons responding to the second pheromone component. Correspondence between input to and output from this compartment is demonstrated by the arborization of an AL projection neuron responding best to antennal stimulation with the second pheromone component. The two smaller ventral compartments in *H. virescens* and the two smaller dorsally located units in *H. zea* received input from two colocalized receptor neuron types, each tuned to a known interspecific signal which interrupts pheromone attraction. Also, in the Eurasian species *H. assulta* the information about the major pheromone component and the interspecific signal is mediated by specific receptor neurons projecting to different MGC compartments, the cumulus and the dorso-medial compartment. Thus, similarities of the MGC in the five heliothinae species are expressed by the structure and function of the cumulus as a relay for transmitting information about the major pheromone component, whereas the structures and functions of the smaller units differ between species.

16. Induction of expression of odorant receptors in olfactory sensory neurons by coculture with olfactory bulb

Stephan Bieri, Monaliza Khan, Michael E. Buckland, Kerry A. Nichol and Anne M. Cunningham

Neurobiology Program, The Garvan Institute of Medical Research, 384 Victoria Street, Darlinghurst, NSW 2010, Australia

The olfactory bulb is known to exert a profound trophic influence on the peripheral olfactory neuroepithelium but the mechanism is undefined. We have used coculture techniques to analyse the effect of olfactory bulb neurons on the expression of odorant receptors by olfactory receptor neurons (ORNs).

ORNs were isolated from neonatal Wistar rats and grown in dissociated primary culture based on a modification of the technique described previously (Cunningham *et al.*, 1998). In the baseline state, these neurons expressed neuron-specific tubulin, G_{olf}, GAP-43 and synaptophysin immunoreactivity. The neurons vigorously extended long neurites and tended to group together in clusters, often associated with S100 or p75 expressing glial cells. Using a polyclonal antibody, AC310, produced to recognize the family of odorant receptor (OR) proteins, we found negligible expression of ORs in the baseline state. However, coculture with dissociated neurons from the olfactory bulb, resulted in an induction of expression of OR immunoreactivity. The positive neurons occurred in clusters comprised of 15–25 cells which were morphologically similar, suggesting they may have undergone neurogenesis *in vitro* from the same progenitor cell. These cells were intermingled with non-immunoreactive clusters of ORNs. Immunoreactivity was not predominantly surface localized but, rather, occurred in dense cytoplasmic accumulations viewed by conventional and confocal microscopy. RT-PCR with a set of degenerate primers based on the conserved MAYDRY region and TM6 of the OR family revealed a significant upregulation of OR mRNA in the ORN-bulb cocultures compared with the baseline ORN cultures, in keeping with our immunocytochemical data. In order to analyse the diversity of OR expression *in vitro*, we cloned and sequenced the RT-PCR products and compared the OR sequences found in the baseline state to those amplified from the ORN-bulb cocultures.

Our results suggest that contact with olfactory bulb neurons directly regulates the expression of ORs in ORNs and we are investigating if this effect is mediated by cellular or secreted factors. By studying the diversity of ORs expressed in the clusters we hope to determine whether individual neuronal progenitor cells are committed to the expression of only one class of odorant receptor.

Supported by the Garnett Passe and Rodney Williams Memorial Foundation and the NH&MRC of Australia.

17. Amiloride-blockable sodium currents in frog taste cells

Albertino Bigiani

Dipartimento di Scienze Biomediche, Università di Modena, via Campi 287, I-41100 Modena, Italy. e-mail: bigiani@unimo.it

In vertebrates, sodium ions in food are thought to be detected, at least in part, through amiloride-sensitive Na^+ channels (ASSCs) localized at the apical membrane of taste receptor cells (TRCs). The biophysical and pharmacological properties of these channels have been characterized by applying patch-clamp techniques to TRCs isolated from frog and mammals. Findings from several studies show that ASSCs in taste cells have a high affinity to amiloride with an apparent inhibition constant (K_i) in the submicromolar range (Lindemann, 1996, Physiol. Rev., 76: 719). Taste organs contain several morphological categories of cells (Roper, 1989, Annu. Rev. Neurosci., 12: 329), but it is unknown whether different cell populations express ASSCs and whether the properties of these channels are similar. To address these questions, we have taken advantage of the frog taste organ (taste disk). In this amphibian, it is readily possible to discriminate different types of taste cells after isolating single cells from the disk (Bigiani *et al.*, 1998, J. Neurosci., in press). We characterized the membrane properties of putative taste supporting cells—called 'wing cells'—in the frog, using patch-clamp recordings. We found

that wing cells express functional ASSCs similar to those in frog TRCs (Avenet and Lindemann, 1988, J. Membr. Biol., 105: 245). However, we now report that in wing cells, K_i for amiloride is an order of magnitude higher (2.9 μM) than in TRCs (0.2–0.3 μM). These findings suggest that the different types of cells in a taste organ can express functional ASSCs with different properties, such as the sensitivity to amiloride. Future directions will be to determine the localization of ASSCs in the membrane (apical/basolateral) of wing cells.

18. The role of olfaction in mother pup recognition in big brown bats, *Eptesicus fuscus*

Johanna M. Bloss

Boston University

Female big brown bats (*Eptesicus fuscus*) from the eastern USA roost in maternity colonies during the summer and give birth to two pups. During the lactation period, a female leaves her pups in the roost while she forages and then must return and suckle her own offspring. Dual choice experiments were conducted to test whether lactating females can discriminate between the odors of their own offspring and same-aged foreign pups. Female big brown bats preferentially chose the scent of their own pups over unrelated individuals. However, using olfaction alone, young pups were unable to consistently discriminate between their mothers and a foreign female. As lactation is an energetically costly process, selection may favor females that use a number of cues, including olfaction, for offspring identification and thereby avoid allocation of valuable resources to unrelated individuals.

19. Modulation of chorda tympani sensitivity with repeated exposure to 'novel' tastants. Acute and long-term familiarization

N. Boireau, A.-M. Pillias and A. Faurion

Neurobiologie Sensorielle, 1, av des Olympiades, F-91744 Massy Cedex, France

The aim of the study was to look for an eventual modulation of chorda tympani peripheral response to taste stimuli with repeated exposure to novel tastants. Three groups of 10 hamsters (*Mesocricetus auratus*) were exposed to novel tastants: dulcine (DUL), potassium glutamate 50 mM (KG5) and guanosine 5′-monophosphate (5′-GMP), as over night two bottle tests during 15 days, prior to whole nerve chorda tympani electrophysiological recording. Two groups were not familiarized to any tastant: a control group had only water in the double bottle test, the other group received a tongue rinse with 5′-GMP 20 min before electrophysiological recording. We recorded the gustatory nerve responses to 21 stimuli repeated six times which corresponds to a 3 h experiment. Response amplitudes for the control group increased significantly for all stimuli between stimulations 1 and 6. On the contrary, CT response amplitudes of groups exposed to, and hence familiarized with, novel tastants did not increase specifically for the familiar stimulus in any case: a modulation of CT sensitivity was observed due to familiarization. In some cases, familiarization also induced changes in response amplitudes to other stimuli (generalization). For example, the group exposed to 5′-GMP generalized this effect to sodium chloride and all stimuli including glutamate anion. On the other hand, we could not observe the reciprocal effect for the group exposed to glutamate. The effect is relatively specific as familiarization to dulcin (sucrose-like to the hamster) did not generalize to sucrose.

Results are discussed in terms of two hypotheses inductibility of chemoreceptor synthesis (*c-fos* expression) or transductional coupling facilitatation.

20. Organization of the subependymal layer in the postnatal rat

L. Bonfanti, P. Peretto[1], A. Fasolo[1] and A. Meringhi

Dip. di Morfofisiologia Veterinaria and [1]Biologia Animale, Università di Torino, Italy. e-mail: merighi@veter.unito.it

In the forebrain of neonatal and adult rodents a high number of cells generated in the subependymal layer (SEL), migrate tangentially towards the olfactory bulb. In the adult, these cells are organized to form chains contained whithin astrocytic glial tubes (Lois *et al.*, , 1996 Science, 271: 978; Peretto *et al.*, 1997, Brain Res. Bull., 42: 2). During neurogenesis, newly generated cells are known to migrate following a radial glia scaffold. By using light microscopic immunocytochemistry with markers of the mature (GFAP) and developing (nestin, vimentin) glia, or antigens expressed by the newly generated cells (PSA-NCAM, stathmin) and electron microscopy, we have investigated the changes occurring in the SEL of postnatal rats. Brains were obtained from two embryos (E20) and 16 young (P 2, 5, 9, 13, 17, 21, 25, 30) Wistar rats.

Up to P13, glial cells expressed vimentin and nestin immunoreactivity; in later stages they were also visible using an anti-GFAP antibody. At E20 typical radial glial cells were prevalent in the forebrain and the SEL was hardly identifiable. From P2, an homogeneous net of thin astrocytic processes was observed in the SEL area, being denser on the SEL perimeter. Some of these processes were tangentially oriented. At P17, thick septa formed by the astrocytic processes were detectable in the medial part of the SEL. An organization reminiscent of adult glial tubes was visible starting from P21. At this stage, PSA-NCAM and stathmin appeared to be restricted to the chain of migrating cells within the SEL. The ultrastructural analysis confirmed that the first appearance of defined clusters of migrating cells and wide intercellular clefts between migrating cells actually occurred in the SEL starting from P21. In thick vibratome sections immunostained for vimentin, some radial glial cells in the SEL clearly displayed several different orientations, including tangential tracts. These results show that the establishment of a unique type of tangential migration into the adult rat brain is associated to structural changes occurring in the first three postnatal weeks, and suggest that modified radial glial cells participate in the formation of glial tubes.

21. Heterogeneity of $GABA_A$ receptors in the rat olfactory bulb

Michela Bonino, Jean-Marc Fritschy[1], Dario Cantino and Marco Sassoè-Pognetto

Department of Anatomy, Pharmacology and Forensic Medicine, University of Turin, I-10126 Turin, Italy and [1]Institute of Pharmacology, University of Zürich, CH- 8057 Zürich, Switzerland

The response properties of the output neurons of the olfactory bulb, the mitral and tufted cells are modulated by reciprocal

connections with two classes of inhibitory interneurons, periglomerular and granule cells. The granule cells and at least one subpopulation of periglomerular cells use γ-aminobutyric acid (GABA) as an inhibitory neurotransmitter. GABA exerts its action in the olfactory bulb by activating two distinct receptor subtypes: $GABA_A$ receptors, which are bicuculline-sensitive chloride channels, and $GABA_B$ receptors, which are coupled to Ca^{2+} and K^+ channels via G proteins and second-messenger systems. $GABA_A$ receptors display an extensive structural heterogeneity based on the differential assembly of a family of at least 13 subunits into distinct heteromeric receptor complexes. Here, we analyzed the distribution of the α1, α2, α3 and α2 subunits of the $GABA_A$ receptor in the rat olfactory bulb, by applying immunocyto-chemistry with subunit-specific antibodies.

All the antibodies produced an intense immunoreactivity both in the main (MOB) and in the accessory olfactory bulb (AOB). However, the staining intensity for the α1 subunit was more elevated in the MOB compared with the AOB. In the MOB, each subunit displayed a unique distribution pattern, pointing to a considerable heterogeneity of $GABA_A$ receptors in the olfactory synaptic circuits. In the external plexiform layer (EPL), the α1 and α2 subunits showed a rather ubiquitous distribution, whereas the α3 subunit was preferentially located in the outer part. This distinct stratification pattern suggests the existence of different functional circuits, which involve specific subtypes of $GABA_A$ receptors. Electron microscopic immunocytochemistry showed that the α1 and α3 subunits are expressed at the dendrodendritic synapses established by granule cells with mitral/tufted cells in the EPL. To assess the colocalization of these subunits, we applied double-label immunofluorescence. The results indicated that in the outer part of the EPL there are at least three subtypes of $GABA_A$ receptors: two subtypes containing either the α1 or the α3 subunit, and one subtype containing both subunits. The heterogeneity of $GABA_A$ receptors in the EPL may be relevant for differentiating the response properties of mitral and tufted cells.

22. Ultrastructure of the taste buds in the bowfin, *Amia calva* (Holostei: Amiidae)

Friederike Boudriot and Klaus Reutter

Department of Anatomy, University of Tübingen, D-72074 Tübingen, Germany

Most of our knowledge about the ultrastructure of fish taste buds (TBs) is concerned to teleostean fish. In other groups of fish there likely exist other or slightly different types of taste buds, as in selachians and dipnoans. In a previous note (Reutter and Witt, 1996, Chem. Senses, 21: 664) we reported about the TBs of a holostean fish, the spotted gar, *Lepisosteus oculatus*. For reasons of comparison and aspects of TB phylogeny in fish we describe here the ultrastructure of the TBs of an other holostean fish, the bowfin, *Amia calva*.

TBs situated in the labial epithelium were investigated. The tissues were fixed in 2% glutaraldehyde-paraformaldehyde and processed conventionally for transmission electron microscopy.

In *Amia calva*, the labial TBs are 70–80 μm high and 55–60 μm wide; they are situated on dermal papillae. The TB's sensory epithelium consists of 40–50 elongated cells, light cells and dark cells. Further, there are basal cells, marginal cells and the axons of a small nerve fiber plexus. Light and dark cells are slender and reach from the TB's base up to the receptor area. Here, the light cells end in one large receptor villus. Light cells are rich in sER, rER, mitochondria, microtubules and intermediate filaments. Apically, dark cells bear several small microvilli and contain rER, mitochondria, microtubules and tonofilaments. Their apical cytoplasm is rich in large, electron dense secretory vesicles. Up to three basal cells lie directly, but without hemidesmosomal attachment, upon the basal membrane. The surface of the basal cells is irregular. Laterally, they protude with some small cytoplasmic lobes between the adjacent basal processes of light- and dark cells and the axons. Basal cells are rich in sER, rER, mitochondria, Golgi systems and vesicles.

Synapses occur between light cells (presynaptic side) and axons (postsynaptic side) and between basal cells (presynaptic side) and axons (postsynaptic side). Marginal cells are non-specialized epidermal cells which mark the TB's sensory epithelium against the epidermis.

Characteristically, in *Amia* TBs the dark cells contain in their apical third densely arranged electron dense vesicles. By exocytosis of these vesicles, the dark cells contribute to the mucus layer covering the receptor area. The basal cells in the TBs of the bowfin resemble those in the TBs of the spotted gar. But, unlike in the TBs of *Lepisosteus*, *Amia* TBs have only one type of light cells.

23. Changes in neurotransmitter release in the main olfactory bulb depend on learning about an odour

Peter A. Brennan, Heather M. Schellinck[1], Keith M. Kendrick[2] and Eric B. Keverne

Sub Department of Animal Behaviour, University of Cambridge, UK,
[1]Department of Psychology, Dalhousie University, Canada and
[2]Department of Neurobiology, Babraham Institute, Cambridge, UK

Olfactory learning is associated with substantial neural changes at the level of the olfactory bulb. Several extensively studied examples of olfactory learning, such as pheromonal learning in mated mice and lamb odour recognition in post-partum sheep, occur during sensitive periods. An important question, therefore, is whether similar neural changes occur in less highly specialized contexts. We have used a simple form of appetitive conditioning to either lemon or peppermint odours in adult mice, and have used *in vivo* microdialysis to measure changes in neurotransmitter levels in the main olfactory bulb in response to the learned odours. Presentation of the conditioned, but not the unconditioned, odour resulted in significant increases in the levels of certain transmitters, including glutamate from the mitral cells, GABA from the granule cells and noradrenaline from the centrifugal projection from the locus coeruleus. Overall, there was a decrease in the ratio of excitatory to inhibitory neurotransmitters in the olfactory bulb in response to the conditioned, but not the unconditioned, odour. Moreover, the magnitude of the decrease in this ratio was correlated with the level of behavioural response to the conditioned odour. These findings suggest that changes in the gain of the reciprocal synapses between mitral neurons and their inhibitory interneurons may be general feature of olfactory learning.

24. Pheromonal representation in the AOB: linking vomeronasal receptors to neuroendocrine effects

Peter A. Brennan, Heather M. Schellinck[1] and Eric B. Keverne

Sub Department of Animal Behaviour, University of Cambridge, UK and [1]Department of Psychology, Dalhousie University, Canada

Recent molecular biological investigations have characterized two classes of putative pheromone receptors expressed in separate populations vomeronasal receptor neurons. As they appear to project to functionally separate regions of the accessory olfactory bulb (AOB) it is likely that they form separate pathways handling fundamentally different types of pheromonal information. We have investigated this question using the expression of the immediate-early gene *egr-1* as a marker for activity of neurons in the AOB of female mice in response to constituents of male urine applied directly to their oronasal groove. A combination of the small molecules 2,3 dihydro-*exo*-brevicomin and 2-*sec*-butyl-4,5-dihydrothiazole has been reported to possess pheromonal activity, inducing oestrous in anoestrous females and promoting aggression in males. We found that this combination elicited expression of *egr-1* in clusters of presumed mitral neurons at the medial and lateral margins of the posterior accessory olfactory bulb. Major urinary proteins (MUPs) are thought to be important pheromonal signals, not only because they bind brevicomin and thiazole, but also because they have been shown to have a pheromonal effect in their own right, accelerating the onset of puberty in pre-pubertal female mice. We have found that these MUPs are also likely to mediate the pregnancy-blocking effects of male urine. Moreover they convey the strain identity of the male pheromone that is vitally important to prevent pheromones from the mating male from aborting his own offspring. Exposure of female mice to MUPs that had been stripped of their ligands predominantly induced expression of *egr-1* in the anterior half of the AOB, suggesting that the V1R class of receptor that projects to this region may be responsive to MUPs. Exposure of females to male pheromones during mating resulted in a different pattern of activity with higher levels of expression of i in the posterior half of the AOB. Therefore different constituents of the pheromonal signal and different contexts in which they are present can induce *egr-1* expression in different populations of neurons in the AOB, indicative of different pathways underlying pheromonal effects.

25. High level expression in *Pichia pastoris* of heterologous putative odorant-binding protein from honeybee *Apis mellifera*

L. Briand, V. Perez, J.-C. Huet, E. Danty[1], C. Masson[1] and J.-C. Pernollet

Unité de Recherches de Biochimie et Structure des Protéines, INRA UR 477, Domaine de Vilvert, F-78352, Jouy-en-Josas Cedex and [1] Neurobiologie Expérimentale et Théorie des sytèmes complexes, CNRS UPR 9081, 16, rue Claude Bernard, F-75231 Paris Cedex 05, France

A honeybee putative general odorant-binding protein Asp2 (Danty *et al.*, 1997, FEBS Lett., 414: 595–598; Danty *et al.*, 1998, Chem. Senses, 23: 83–91) has been expressed in the methylotrophic yeast *Pichia pastoris*. The Asp2 coding region, which includes an endogenous secretion signal, was amplified by polymerase chain reaction and subcloned into the *Pichia* expression vector pHILD2. Yeast cells were transformed with a construct using standard spheroplast transformation method. The recombinant Asp2 was secreted into the buffered minimal medium using the native signal peptide. Samples of the extracellular medium, taken at various time intervals of the fermentation, were separated by reversed phase HPLC. The major peak was analysed by electrospray ionization mass spectrometry and N-terminal sequencing. Mass spectrometry showed a protein with a mass of 13 695.2, which is in perfect agreement with the molecular mass measured on the native protein showing native-like processing and formation of the three disulfides bridges. N-terminal sequencing confirmed that the native signal peptide was properly cleaved. HPLC revealed an additional peak adjacent to the main peak due to proteolytic degradation probably caused by extracellular proteases during the fermentation at pH 6.0. A detailed analysis showed amputation of eight amino acids on the C-terminal end of the protein. This degradation was overcome by buffering the culture medium to pH7 and the final expression yield was 100 mg/l of culture. The recombinant protein was then purified by ion-exchange chromatography and gel filtration in a highly pure form. *Pichia pastoris* producing high yields recombinant Asp2 should allow ligand binding and mutational analysis in order to understand the relationship between structure and biological function of the protein. Crystallogenesis of the recombinant Asp2 is currently under investigation.

26. WA point mutation (C460S) in the olfactory cyclic nucleotide-gated channel abolishes the activation by nitric oxide

Marie-Christine Broillet[1,2] and Stuart Firestein[1]

[1]Institute of Pharmacology and Toxicology, University of Lausanne, CH-1005 Lausanne, Switzerland and [2]Department of Biological Sciences, Columbia University, New York, NY 10027, USA. e-mail: marie-christine.broillet@ipharm.unil.ch; sjf24@columbia.edu

We have recently demonstrated that nitric oxide (NO) generating compounds can directly activate the olfactory cyclic nucleotide-gated (CNG) channels in addition to activation by their natural ligand cAMP. This reaction appeared to involve the free SH group of a cysteine residue located on the intracellular face of the channel. To further identify the target site(s) of NO, we mutated the intracellular cysteine residues C460, C484, C520 and C552 of the rat a (OCNC1) CNG channel into serine residues and studied the respective single channel properties and activation by cyclic nucleotides and NO of the mutant channels after their expression in HEK 293 cells. We found that while all the mutant channels can still be activated by cAMP with single channel characteristics similar to the wild type homomeric a (OCNC1) channel, the mutant C460S a (OCNC1) channel exhibits a complete loss of sensitivity to NO. This result shows that this amino acid residue is clearly involved in the NO activation mechanism. The lack of effects of the other mutations indicates that there is no contribution(s) or collaboration(s) of the other intracellular cysteine residues in the NO activation of the channel. Using macroscopic currents, we also determined that the half maximal activation of the a (OCNC1) channel occurs at SNC concentrations of 103 ± 7 mM (n = 6) and that the activation by NO shows a clear cooperativity. The Hill coefficient ranges between 1.5 and 3.2, suggesting that the opening of a single channel depends on the cooperative binding of at least three NO molecules. These

data demonstrate that NO is likely to play a role in CNG channel modulation and that the mechanism is through a direct interaction with the channel protein.

27. Effects of short-term odor exposure on the olfactory bulb reactivity in the adult rat

N. Buonviso, N., A. Helme-Guizon[1], M. Chaput, M. Chanlansonnet, R. Gervais[2] and J. Mallet[1]

Neuroscience & Olfaction, CNRS-University Lyon I, F-69622 Villeurbanne Cedex, [1]Laboratoire de Génétique de la Neurotransmission, Hopital de la Pitié, F-75013 Paris and [2]Institut des Sciences Cognitives, CNRS, F-69675 Bron, France

In almost all sensory systems, functional modifications of cortical maps have been reported in adult life following manipulations of the sensory input (deafferentations, stimulations etc.). When observed in the olfactory system, such changes were always associated to a learning paradigm. No study reported the existence of modifications of the bulbar reactivity following a very simple manipulation of the olfactory input as an exposure to an odorant. This study tested this possibility. Rats were exposed for 20 min per day during 6 consecutive days to a litter odorized by either cineole (CIN), methyl-amyl ketone (MAK) or isoamyl acetate (ISO), or with no odor in the control group (CTRL). On day 7, rats were anesthetized and we recorded the spontaneous activity of mitral/tufted cells along with their responses to the familiar odor and to four novel odors. Results revealed that (i) the spontaneous discharge frequencies were not significantly different in the four groups; (ii) the average odor-evoked/spontaneous frequency ratio was ~1 in the exposed groups but ~2 in the CTRL group; (iii) the proportion of excitatory responses was considerably decreased in the exposed groups while the number of non-responses was significantly enhanced; and (iv) excitatory responses were decreased not only to the familiar odor but also to four other novel odors, so that the number of cells excited by none of the five test odors was dramatically increased in the exposed groups. The persistence of these effects was then tested by recording cells in MAK and ISO groups 10 days after the end of exposure. Whereas responsiveness retrieved CTRL levels in the MAK group, it remained depressed in the ISO group. Finally, we wondered if such functional modifications could be accompanied by changes in molecular markers of synaptic plasticity. We used hybridization *in situ* technique to study the distribution of mRNA coding different proteins of the exocytosis complex in rats exposed to ISO and in CTRL rats. Results revealed a significant increase in the optical density of the mitral cell layer labeling in exposed rats for mRNAs coding synaptophysine and synaptotagmine proteins.

This study reveals that a simple short-term exposure to one odor induces a drastic and non-specific decrease in the olfactory bulb reactivity, along with an increase in the synthesis of mRNA coding proteins involved in synaptic transmission. The high reactivity of cells in naive rats could result from a restricted olfactory environment. Changes induced by odor exposure could be triggered by the action of centrifugal fibers, leading to permanent changes in intra-bulbar synaptic efficacy.

28. Stability and interactions of porcine odorant-binding protein

T. V. Burova[1], Y. Choiset, A. Meynier, C. K. Jankowski[2] and T. Haertlé

Laboratoire d'Etude des Interactions des Molécules Alimentaires, Institut National de la Recherche Agronomique, B.P. 71627, F-44316 Nantes Cedex 03, France and [1]Faculté des Etudes Supérieures et de la Recherche, Université de Moncton, Moncton NB, Canada E1A 3E9

[1]On leave from the Institute of Biochemical Physics of RAS, Vavilov Str. 28, Moscow 117813, GSP-1, Russia

Apparently homogenous odorant binding protein (OBP) purified from pig nasal mucosa displayed subunit molecular weights of 17.223, 17.447, 17.689 (major component) kDa as estimated by ESI/MS. According to gel-filtration this protein, its truncated forms and/or its variants are homodimeric in physiologic conditions (pH 7.0, 0.1 M NaCl). The dimer–monomer equilibrium shifts towards prevalent monomeric form at pH <4.5. High-sensitivity differential scanning calorimetry (HS-DSC) shows that the unfolding transition of pOBP at neutral pH is completely reversible, being characterized by the transition temperature of 69.2°C and enthalpy of 391.0 kJ/mol per monomer. Surprisingly, despite previously demonstrated by crystallographic analysis of its 3-D structure, domain swapping, the transition curve of pOBP is well approximated by the two-state model on the level of subunit indicating that two monomers behave independently. This means that temperature induced dissociation of homodimer occurs without enthalpic contribution and precedes the unfolding. The isothermal titration calorimetry (ITC) shows that under physiological conditions pig OBP binds 2-isobutyl-3-methoxypyrazine (IBMP) and 3,7-dimethyloctan-1-ol (DMO) with association constants of 3.2×10^{-6} M^{-1} and 4.7×10^{-6} M^{-1} respectively. The binding stoichiometry of both ligands corresponds to one molecule of ligand per homodimer of pOBP. The binding stoichiometry decreases at acidic pH following the shift in dimer ↔ monomer equilibrium towards monomeric form. According to HS-DSC data, the binding of IBMP leads to substantial stabilization of the pOBP folded structure that is manifested by an increase of the unfolding temperature and of enthalpy. Both ITC and HS-DSC data revealed that the binding of studied ligands to pOBP is an enthalpically driven process with unfavourable change of the entropy. This result allows us to conclude that mechanism of binding of the studied odorants to pOBP is not only driven by hydrophobic effect but may be also regulated by electrostatic interactions.

29. The shape of the olfactory space: a tribute to Andrew Dravnieks

Pascal Callegari, Dominique Valentin[1] and Paul Laffort

CNRS, European Centre for Chemical Senses and [1]ENSBANA, University of Bourgogne, Dijon, France

Previous attempts at defining the structure of human olfactory space have yielded contradictory results. For example, the Jeltema and Southwick (1986, J. Sens. Stud., 1: 123–136) factor analysis of 146 descriptors performed on 415 profiles (data unfortunately unpublished) gave rise to a rather cumbersome 17-dimensional space explaining 89% of the variance. Other studies based on perfumery classification systems such as the Discodor of Haarman and Reimer (1979), the rosace of Firmenich (1989) and

the Champs des odeurs of Jaubert *et al.* (1987, Parf. Cosm. Arômes, 77: 53–56 and 78: 71–82) yielded 2- or 3-D olfactory spaces. However, the lack of clarity of the experimental methodology used in these three latter studies makes it difficult to determine their validity. Other studies, using similarity data, also yielded a low dimensional olfactory space. For example, in an often cited study, Woskow [1968, in N. Tanyolaç (ed.), Theories of Odor and Odor Measurements. Proceedings of the Nato Summer School, Istanbul 1966, Maidenhead, Technivision, pp. 147–188] showed that a multidimensional scaling (MDS) analysis of the similarity data of 25 odours yielded a 3-D space explaining 86% of the variance. The problem, however, with similarity studies, is that they can be based only on a limited number of odours. Thus, the greater coherence of the resulting space can be due to the small size of the sample examined.

In the study reported here factor analysis (correspondence analysis) and similarity analysis were used to examine the shape of the olfactory space derived from the Dravnieks' (1985, Atlas of Odor Character Profiles, ASTM Data Series 61, Philadelphia) atlas (141 odours × 146 semantic descriptors). The correspondence analysis using the original data, yielded results similar to that of Jeltema and Southwick (1986): the first three dimensions explain only 34% of the total variance and 17 dimensions are needed to explain 80% of the variance. Observation of the 3-D subspace reveals the presence of a U-shaped structure with a peak at the basis of the U. The proximity analysis was based on similarity data derived from the odour profiles compiled by Dravnieks using a Callegari (1998, thesis, University of Boulogne) algorithm. This algorithm, based on the ratio model of categorization (Tversky, 1977), leads to similarities comparable to that obtained directly by human subjects. A MDS of the similarity data yielded results comparable to those of Woskow (1968). Thus, the first three dimensions explain 88% of the variance, revealing a more coherent space than the correspondence analysis of the profiles. Examination of the 3-D subspace reveals a basin-like shape somewhat similar to that of Woskow (1968). This last result is reminiscent of the topographical coding hypothesis of olfactory information at the olfactory bulb level.

30. How does HSV-1 reach the brain?

P. Calvi, A. Cavaggioni, C. Mucignat and G. Palù[1]

Istituto di Fisiologia dell'Università di Padova and [1]Istituto di Microbiologia dell'Università di Padova, I-35131 Padova, Italy. e-mail: andreac@ipdunidx.unipd.it

Herpes simplex virus type 1 (HSV-1) is a common guest which occasionally reactivates from latency in the sensory ganglia of humans. The brain is protected from HSV-1 except for rare cases of herpetic encephalitis, but viral genomic sequences have been amplified post-mortem by PCR from olfactory bulbs, temporal lobes and hippocampus, suggesting that HSV-1 is a common and asymptomatic guest also of our brain. How does HSV-1 reach the brain? To try to answer this question we turned to an animal model. Mice appear to be resistant to infection of HSV-1 inoculated intranasally; only suckling mice and adult mice of some strains develop encephalitis by retrograde diffusion of the virus in the olfactory circuits. We took infection of resistant mice (strain Swiss) as a model to see whether the turnover of the olfactory neuroepithelium facilitates infection of the brain through the nose.

Acute experiment. Degeneration and a mitotic wave was induced in the olfactory epithelium of twelve adult Swiss mice by intracranial axotomy of the right fila olfactoria. HSV-1 of strain KOS was given intranasally at a dose of 500 000 pfu, on day 3, 5, 7, 14 and 22 post-axotomy. The mice were observed for signs of encephalitis and the survival time was scored. It was concluded that olfactory axotomy facilitates HSV-1 infection in a short time window of a few days post axotomy. Interestingly this period is coincident with the induced turnover of neural olfactory cells. *Long-term experiments*. The infection model was iterated in the third post-axotomy day in batches of six adult Swiss mice using different viral doses and strains, and compared with non-infected controls. HSV-1 of strain KOS at a reduced dose of 65 000 pfu induced signs delayed by several weeks. Behavioral signs appeared first, namely, reduced exploratory activity in arena test, reduced social interaction, crouched posture and ruffled fur, later there was a consistent weight loss and neurological signs appeared last, shortly before death. Histological examination of the brain disclosed signs of diffuse atrophy in some mice but neither infiltrates nor reactive cells were observed. HSV-1 of strain F (350 000 pfu) produced only cutaneous sores on the head skin suggesting productive infection of the trigeminal sensory ganglia of experimental animals. HSV-1 of strain 3616 (60 000 pfu), a virus engineered for 'attenuated' neurovirulence gave two cases of delayed acute panencephalitis with hind limb paralysis and, histologically, with subpial and perivascular infiltrates of reactive cells as well as focal necrosis in the brain. By comparison with controls it was concluded that olfactory axotomy facilitates the long term infection of the brain, the signs depending on the HSV-1 strain.

These experiments on the axotomized mouse model of HSV-1 infection suggested that the olfactory neuroepithelium with its physiological, ongoing and lifelong cell turnover is a natural gate to the brain for HSV-1.

31. Expression of stathmin related proteins during olfactory neurogenesis

Paola Camoletto, Alessandra Colesanti, Aldo Fasolo

Department of Human and Animal Biology, University of Torino, via Accademia Albertina 17, I-10123 Torino, Italy

Stathmin related proteins (stathmin, SCG10, XB3, RB3) are significantly expressed in the developing nervous system and their phosphorylation is correlated with the action of multiple extracellular stimuli regulating cell proliferation and differentiation.

Stathmin is an ubiquitous 19 kDa cytosolic phosphoprotein, which is a substrate for protein kinase A, MAP kinase, and cdk kinase, and possibly a marker for cellular activation. SCG10 is a neuron-specific growth-associated protein that is concentrated in the growth cones of developing neurons and is involved in the control of neuronal differentiation. We have previously found that stathmin expression in the adult olfactory system of rodents is restricted to newly-generated, undifferentiated olfactory receptor neurons (ORNs).

To understand the role of these proteins during neurogenesis we have analyzed the immunocyto-chemical distribution of stathmin and SCG10 throughout development of the olfactory epithelium (OE) of the mouse and in adulthood. At early stages (E13–E16) stathmin and SCG10 are largely coexpressed in the OE and in the

vomeronasal organ and some of them are altogether positive for OMP (olfactory marker protein).

Later on (E17–P7) anti-stathmin labels several rows of ORNs in the OE, including basal cells, whereas SCG10 and neuron specific tubulin (NST) are mainly expressed in immature ORNs.

In the adult mouse, stathmin and SCG10 immunolabelings are restricted to few immature ORNs, whereas after regeneration following peripheral lesion, they are again strongly expressed and by large colocalize with NST.

Financial support from Cavalieri Ottolenghi*, CNR, MURST.

32. Changes in olfactory perception and dietary habits in the course of pregnancy: a questionnaire study

Patrizia Cantoni, Robyn Hudson, Hans Distel and Matthias Laska

Department of Medical Psychology, University of Munich Medical School, Goethestraße 31, D-80336 Munich, Germany. e-mail: laska@imp.med.uni-muenchen.de

Marked changes both in dietary habits and in the perception and hedonic evaluation of odors are frequently reported to occur during pregnancy. In order to assess possible correlations between these two phenomena, we conducted a retrospective questionnaire study in which 500 women who had successfully completed at least one pregnancy participated. Our main findings were as follows:

Sixty-eight percent of the women reported having experienced changes in olfactory perception during their pregnancies. These changes were most marked during the first trimester and were almost always perceived as an increased general responsiveness to odors.

Seventy-four percent remembered odors that were perceived as less pleasant than prior to or after their pregnancies, and 58% stated that certain everyday odors even caused nausea. In response to both questions, perfume, cigarette smoke, meat, food in general, diesel exhaust and sweat were named by at least 10% of the women. In contrast, only 21% mentioned odors as being more pleasant during pregnancy, with flowers, fruits, woodlands and perfume as favorites.

Seventy-five percent of the women changed their dietary habits during pregnancy, with an approximately equal number reporting an increase or decrease in the consumption of certain foods. Again, these changes were most marked during the first trimester.

Food items like coffee, meat, alcohol, sausage, sweets and certain vegetables were named most frequently as being less pleasant during pregnancy, and consequently consumed to a lesser degree. A similar percentage of women recollected food items as being more pleasant during pregnancy, with sweets, fruits and dairy products as the favorites which then were consumed more frequently.

Fifty-eight percent of the women suffered from 'morning-sickness', i.e. pregnancy-related nausea which, again, was most marked during the first trimester.

The well-known longevity of odor memories was confirmed by the finding that the number of odors remembered as being more pleasant or less pleasant during pregnancy did not decreaase with increasing time since delivery.

Statistical analyses revealed significant positive correlations between the frequency of changes in olfactory perception and (i) changes in dietary habits and (ii) pregnancy-related nausea.

Taken together, our results suggest that these phenomena may not only coincide in the course of pregnancy but may be functionally linked to each other.

Supported by the Deutsche Forschungsgemeinschaft (La 635/6-1 and 6-2).

33. Cortical activation related to both gustatory and lingual somatic stimulation in the human: a fMRI study

B. Cerf, P.-F. Van de Moortele[1], D. Le Bihan[1] and A. Faurion

Neurobiologie Sensorielle, F-91744 Massy Cedex and [1]SHFJ, CEA, 91406 Orsay, France

Pure gustatory and lingual somatic sensitivity collaborate and form, together with retronasal olfaction, a global perception improperly named 'taste'. The aim of this study was to look for the contribution of lingual somatic sense to taste perception at cortical level in the Human. Twelve subjects participated in the experiment. Taste stimuli were NaCl, aspartame, quinine hydrochloride adn HCl pH 2.0, whereas somatic stimuli were HCl pH 1.5 (pungent) and aluminum potassium sulfate (astringent and acid). Functional magnetic resonance imaging (fMRI) was performed with a 3 Tesla whole body MR scanner (Bruker) allowing echo planar imaging (repetition time: 5 s, 22 slices 5 mm thick; resolution: 64 × 64 pix; field of view: 22 × 22cm²). Five minute experiments alternated water as reference (75 s) and stimulus (18 s), delivered as bolus of 50 µl every 3 s and freely swallowed by the subject. Data were processed with Spm96 using the mean subject's perception profile as extracting filter ($F < 0.01$, $P < 0.01$ on the voxel, $P < 0.2$ on the cluster). Results were analyzed for each subject individually to take into account the great inter individual differences of sensitivity which characterize taste chemoreception. Both somatosensory and taste activations were localized in insula, pre- and post-central gyrus (basis of SI), frontal, temporal opercula, in accordance with electrophysiological data on monkey (Norgren, 1990, in G. Paxinos (ed.), The Human Nervous System, Academic Press, pp. 845–861). This similarity of activation for taste and somatosensory stimuli is in accordance with the well known psychological confusion between these sensory modalities. However: (i) for somatosensory data, activation in the right hemisphere lower part of insular cortex was discriminated from activation in other peri-insular regions, whereas for taste data, activation in both left and right lower insula was discriminated from other peri-insular regions; (ii) for taste data, the superior part of insular cortex was activated correlatively with the rolandic operculum (basis of SI or pre- and post-central gyrus), which was not the case for somato sensory data; and (iii) for taste data, the lower part of left insula was associated with the left angular gyrus (speech comprehension), whereas it was not the case for somatosensory data.

34. Social odor signals in the Alpine marmot, *Marmota marmota* (L.): complexity of the chemical odorant message, scent-marking behavior and olfactory receptors

J.L. Clement, A. Tirard, M. Renucci, A.G. Bagneres, V. Matarazzo, M.C. Bel[1] and A. Belaich[2]

CNRS-UPR 9024, Laboratoire de Neurobiologie, [1]UCB-Lyon I, Laboratoire de Biologie des Populations d'Altitude CNRS-UMR 553, 43 Bd du 11 Novembre 1918, F-69622 Villeurbanne and [2]CNRS-UPR9036 Bioenergetique et ingenierie des proteines, 31 chemin Joseph Aiguier, F-13402 Marseille, France

The Alpine marmot marks out its territory using chemosignals secreted by a gland located near the eye. The purpose of this study is to describe the composition of this secretion, the behavior and olfactory protein receptors.

Field bioassays were performed based upon typical responses of territorial resident marmots towards scent-marks from potential intruders were performed. Marmots were found to sniff longer and to mark more glass tubes covered by foreign scent-marks than clean ones.

GC/MS analysis revealed a group of 30 compounds in each individual temporal secretion, including acids, ketones, alcohols and amino acid in relative proportions.

Using the nasal epithelium of the Alpine marmot and various sets of degenerated primers corresponding to consensus sequences of odorant receptors, we were able to amplify by reverse transcription polymerase chain reaction (RT-PCR), clone, and obtain the partial sequences of 14 new odorant receptor gene products. The amino acid translations exhibit a high similarity with previously reported mammals olfactory receptor protein sequences, and classify them unambiguously as members of the same superfamily of seven transmembrane domain receptors. Transmembrane helical regions II, III, IV and V, as well the extracellular loop regions, were delineated using an hydropathy plot. Comparisons with olfactory receptor sequence from other species suggest that the marmot sequences determined in this study belong to three different subfamilies.

35. The odor-specificities of a subset of olfactory receptor neurons are governed by the *acj6* gene of *Drosophila*, a POU domain transcription factor

P. Clyne, M. De Bruyne and J.R. Carlson

Department of MCD Biology, Yale University, New Haven, CT 06520-8103 USA. e-mail: john.carlson@yale.edu

Little is known about how olfactory receptor neurons acquire their odor-specificities. Using single-unit electrophysiology, we have learned that one olfactory organ of *Drosophila* contains six classes of neurons, paired in three types of sensory hairs according to a strict pairing rule. Some of these neurons appear to be narrowly tuned to a small subset of odors, whereas others appear more broadly tuned. One of these neurons is excited by one odor and inhibited by others. We have found that neuronal identity relies on the *abnormal chemosensory jump 6* (*acj6*) gene. The original *acj6* mutant was isolated by virtue of a defect in olfactory behavior. Physiological analysis of individual olfactory neurons showed that in *acj6* mutants, a subset of neurons acquires a different odorant response profile. Molecular analysis of *acj6* shows that it encodes a POU domain transcription factor. Other members of this class of transcription factor have previously been shown to act in the development of mammalian visual and auditory neurons.

Our data suggest a model in which the odor response spectrum of an olfactory neuron, and perhaps the choice of receptor genes, is determined through a process requiring the action of POU domain transcription factors.

36. The intrinsic nervous system of the CP/VEG complex

Caterina Crescimanno, Andrea Sbarbati, Carlo Zancanaro, Paolo Bernardi and Francesco Osculati

Institute of Anatomy and Histology, University of Verona

The intrinsic nervous system of the circumvallata papilla (CP) and von Ebner glands (VEG) was investigated in rat tongue. Cells involved in the production of nitric oxide (NO) were identified by immunohistochemical [neuron specific nitric oxide synthase type 1 (NOS-1)] and cytochemical techniques (NADPH-diaphorase) at the light and electron microscopy levels. Nerves containing calcitonin gene-related peptide (CGRP), vasoactive intestinal peptide (VIP) and substance P (SP), which have been previously described as involved in taste systems, were also studied. A ganglion of ~180–190 neuronal cells was present between the CP and VEG (CP/VEG ganglion). These cells scored as positive to NOS-1 and to NADPH-diaphorase, but did not contain the neuropeptides used in this study, i.e. CGRP, SP and VIP. From the CP/VEG ganglion leave four nervous branches: the first runs in the lamina propria of the receptor free mucosa surrounding the CP; the second is ramified below the regions of epithelium in which taste buds (TBs) were embedded; the third is directed towards the VEG where it ramifies among the adenomeres; the last follows the vascular system of the CP. We supposed that the nerve plexus below the TBs is composed of two layers. The superficial layer is rich in peptidergic fibers while the deeper layer is rich in nitrergic fibers. In the CP, CGRP immunoreactive (CGRP-IR) fibers are regularly associated to the TBs. CGRP-IR cell bodies are not visible in TBs, in the ganglion and in the VEG. Substance-P-immunoreactive (SP-IR) fibers are visible at the base of the TBs with a distribution pattern quite similar to those of CGRP-IR fibers.

Our study demonstrates that a common intrinsic nervous system is present in the CP and VEG. The nitrergic CP/VEG ganglion cells may mediate interactions between chemoreceptorial systems in the CP and secretory cells in the VEG. These data suggest that NO could be involved in the regulation of blood supply to the CP and in the regulation of the paracrine system of the VEG which is mainly composed of VIP or SP-IR cells.

37. Two novel members of OBP family identified in the honeybee: sexual dimorphism and development studies

E. Danty, C. Michard-Vanhée, D. Huet, J.-C. Huet[1], G. Arnold, J.-C. Pernollet[1] and C. Masson

Laboratoire de Neurobiologie Expérimentale et Théorie des Systémes complexes, CNRS, UPR9081, INA, 16, rue C. Bernard, F-75005 Paris, Centre Européen des Sciences du Goút CNRS, 15, rue H. Picardet, F-21000 Dijon and [1]Biochimie et Structure des Protéines, INRA UR 477, Domaine de Vilvert, F-78352 Jouy-en-Josas, France

In the honeybee, crucial behaviors such as mating, foraging and social interactions are merely based on chemical communication mediated by olfactory cues, species-specific (pheromones) or not (allochemicals). How odor ligands are screened and detected represents an important step underlying odor transduction and coding. Airborne odorant molecules are thought to be translocated through the sensillum lymph to the receptor proteins by small water soluble extracellular proteins, the odorant-binding proteins (OBPs). In adult bees, we have recently reported the biochemical characterization of a large group of antennal specific proteins (ASP) subdivides, on the basis of N-terminal microsequencing, into three subclasses, namely ASPI, ASP2, ASP3 (Danty *et al.*, 1997, FEBS Lett., 414: 595–598; Danty *et al.*, 1998, Chem. Senses, 23: 83–91). The complete CDNA sequence of ASPI and ASP2 was obtained by RACE 3′, using partially degenerated oligonucleotides selected in the N-terminal sequences, and by antennal CDNA library screening. ASPL and ASP2 represent two novel members of the large family of insect OBP: they share the common biochemical properties (molar mass, acidity, solubility), tissue specificity, conservation of the six cysteines involved in three disulfide bridges formation and a highly hydrophobic signal peptide cleaved during maturation processing. Nevertheless ASPI and ASP2 present only 15% sequence homology. They are quite different from other members of the family identified in Diptera or in Lepidoptera. *In situ* experiments show a differential localization of both MRNA, in two distinct olfactory sensilla types and confirm the quantitative sexual dimorphism which will be discussed in the context of the coding of biological odor signals depending on sex. A preliminary developmental profile of ASP also suggest a role of these proteins in olfaction. Protein expression in yeast is in progress to study their structural and binding properties (see the poster of Briand *et al.*).

38. Rodent taste cell responses to pH and osmotic pressure

John A. DeSimone, Vijay Lyall, Robert E. Stewart[1], Gerard L. Heck and George M. Feldman[2,3]

Departments of Physiology and [2]Medicine, Virginia Commonwealth University, Richmond, [1]Department of Psychology, Washington and Lee University, Lexington and [3]McGuire Veterans Affairs Medical Center, Richmond, VA, USA

pH-sensitive chemoreceptor cells occur in the brainstem (NTS), in carotid bodies and in lingual taste buds. In each case intracellular pH (pH_i) is a linear function of imposed changes in extracellular pH (pH_o), with a high slope (>0.7). In these systems perturbations in pH_i do not initiate restoration of pH_i. In contrast, changing an extracellular buffer (e.g. CO_2, HCO_3^-, NH_4^+, propionate) under isohydric conditions causes only a transient change in pH_i because restoration of pH_i is initiated. We have studied acid-evoked taste responses in the chorda tympani (CT) of rats and hamsters and utilized cell imaging to study changes in pH_i in isolated cells and taste bud fragments. CT acid responses were typically insensitive to perturbations in field voltage, and to the Na^+ channel blockers, amiloride and benzamil, and to the Na^+/H^+ antiporter blocker, MIA. In isolated taste cells the pH_i versus pH_o relation was also unaffected by amiloride, Na^+ replacement and MIA, and recovery from isohydric perturbation of pH_i was also Na^+-independent. Transepithelial lingual potentials, recorded during CT responses, show a positive-going transient when acids are removed, suggesting that fixed negative charges are titrated by H^+ ions diffusing into paracellular spaces. Direct measurement of the basolateral microenvironment pH of a polarized rat fungiform taste bud demonstrated a small pH drop for a large pH drop on the apical side. These changes were rectified and inhibited by apical Ca^{2+}. It appears acid sensing involves intracellular pH-tracking while paracellular pH changes are attenuated by as yet undefined mechanisms.

While taste receptors respond to low pH, their response to osmotic pressure is more complex. Direct measurement of cell volume showed that increases in tonicity resulted in decreases in volume. In contrast, CT responses were selectively affected. Specifically, rat CT responses to a fixed concentration of NaCl were increased by as much as 50% by the additional osmotic pressure induced by mannitol or cellobiose (poor CT stimuli). It appears that cell volume changes may modulate responses to tastants. The results indicate that strong stimulation of salt-best NTS units attributed to certain saccharides may actually represent osmotically enhanced responses to salt contaminants.

Supported by NIH Grants DC02422 and DC00122, and the Department of Veterans Affairs.

39. How eucaryotic cells sense chemical gradients

Peter Devreotes, Carole Parent, Zhan Xiao, Saskia Van Es, Jane Borleis, Wendy Froelich, Ning Zhang, Tian Jin, Yihong Yao, James Silverman, Chuck Brogan and Yu Long

Johns Hopkins University, School of Medicine, Baltimore, MD 21205, USA

Chemotaxis is an integral part of immune response and plays a key role in wound healing, angiogenesis, and embryogenesis. This fundamental cellular process is found in all eukaryotes and has remained essentially unchanged throughout evolution. Research in the last fifteen years in *Dictyostelium discoideum* has shown that chemoattractants are sensed by the same basic signal transduction mechanisms as are many hormones, neurotransmitters, odorants. The receptors for these cell–cell signaling molecules activate heterotrimeric G-proteins that regulate phosphodiesterases, phospholipases, ion channels and adenylyl cyclases. Our strategy is to exploit the genetic advantages of *D. discoideum* to discover mechanisms of sensing chemoattractant gradients and to apply this information to higher eukaryotic cells.

We have identified many of the genes involved in these processes. There are four cell surface cAMP receptors, eight G-proteins α-subunits, unique β- and γ-subunits, and an adenylyl cyclase. Targeted gene disruptions have shown that many of these genes are essential for development and play central roles in chemotaxis, cell–cell signaling and agonist-induced gene expression. Complementation of these null cell lines with randomly

mutagenized libraries has allowed us to identify point mutations that alter specific functions of the receptors, G-protein subunits or adenylyl cyclase. Current work is focused on biophysical studies of these mutant proteins. Complementation also provides a simple genetic test of the function of GFP-tagged proteins. With this approach, we are studying the dynamic distribution of receptors and G-protein subunits in single living cells during chemotaxis and persistent stimulation.

By screening gene-tagged cell lines for mutants with phenotypes similar to those lacking the known signal transduction components, we are identifying novel genes involved in these pathways. For example, activation of adenylyl cyclase requires two cytosolic proteins, Crac and Pianissimo, in addition to receptor/G-protein/adenylyl cyclase. Crac is rapidly and transiently recruited to the cell membrane during chemoattractant stimulation. Recent work has identified novel genes specifically required in the pathway leading to new actin filament formation in extending pseudopods.

40. Temporal variation of olfactory responses of the Colorado potato beetle to volatiles emitted by potato plants

Joseph C. Dickens

US Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Plant Sciences Institute, Vegetable Laboratory, Beltsville, MD 20705 USA

Behavioral responses of insects to host plants depend on the physical characteristics of the plants and the chemical signals they emit. Volatile chemical signals are detected by receptors which undergo a maturation process in which neurophysiological responses to odors increase to a point with age. This increase may be correlated with behavior of the insect. A degree of plasticity occurs in some insects in which neural and behavioral responses to odors may be modified by sensory experiences of adults. Colorado potato beetles (CPB), *Leptinotarsa decemlineata*, are attracted by volatile chemical signals emitted by mature potato plants. Feeding by CPB induces emission of a number of volatiles by the plant over the short term, while over the long term there is a systemic release of compounds by the plant that is thought to be in response to salivary secretions of the beetles. I used electroantennograms (EAGs) to investigate maturation and temporal variation of responses of antennal chemoreceptors of five age groups of both sexes of CPB to 22 volatiles emitted by potato plants. EAGs to a standard odorant, (*Z*)-3-hexenyl acetate, increased from day of emergence through 12–14 days of adulthood. This rate of this increase was similar to the time course for ovarian development in females. Eight volatiles elicited EAGs that were >25% of the standard. To obtain further information about the relative effects of these eight volatiles on younger (0–1 and 2–4 days old) versus older (12–14 and 21–23 days old) insects relative to those approaching sexual maturity (6–8 days old), a new statistic called 'linear age-skew' was created. 'Linear age-skew' values were plotted against mean responses to chemicals to give an indication of chemicals that elicit similar overall responses as well as have similar effects across age groups. Three distinct classes which were apparent from 'linear age-skew' values are discussed with regard to their presence among volatiles released by potato plants over the short term and those released systemically in response to CPB feeding. Our results help to explain behavioral responses of CPB to chemical signals and provide a basis for investigating interactions of the CPB with its host plant, conspecifics and associated insects.

41. Localization of odor-induced neuronal activity in the antennal lobes of *Calliphora vicina* by high-resolution 2-deoxyglucose autoradiography

P.G. Distler

Institut für Zoologie, Universität Regensburg, D-93040 Regensburg, Germany. e-mail: paul.distler@biologie.uni-regensburg.de

The distribution of odor evoked neuronal activity in the antennal lobes of the blowfly *Calliphora vicina* has been studied by means of [^{3}H]2-deoxyglucose (2-DG) uptake. 2-DG injected flies were stimulated with natural attractants like odor from meat or with single odorants (e.g. *n*-aliphatic alcohols, *n*-aliphatic acids, aromatic compounds, terpenes). Control animals were stimulated with filtered air. Meat which contains many odorous compounds induced uptake of 2-DG in a large number of glomeruli. With stimulation by a single odorant a smaller number of glomeruli were labeled. In the control brains, the majority of glomeruli showed low 2-DG uptake. The distribution of labeled glomeruli was odor-specific and consistent in different specimen as well as in the two antennal lobes of a given specimen. The labeling patterns evoked by different odors overlapped partially but were clearly different. The highest amount of label within the glomeruli was found in the arriving axons of antennal receptor neurons. The present data provide evidence that different odors are represented as defined spatial patterns of activity across the antennal lobe glomeruli. The overlapping activity patterns suggest that certain glomeruli participate in the nervous processing of different odors.

42. Spontaneous activity, adaptation and sensitization in pheromone-sensitive olfactory receptor neurons of the hawkmoth *Manduca sexta*

Jan Dolzer, Karin Bittmann and Monika Stengl[1]

Institut für Zoologie, Universität Regensburg, D-93040 Regensburg, Germany. e-mail: jan.dolzer@biologie.uni-regensburg.de

[1]*Current address: Fachbereich Biologie, Tierphysiologie, Philipps-Universität, D-35032 Marburg, Germany*

Adaptation, the adjustment of sensitivity in response to adequate stimulation, enlarges the dynamic range of an olfactory receptor cell without loss of resolution, and provides an early step in the processing of sensory information. Using the tip recording technique [Kaißling, 1974, in Jaenicke (ed.), Biochemistry of Sensory Functions, Springer], we investigated the electrical activity of pheromone-sensitive olfactory receptor neurons (ORNs) innervating long sensilla trichoidea on the antennae of the male hawkmoth *Manduca sexta*. First we examined the spontaneous activity and tested, whether it underlies circadian oscillations. For the investigation of olfactory responses in different adaptation states test stimuli of bombykal, the main component of the conspecific pheromone blend, were applied at different periods after conditioning stimuli (CSs) of various intensity.

The spontaneous activity of both ORNs in each sensillum consisted of bursts of action potentials with a clear spike frequency maximum in the range of 30–100 Hz. Therefore, we assume that the ORNs themselves can act as oscillators,

modulating or even partly causing the oscillations found in the CNS after olfactory stimulation (Laurent, 1996, Trends Neurosci., 19: 489–496).

Odor responses to a test stimulus elicited shortly after CSs of high intensity were compared with those without preceding CS. After a strong CS, the amplitude of the sensillar potential and the slope of its rising phase were reduced, as well as the number and frequency of the action potentials. Thus, the dose–response curve in the adapted state was shifted ~2 log units to higher stimulus intensities. The adaptation of the action potential response was more pronounced than that of the sensillar potentials. The different adaptation rates suggest at least two distinct olfactory adaptation mechanisms with different target sites.

In contrast to the adapting effect of strong CSs on sensillar- and action potentials, weak CSs appeared to sensitize the action potential generator. The sensillar potentials remained virtually unaffected by a CS of an intensity ten times lower than that of the test stimulus. The number and frequency of the action potentials, however, appeared to be increased by the preceding weak CS.

Further experiments are in progress to investigate the time courses of adaptation and sensitization.

Supported by DFG STE 531/5-1, 10-1 and 10-2.

43. Effects of 8-bromo cyclic GMP on ion channels in cultured olfactory receptor neurons of the hawkmoth *Manduca sexta*

Jan Dolzer and Monika Stengl[1]

Institut für Zoologie, Universität Regensburg, D-93040 Regensburg, Germany. e-mail: jan.dolzer@biologie.uni-regensburg.de

[1]*Current address: Fachbereich Biologie, Tierphysiologie, Philipps-Universität, D-35032 Marburg, Germany*

A delayed and sustained increase of cyclic GMP (cGMP), that was observed in moth olfactory sensilla after strong pheromone stimulation, has been suspected to be involved in adaptation (Ziegelberger *et al.*, 1990, J. Neurosci., 10: 1217–1225; Boekhoff *et al.*, 1993, Insect Biochem. Mol. Biol., 23: 757–762). In cell-attached and inside-out patch clamp recordings from cultured ORNs of *Manduca sexta* we investigated the influence of the membrane-permeant cGMP analogue 8-bromo cGMP (8bcGMP) on ion channels.

Voltage-gated Na^+ and K^+ channels were blocked with 10^{-8} M tetrodotoxin in the bath and 160 mM Cs^+ in the pipette solution. Under these conditions we regularly observed three classes of ion channels: small, medium-sized and large.

For reasons of brevity, only the medium-sized channels will be described in more detail here. At least three populations with a conductance of the order of 50 pS but different pharmacology were recorded. Because of their reversal potentials around 0 mV, we assume that they are non-specific cation channels. Their openings were usually flickering, but dwell times of up to many seconds were also observed. In most of the experiments one type of medium-sized channel was activated by pressure ejection of 8bcGMP, typically after a delay of many s up to several min. The activation appeared to be faster and more pronounced after the application of 10^{-7} M phorbol esters and/or in the presence of several mM ATP, suggesting a protein kinase C-dependent channel phosphorylation. In some of the experiments these channels, which always occurred in at least three copies per patch, were blocked by 20 mM tetraethylammonium (TEA), while another type of medium-sized channel was generally not TEA-blockable. This channel type displayed flickering openings, and many copies of it activated without any obvious stimulation except seal formation or patch excision. It appeared to be unaffected by 8bcGMP and was reversibly blocked by 10 mM, but not by 1 mM Zn^{2+} in the bath or pipette solution, while the 8bcGMP-activated channels remained active. A third type of medium-sized channel, which displayed strong outward rectification under the given ionic conditions, was transiently inactivated by a high concentration of 8bcGMP.

We currently attempt to further distinguish and characterize the different types of medium-sized non-specific cation channels. Their simultaneous presence in large numbers in the same patch, together with their usually very short open times prevent a reliable statement about their conductances and I-V relations at the moment. The different influence of 8bcGMP, TEA and Zn^{2+}, however, should allow their more detailed description in the future.

Supported by DFG STE 531/5-1, 10-1 and 10-2.

44. Olfactory adaptation in *Manduca sexta*

Jan Dolzer[1], Karin Bittmann[1] and Monika Stengl[1,2]

Institut für Zoologie, Universität Regensburg, D-93040 Regensburg, Germany. e-mail: jan.dolzer@biologie.uni-regensburg.de

[1]*Current address: Fachbereich Biologie, Tierphysiologie, Philipps-Universität, D-35032 Marburg, Germany*

In response to strong stimulation sensory organs reduce their sensitivity to subsequent stimuli. This process has been termed adaptation. In moth olfactory sensilla adaptation is achieved by reducing the responsiveness of at least two levels in the chemo-electrical transduction machinery. One is the generation of receptor potentials, the other one is their transformation into action potentials. We investigated the mechanisms of adaptation in pheromone-sensitive olfactory receptor neurons (ORNs) of the hawkmoth *Manduca sexta* using different experimental approaches.

By means of extracellular tip recordings (see poster abstract in this volume) we examined adaptation processes, as well as their time course in individual olfactory sensilla. This technique allows the extracellular recording of receptor potentials (sensillar potentials) and action potentials. Both these components of the response are superimposed but are nevertheless clearly distinguishable. Therefore, sensitivity shifts—in either direction—can be investigated for the two transduction levels separately.

Histochemical experiments were employed to investigate the localization of components, which have been suspected to be involved in transduction and/or adaptation. Biochemical and physiological experiments have shown a rise of cyclic GMP (cGMP) and Ca^{2+} levels after strong pheromone stimulation. Both occurred after a delay and persisted for minutes. With NADPH diaphorase histochemistry and a cGMP antibody we investigated whether a nitric oxide (NO) synthase or an NO-dependent guanylyl cyclase are present in ORNs. A subpopulation of the ORNs were NADPH diaphorase-positive only after pheromone exposure for several minutes, but not seconds or hours. No NO-dependent upregulation of cGMP was detected, however. Thus, ORNs appear not to contain NO-dependent guanylyl

cyclase. Whether the NADPH diaphorase activity indicates the presence of NO synthase in ORNs remains to be evaluated.

To investigate the physiological effects of cGMP on ion channels possibly involved in the transduction cascade, cell-attached and inside-out patch clamp recordings were performed (also see poster abstract in this volume). Several channel types were found, which are affected by the application of the membrane-permeant analogue 8-bromo cGMP in different ways. Their physiological relevance remains one of the central questions of our future research.

Supported by DFG STE 531/5-1, 10-1 adn 10-2.

45. The life and works of Ludvig Jacobson, discoverer of the vomeronasal organ

Kjell B. Døving and Didier Trotier[1]

Department of Biology, University of Oslo, Norway and [1]Laboratoire de Neurobiologie Sensorielle, EPHE, Massy, France and Physiologie Oro-Faciale, JE 359, Université Paris 7, 2 place Jussieu, F-75251 Paris, France

Ludvig Jacobson (1783–1843) was born in Copenhagen. He studied medicine and was recognized as an eminent anatomist and teacher. King Frederik VI of Denmark demanded that Jacobson followed the French army to gain experience about medical services in wartime. Jacobson joined the French army, but at the battle at Leipzig in 1813 he was badly beaten up and robbed. He was hospitalized in Leipzig, but recognized and promoted to physician in the English Hannoverian Legion until his return to Copenhagen in 1814. When he returned to Copenhagen he was disappointed to find that the University did not want him as a professor of anatomy, because he was a Jew. King Fredrik VI later gave him a professorship.

The discovery of the vomeronasal organ must have been a surprise, but also a source of envy. Jacobson sent a description of the organ to Cuvier. However, Cuvier, the despot of zoology of that time, wrote a short notice (Cuvier, 1811, Ann. Mus. d'Hist. Nat., 18: 412–424) and let the article collect dust in the archives at the Musée d'Histoire Naturelle in Paris. These circumstances and the fact that the original description of Jacobson was hidden in a local Danish veterinary journal, written in Danish with Gothic letters, made Jacobson's findings hard to access and forgotten. The article (Jacobson, 1813, Veterinair = Selskabets Skrifter, 2: 209–246) includes excellent illustrations. We recently made translation of the article based on the Danish and French originals (Trotier and Døving, 1998, Chem. Senses, in press). In his description of the organ Jacobson makes a series of distinct observations: the numerous glands that are sometimes ignored in the modern literature, the massive innervation of the organ 'from' the accessory olfactory bulb, the trigeminal innervation and the innervation of the autonomous nervous system. As to the function of the organ, he considered it a secretory organ, but he also advocated that it could be sensory.

46. Odor response properties of neuroreceptor cells in rats

P. Duchamp-Viret, M. Chaput and A. Duchamp

Neurosciences et Systèmes Sensoriels, CNRS-ESA 5020, Université Lyon 1, 43 Bd du 11 novembre 1918, F-69622 Villeurbanne Cedex, France

Recent molecular genetic studies have identified a multigene family encoding for odor receptor proteins in mammals. However, their results cannot be easily interpreted in functional terms since very little is known on neuroreceptor cell responsivity to odors in mammals. To date, at the peripheral stage, odor coding, namely quality and intensity coding, has been quasi exclusively although extensively studied in low vertebrates. In the frog, neuroreceptor cells have been found to be well responsive to pure chemical compounds and their quality discriminative properties were utilized to propose the concept of 'odor qualitative group' for odors displaying common stimulating properties. The lack of functional data obtained in mammals led us to investigate olfactory neuroreceptor cell response properties in rats, despite experimental difficulties.

Single-unit recordings of neuroreceptor cells are performed in the endoturbinate II and septum of anesthetized freely breathing or tracheotomized adult rats. Odor stimuli are pure chemical compounds chosen among those previously tested in the frog. Stimuli are delivered using a dynamic flow multistage olfactometer providing discrete dilutions of the saturated vapor in the range of 10^{-4} to 5×10^{-1} of saturation. At all dilutions, the stimulus is a reproducible 2 s square odor pulse delivered to the uncovered epithelium at a constant flow rate of 200 ml/min.

Neuroreceptor cells are recorded using a metal filled microelectrode of 2–5 MΩ impedance at 1000 Hz (1–3 μm diameter), in the 40–150 μm depth range from the surface. EOG signal is simultaneously recorded with a micropipette of ~50 μm diameter filled with Ringer + gelatin on the same epithelial site. Extracellular spikes generally display a signal-to-noise ratio of 5–15. In most experiments, cell units are recorded and stimulated by odors for >1 h despite of respiratory movements.

Preliminary results show that neuroreceptor cells are well responsive to the pure odor chemicals previously tested in the frog. Further experiments are attempting to establish precisely the degree of selectivity of rat neuroreceptor cells and to appreciate their sensitivity. Such experiments will likely allow us to appreciate the degree of specificity of interaction between odors and molecular receptor proteins. In addition, our data will certainly gained to be compared with those previously obtained in the frog.

47. MHC and olfactory communication in humans

Frank Eggert, Roman Ferstl and Wolfgang Müller-Ruchholtz[1]

Departments of Psychology and [1]Immunology, University of Kiel, D-24098 Kiel, Germany

The major histocompatibility complex (MHC) is a gene cluster that constitutes the main factor in determining immunological individuality. The most prominent feature of the MHC in natural populations of most species is its extraordinary genetic diversity and its high level of heterozygocity in natural populations. This high level of heterozygocity seems to be maintained by behavioural factors which are actively engaged in controlling reproductive success and influencing the formation of social groups. The first hints of similar effects of the MHC on the social behaviour in humans came from two types of studies: a series of population studies on the similarity or dissimilarity of the HLA genes in couples and a number of studies examining the occurrence of shared HLA types in couples with spontaneous abortions. One of the goals of our own studies was to demonstrate an HLA-associated odour expression in humans and its effects on social perception. Descriptive results from three field studies concerned

with a possible association between genetical similarity with regard to the MHC and the hedonics of body odours as well as the degree of acquaintance yielded a similar pattern of results: The similarity in class I HLA-A and -B alleles appears to be associated with the hedonics of another person's body odour and with the degree of acquaintance or familiarity between persons. The direction of these associations appear to depend on whether the persons are of the same sex or of opposite sexes. Repeated measurements of the degree of acquaintance point to the conclusion that formation of groups and social choice between people may be influenced by their HLA-similarity.

The studies described were supported by a grant from the Volkswagen-Foundation.

48. Observations on adult human vomeronasal organs

C. Eloit, M. Wassef[1], J. Ferrand[1], J.L. Bensimon and D. Trotier[2]

Service ORL and [1]Service Anatomie et Cytologie Pathologiques, Hôpital Lariboisière 2, rue Ambroise Paré, F-75475 Paris Cedex 10, [2]Neurobiologie Sensorielle, 1 avenue des Olympiades, F-91300 Massy and Physiologie Oro-Faciale, Université Paris 7, 2 place Jussieu, F-75251 Paris, France. e-mail: trotier@bisance.citi2.fr

We observed the nasal septum of 564 adults (16–84 years; mean: 47 years; 315 females, 249 males) using nasal endoscopy. The vomeronasal vestibule, which appeared as a funnel-like hollow up to ~2 mm in diameter, located on the anterior one-third of the nasal septum, was observed bilaterally (45 subjects) or monolaterally (127 subjects). Despite a careful inspection, the vestibules could not be observed on 336 subjects. For 56 patients the presence of the vestibules was uncertain.

The movement of the mucus at the surface of the respiratory epithelium covering the vestibule was apparently not directed towards the lumen of the cavity. When the opening of the vestibule was large enough, the injection of a drop of Iopamiron®, a hydrosoluble contrast substance, allowed a precise localization of the vomeronasal cavities using computer tomography scannings (nine subjects). The cavities were oriented antero-posteriorly over a length of ~2–5 mm.

We examined the cytoarchitecture of the epithelium covering the vomeronasal cavities obtained from adults by autopsies or biopsies during nasal surgery. The cavity was lined with a columnar epithelium on both sides of the lumen which apparently contained mucus. Blood vessels and secretory glands were observed around the cavities.

Attempts to identify the presence of neuron-like cells in the adult vomeronasal epithelium, using neural-tissue markers (neurofilaments, neuron-specific enolase, synaptophysin, chromogranin, Tau protein, S 100 protein) were unsuccessful. An antibody against the olfactory marker protein (OMP) failed to reveal neuron-like OMP-expressing cells.

This study illustrates the difficulty to demonstrate the presence of neurons in the adult human vomeronasal organ. Therefore, it is difficult to affirm that the epithelium which covers the vomeronasal cavity of adult humans acts as a sensory system connected to the brain, as in other mammals.

Many thanks to Prof. F. Margolis (Anatomy and Neurobiology, University of Maryland, Baltimore) for his generous gift of anti-olfactory marker protein antibody.

49. Functional activation of epidermal growth factor receptor and mitogen-associated protein kinase in the olfactory epithelium by transforming growth factor-alpha *in vivo*

Patrick I. Ezeh and Albert I. Farbman

Northwestern University, Evanston, IL 60208, USA

Transforming growth factor-alpha (TGF-alpha) and epidermal growth factor (EGF) are members of the EGF family of growth factors. They have a common receptor, the EGF receptor. EGF receptor belongs to the tyrosine kinase group of receptors referred to as the ErbB family which also includes ErbB-2, ErbB-3 and ErbB-4. Binding of a ligand to an ErbB receptor elicits an increase in tyrosine kinase activity resulting in the autophosphorylation of the receptor followed by a phosphorylation cascade of other tyrosine kinase substrates including mitogen-associated protein kinase (MAPK). TGF-alpha and EGF have been shown to stimulate cell division in the olfactory epithelium *in vitro* (Farbman and Buchholz, 1986, J. Neurobiol., 30: 267) and may regulate cell division *in vivo*. To investigate whether exogenous TGF-alpha or EGF has any functional effect on the olfactory mucosa *in vivo* we administered each of the growth factors to rats via the carotid artery. After 2 min olfactory mucosa and liver samples were collected, homogenized and immunoprecipitated with antibodies to the ErbB receptors. The immunoprecipitates were subjected to SDS–PAGE and Western immunoblotting. Using phosphotyrosine antibody the receptors were probed for activation. Activation of MAPK was also investigated. Exogenous TGF-alpha activated EGFR, ErbB2 and MAPK, whereas EGF activated only its receptor, EGFR. TGF-alpha was a more potent activator of EGFR than EGF. Neither ligand had an effect on ErbB-3 or ErbB-4 receptors, both of which were found in olfactory epithelium. Neither the receptors nor MAPK were activated in tissues from animals receiving the growth factor vehicle without the growth factor. These results are consistent with the notion that binding of TGF-alpha to EGFR may play a role in olfactory cell division *in vivo*.

50. Learning related changes of neural odour representations in the insect brain

Till Faber, Jasdan Joerges and Randolf Menzel

Institut für Neurobiologie, FU-Berlin, Königin-Luise-Straße 28–30, D-14195 Berlin, Germany

Learning and memory are crucial for discriminating behaviourally relevant stimuli. Effects of learning on neural activity of large ensembles and single neurons have been studied extensively, but it is not well understood if and in which way distributed neural representations of the external world are altered by learning. We investigated the effect of learning on neural activity patterns by functional brain imaging in the honeybee. Animals were trained using a differential conditioning procedure, in which bees learn to discriminate a rewarded odour (CS+) from an unrewarded odour (CS–). Neural representations of the odours were registered before and after training using Ca^{2+}-imaging of the antennal lobe (AL), the analogue of the mamalian olfactory bulb.

We show here that learning leads to increased activity for the representation of the CS+, but not for the CS–. A weak activity increase was found for a non-trained control odour (S), indicating

a generalization effect. Furthermore, training leads to qualitative changes of odour representation: activity patterns for CS+ versus CS– became more dissimilar. Our results show experience-dependent changes in a primary sensory center *in vivo*, and indicate that the internal, neural representations of the environment are modified through associative learning.

51. A pheromone-binding protein from pig nasal mucosa

M. Fantacci, A. Scaloni[1], M. Monti[1], L. Ferrara[1], S. Marchese and P. Pelosi

Dip. Chimica e Biotecnologie Agrarie, via S. Michele, 4, I-56124 Pisa and [1]CISESMA- IABBAM, CNR, Napoli, Italy. e-mail: ppelosi@agr.unipi.it

An odorant-binding protein (OBP-I) of ~18 kDa is known to be highly expressed in the nasal epithelium of the pig. It is similar to bovine OBPs in its amino acid sequence and binding affinity to 2-isobutyl-3-methoxypyrazine and other ligands.

Here we report the identification and purification of a second protein of this class (OBP-II) from the nasal mucosa of the same species. This protein presents a molecular mass of 17 490 Da and does not bind 2-isobutyl-3-methoxypyrazine and the other ligands of the first OBP. Instead it showed affinity in the micromolar range to 5α-androstan-3-one, an analogue of the pig sex pheromone.

Its internal sequence (13 residues), obtained after incubation of the protein with trypsin, is identical to that of VEG protein of the same species. MALDI mapping of a tryptic digest revealed several fragments of identical sequence between OBP-II and VEG. However, the two proteins present structural differences as revealed by their molecular mass measurements, obtained by electrospray mass spectrometry.

VEG proteins have been so far identified in the von Ebners' glands of the tongue, in the lachrymal glands, in the prostate and in the nasal area. Their physiological function is still elusive: the binding to a sex pheromones here described suggests a role in chemical communication between sexes and for the first time establishes a link between OBPs and VEGs.

52. Expression and structural characterization of a recombinant mouse major urinary protein

E. Ferrari, L. Franzoni, T. Lodi[1], R.T. Sorbi[2], R. Tirindelli[3], H. Rüterjans[4], A. Cavaggioni[5] and A. Spisni

Istituto di Chimica Biologica, [1]Istituto di Genetica, [2]Dipartimento di Fisica, Sezione di Biofisica, [3]Istituto di Fisiologia Umana, Università di Parma, Italy, [4]Institute of Biophysical Chemistry, W. Goethe-University, Frankfurt-am-Main, Germany and [5]Istituto di Fisiologia Umana, Padova, Italy

The proteins of the mouse major urinary protein complex (MUP), members of the lipocalin family, bind volatile pheromones and interact with the vomeronasal neuroepithelium of the olfactory system. We report the expression of a MUP protein using its native signal sequence for secretion in the methylotrophic yeast, *Pichia pastoris*. Mature recombinant MUP (rMUP) was secreted at the concentration of 270 mg/l in minimal medium and isolated from the culture supernatant by one-step ion-exchange chromatography in a nearly pure form. Binding activity, tested with an odorant molecule which displays high affinity for native MUP, indicates that rMUP has a behaviour similar to the native one.

To determine the solution structure of this protein, we took advantage of the capacity of *P. pastoris* to grow on defined minimal salt media to produce uniformly $^{13}C/^{15}N$ double enriched and ^{15}N-labeled rMUP. These isotopically labeled protein samples have been successfully used to perform multidimensional multi-nuclear NMR experiments. The analysis of the data reveals a secondary structure in good agreement with the native MUP X-ray structure. The preliminary tertiary structure confirms that the global fold is the expected one presenting the typical eight -stranded antiparallel β-barrel and the hydrophobic cavity.

Moreover, the stability with respect to denaturant agents, revealed by CD measurements, indicates the existence of strong intramolecular interactions that render the protein conformation rather stable and thus suitable for its specific biological function.

53. Opposite regulation of mGluR1a and NMDAR1a mRNA expression in the rat olfactory bulb following NMDA receptor blockade

N. Ferraris, I. Perroteau, G. Maffiodo, A. Fasolo, A. Contestabile[1] and P. Bovolin

Department of Animal and Human Biology, University of Turin and [1]Department of Biology, University of Bologna, Italy. e-mail: bovolin@dm.unito.it

Glutamatergic input loss regulates the expression of the metabotropic glutamate receptor mGluR1a mRNA and protein in projection neurons of the rat olfactory bulb (OB). On the contrary, it does not affect the expression of the NMDA receptor subunit NMDAR1a, expressed by both projection neurons and peri-glomerular (PG) interneurons. Glutamate released by olfactory receptor neurons activates NMDA receptors expressed at the glomerular level, resulting in an increase in intracellular calcium concentration, which could trigger modifications in cellular phenotype. We investigated this possibility by monitoring the changes in mGluR1a and NMDAR1a mRNAs in adult rats acutely (1 day) and chronically (6 days) treated with a competitive antagonist of the NMDA receptor, CGP39551. OBs from control and treated (25 mg/kg, i.p.) rats were dissected out 24 h, 48 h and 16 days after acute and chronic drug administration, and analyzed by semiquantitative RT-PCR. Acute NMDA receptor blockade results in a sustained decrease of mGluR1a mRNA both at 24h (–27%) and 48h (–40%) and, at 16 days, it goes back to control levels. Chronic blockade causes a progressive decrease in mGluR1a mRNA (–25%). These changes in mGluR1a mRNA levels only partially reflect those observed following OB deafferentation. On the other hand, acute blockade results in a marked increase in NMDAR1a mRNA starting at 24 h, reaching a peak at 48 h (+38%) and returning at control levels at 16 days. Chronic treatment, like OB deafferentation, does not affect NMDAR1a mRNA expression. These data clearly indicate that mGluR1a and NMDAR1a mRNA expression, following acute NMDA receptor blockade, are regulated in an opposite manner. This hypothesis is reinforced by the observation that chronic treatment influences mGluR1a but not NMDAR1a mRNA expression. In addition, the different results obtained with chronic exposure to CGP39551 and by OB deafferentation suggest that, in the deafferented OB, the modulation of mGluR1a mRNA expression could be due not only to the loss of glutamate-mediated activation of NMDA receptors but also to additional factors, such as a loss of release of trophic factors from the olfactory nerve terminals.

Supported by MURST. N.F. is recipient of a fellowship by the Accademia dei Lincei Foundation.

54. Expression of Sonic hedgehog and related signaling molecules in developing taste papillae of the mouse

Thomas E. Finger, Joshua M. Hall, Karl Anderson and Joan E. Hooper

Rocky Mountain Taste & Smell Center and Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262, USA

The fungiform and vallate taste papillae in rodents undergo the early stages of their development even in the absence of innervation. Accordingly, the initial signals for taste papilla differentiation and location must be intrinsic to the tongue primordium. In order to begin to understand the early events leading to papillary formation, we have used *in situ* hybridization to study the early expression of Sonic hedgehog (Shh), a developmental signaling molecule, previously reported present in developing taste papillae. Also by examining the expression of Patched (Ptc) and Gli1, both indicative of the receptor for Shh, we could determine which tissues were receiving the Shh signal. At E12, all three molecules occur throughout the primordial tongue epithelium. Over the next 24–36 h, first Shh and shortly thereafter Ptc and Gli1 become restricted to the sites of incipient taste papillae. By E14, the time of epithelial innervation, Shh expression has become limited to the central epithelial cells of the developing papillae while Ptc and Gli1 are expressed in a more extensive set of epithelial cells surrounding the region of high Shh. These latter molecules also are expressed by mesenchymal tissue underlying the epithelial areas of high Shh expression. Thus diffusable Shh may provide the signal the underlying mesenchyme to build a papilla. These results also show that Shh and related epithelial signaling molecules are present in the right time and place for them to play a role in determination of papillary position, or possibly in determination of taste buds.

55. Functional expression of odor receptors

Stuart Firestein

Department of Biological Sciences, Columbia University, New York, NY 10027, USA. e-mail: sjf24@columbia.edu

Odor receptors are encoded by a gene family that may contain as many as 1000 separate genes in mammals. Although the receptor genes were cloned in 1991 by Buck and Axel, expression of functional protein has remained elusive. Several features of the odor receptors complicate the expression problem. Firstly, they must be appropriately targeted to the membrane, possibly requiring some specialized cellular machinery; secondly, because of the enormous and varied stimulus set, functional activation or binding must be measured in a system in which many odors can be tried and the response is measured as a physiological change. This requires, thirdly, that the expressed receptors couple to an appropriate second messenger cascade to produce a measurable response. We have developed an adenovirus-based system for inducing expression of particular cloned odor receptors in normal olfactory neurons. These normal neurons have the full capacity to express the cloned receptor contained in a recombinant adenovirus. Measuring responses with the EOG, we find that in a rat infected with an adenovirus containing the rat receptor I7 there is an increase in the response to octyl aldehyde and closely related saturated, aliphatic, short chain aldehydes. This method should allow us to examine a large number of odor receptors for their specific ligand binding characteristics in a program designed to develop a molecular receptive field map for the odor receptors.

56. Standardization of human subjects for gas chromatography–olfactometry (GC/O)

Jane E. Friedrich and Terry E. Acree

Department of Food Science and Technology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA. e-mail: tea2@cornell.edu

In order to identify the 'odor-important' chemicals in a sample we must use a bioassay that detects odorants in terms of their smell activity and quality. Most flavor chemists use gas chromatography—olfactometry (GC/O) for this purpose. GC/O combines olfactometry with gas chromatographic (GC) separation of volatiles from mixtures and utilizes humans to access odor activity. A persistent problem with GC/O data is the irreproducibility of an individual sniffer's response. Normal olfactory acuity measured as thresholds normally varies less than two standard deviations while further deviation results in specific anosmia. To avoid the bias caused by specific anosmia, researchers sometimes average data from two or more sniffers. In rats, it has been shown that increased expression of a single gene leads to greater sensitivity to a small subset of odorants, not to a single compound. Thus, the receptor sites of mammals seem to be broadly tuned across different chemicals to create a specific aroma class. If humans do have a limited set of receptors, a standard set can be designed to test all receptor sites and odor classes. The objective of this research is to design a standard set of chemicals based on aroma class instead of chemical class that can be used to determine the acuity of individuals to specific aroma classes.

A flavor genus of 26 has been created based on ASTM DS 66 with the addition of three classes for non-food smells. The genera are: maillard, dairy, edible oil, fermented, fishy, shellfish, berry, citrus, pome, stone, tropical, grain, cured meat, fabricated meat, processed meat, raw meat, herbs, peppers, root spices, seed spices, sweet spices, aromatic, vegetable, floral, animal and mineral. For this study a standard set of 104 chemicals were chosen and divided into mixtures to avoid chromatographic interference. Using this standard set of chemicals we assume that individuals can be screened for specific anosmia, obtain coefficients of response to specific aroma classes and eliminate sniffers with general anosmia. Publishing the coefficients of sniffers used in GC/O experiments would allow us to compare data from different sniffers and different laboratories eliminating the need to use multiple sniffers.

57. cAMP-gated channels of olfactory sensory neurons: subunit composition, Ca^{2+} permeation and modulation of ligand sensitivity

S. Frings, C. Dzeja, W. Boenigk, F. Mueller, J. Bradley, G. Ronnetti and U.B. Kaupp

Institut für Biologische Informationsverarbeitung, Forschungszentrum, Jülich, D-52425 Jülich, Germany

Signal transduction in vertebrate olfactory sensory neurons (OSNs) is mediated by cAMP-gated cation channels in the

membrane of sensory cilia. We have investigated the subunit composition of these channels by analyzing the expression of three distinct channel polypeptides (designated alpha3, alpha4 and beta1b subunits) in rat OSNs, and by comparing functional properties of native channels with those of heterologously expressed heteromeric channels with various subunit compositions. In-situ hybridization and immunoprecipitation analysis revealed that the three subunits form a common protein complex in sensory cilia. Single-channel analysis showed that channels consisting of all three subunits closely resemble native channels from rat OSNs.

Our data suggest that native channels are composed of all three subunits. We have analyzed specific contributions of each subunit to functional properties of the native channel. We measured the Ca^{2+} contribution to the channel current and found that the olfactory CNG channel conducts Ca^{2+} very efficiently. We show that the cAMP sensitivity of the channel can be increased by phosphorylation of a single serine residue and decreased by binding of calmodulin. Our results suggest that the channels serve as Ca^{2+} entry pathways and generate a Ca^{2+} signal that determines both amplitude and time course of the receptor current.

58. Evolution of olfactory receptors

Joachim Freitag, Günther Ludwig and Heinz Breer

University of Stuttgart-Hohenheim, Institute of Physiology, D-70593 Stuttgart, Germany

The chemospecificity of olfactory sensory neurons is supposed to be determined by their specific receptor types which are capable of interacting with structurally distinct odor molecules. In mammals the repertoire of olfactory receptor types is extremely large, whereas in fish the number appears to be considerably smaller. Sequence comparisons revealed that receptors from both groups share some conserved sequence motifs but exhibit only moderate sequence conservation and form separate, non-overlapping receptor families. It was unclear wether the structural and numerical differences of the olfactory receptor gene repertoire may reflect only the phylogenetic distance between fish and mammals or may be the result of adaptive processes, allowing the fish to deal with a limited number of water-soluble molecules and mammals to discriminate the large variety of hydrophobic, volatile compounds.

To gain some insight in the evolution and functional implication of olfactory receptor diversity, receptor genes were studied in species representing intermediate stages of vertebrate evolution. The identification of both fish-like and mammalian-like receptor genes in amphibia, expressed in functional different compartments of the nose, gave rise to the concept that aquatic and terrestrial odorants may be detected by two different classes of olfactory receptors (Freitag *et al.*, 1995, Neuron, 15, 1383–1392). Comparing the receptor sequences of both classes, it showed that although originating from common ancestral genes, both receptor classes apparently evolved differently. Analyses of the receptor genes from additional aquatic and semiaquatic vertebrates gave some insight into the phylogenetic evolution of the vertebrate olfactory receptor gene family and indicated that adaptation of vertebrate species to a novel environment may have influenced the structural and numerical changes in the olfactory receptor gene repertoire.

59. GC-olfactometry. A powerful tool for coffee aroma research

G. Full and F. Suggi Liverani

Aromalab illycaffe' s.p.a., v. Flavia 110, Trieste, Italy

GC-olfactometry, generally speaking, means using the human nose as a very sensitive detector for gas chromatography. In cases where only traces of potent flavour compounds are present, conventional detectors for gas chromatography (e.g. flame ionization detectors or mass spectrometry) cannot detect anything but the human nose can still smell these compounds very well. This offers an excellent approach to locate interesting compounds that are important contributers to the characteristic aroma of a particular food. By applying the concept of aroma extract dilution analysis (AEDA) it is possible to find the compounds that contibute most to the aroma. After locating these compounds of interest by GC-olfactometry, the next step is to enrich, isolate and identify them. Application of this techniques in coffee research has led to the identification of various compounds that are detrimental for the coffee aroma. Examples for such compounds are trichloroanisol, 2-methoxy-3-isopropylpyrazine and methylisoborneol. Trichloroanisol is the compound responsible for the so-called 'Rio coffee'. The typical taste of a Rio coffee is described as earthy and mouldy. Trichloroanisol is one of the most potent known flavor compounds. Its threshold—that is, the lowest concentration that can be perceived—is ~0.05 ng/l of beverage. Another off-flavor in coffee is 2-methoxy-3-isopropylpyrazine, which is naturally present in green coffee. If the concentration of this compound is above a certain level, it causes a 'peasy' coffee taste. The last example, methylisoborneol, is causing an earthy 'Robusta like' note. The concentration of this compound is ~4 times higher in Robusta coffee than in Arabica. Of course, not only the bad flavours can be analysed by this technique, but also trace compounds that contribute to the overall, pleasant, roasty character of coffee. Examples for these are sulphur compounds like 3-mercapto-3-methylbutylformate.

60. Putting odours to music

C.G. Galizia and R. Menzel

Institut für Neurobiologie, Fachbereich Biologie, Freie Universität Berlin, Königin Luise Straße 28–30, D-14195 Berlin, Germany. e-mail: galizia@zedat.fu-berlin.de

Odours are represented as glomerular activity patterns in the the antennal lobe (AL) in insects and the olfactory bulb in mammals. Each odour elicits a unique pattern of activated glomeruli, and each glomerulus takes part in the olfactory code for more than one odour. The question arises about how higher order brain centres decode this information. To approach this question we developed an unconventional method of looking at distributed spatio-temporal activity patterns. The basic idea is simple: if each glomerulus is attributed a particular and unique frequency, and stronger activity is translated into a louder sound, than detecting an odour evoked activity pattern is equivalent to recognizing a musical chord. Changing just one note in the chord completely changes its musical character (for instance, changing G to A converts a C-major into an a-minor chord, if the other notes C and E are left unchanged).

We applied this idea to our measurements of odour-evoked activity patterns in the AL of the honeybee (*Apis mellifera*). In

doing so we experienced some very interesting phenomena which may shed new light on our understanding of complex brain maps in general and on olfactory coding in particular.

First, we had to introduce a lower threshold and a nonlinear transformation which emphasizes high values because our ear is very sensitive to even weak notes within a chord. Both activity thresholds (like gating) and nonlinear transfer functions (as achieved through lateral inhibition) are basic properties of brains.

Second, the musical transformation emphasizes the temporal dynamics and the configuration of the elements as a unique percept.

Third, when mapping individual glomeruli to particular notes, we realized that this attribution must not be random in order to allow our ear to clearly distinguish the different odours.

Fourth, again depending on the mapping of glomeruli to particular notes, musical odour representations could be divided into pleasant (consonant) and unpleasant (dissonant) odours.

We propose that mapping physiological responses to odours as a sequence of musical tunes gives new insight into the way the brain handles olfactory information.

61. Aspects of chick taste bud development

Judith. R. Ganchrow, Donald Ganchrow[1] and Martin Witt[2]

Department of Oral Biology, The Hebrew University—Hadassah Faculty of Dental Medicine Founded by the Alpha Omega Fraternity, Jerusalem, [1]Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel and [2]Department of Anatomy, Technical University of Dresden, Dresden, Germany

The perihatching period is particularly dynamic for chick taste bud development. Anterior mandibular and palatal bud primordia first appear on embryonic day (E) 16 as spherical clusters of cells in basal epithelium adjacent gland duct openings, and expand to an ovoidal shape with taste pores opening at E19, i.e. 2 days prehatching. At hatching, the adult complement of taste buds is present although gemmal cells are added posthatch commensurate with increasing bud width. At perihatching ages, we have examined gemmal immunoreactivity to vimentin (V3B4), fibronectin, neural cell adhesion molecule (NCAM–5e), neuron-specific enolase (NSE), tenascin, and calcitonin gene-related peptide (CGRP) antibodies. Vimentin was expressed in many gemmal cells, with individual immunoreactive (IR) cells observed in a rosette pattern surrounding the bud lumen, similar to alternating bud cell types as arranged ultrastructurally. Basally located, elongate IR gemmal cells also were present at the basement membrane epithelial interface with intensely IR mesenchyme. These characteristics were present in spherical as well as more mature buds. Vimentin is normally expressed by mesenchymal derivatives (e.g. fibroblasts, endothelial cells), and also by epithelial cells with high proliferation rates as occurs during bud formation and renewal. NCAM was expressed in nearly all early posthatch bud cells as well as intragemmal and perigemmal fibers. Terminal segments of axons were characterized by varicosities or club-like endings similar to ultrastructural profiles at these ages. Gemmal cells generally were NSE-immunonegative whereas intra- and perigemmal axons were NSE-immunopositive, particularly in more developed buds. NSE is known to be expressed in maturing nerve cells. Fibronectin was expressed at posthatching, subjacent to the bud base, and within the bud lumen. Tenascin also was observed in the bud lumen at this age. Taste buds were CGRP-immunonegative. Further ultrastructural analysis of bud immunoreactivity at perihatching ages may key into marker-selective, functional events in developing buds.

62. Organization of sensory ganglion cells innervating, and pattern of innervation of, fungiform papillae in the hamster

Donald Ganchrow, Judith R. Ganchrow[1], Biguang Yao[2] and Mark C. Whitehead[2]

Department of Anatomy and Anthropology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, [1]Department of Oral Biology, The Hebrew University—Hadassah Faculty of Dental Medicine Founded by the Alpha Omega Fraternity, Jerusalem, Israel and [2]Division of Anatomy, Department of Surgery, School of Medicine University of California, San Diego, CA, USA

Individual fungiform papilla taste buds may receive innervation from more than one chorda tympani nerve fiber; conversely, single gustatory fibers branch to innervate more than one bud. The number and topographic distribution of geniculate ganglion cells innervating single taste buds were determined in hamsters using an iontophoretic fluorescent method. Single fungiform papillae including the gemmal pore (n = 16 cases), located either at the tongue tip (0.5–1.5 mm from the tip) or more posteriorly (1.5–3.0 mm from the tip), were successfully injected with 3.3% tetramethylrhodamine dextran amine. After 4–7 days survival, the geniculate and trigeminal ganglia, and anterior tongue (40 μm sections) were examined for labelled cells and fibers, respectively. Retrograde labelling indicated an average of 15 ± 4 (SD) geniculate ganglion cells converges to innervate a single fungiform taste bud. Buds at the tongue tip are more densely innervated (26 ± 5 ganglion cells, range 13–35) than more posterior-lying buds (8 ± 3 ganglion cells, range 5–12), which in part may be related to areal size (μm^2) of the ganglion soma, and possibly the taste bud. In addition, ganglion somata innervating a single taste bud were scattered widely within the geniculate ganglion.

Each fungiform papilla receives an average of 13 ± 4 (range 3–33 somata) labelled trigeminal ganglion cells widely distributed within the mandibular division of the ganglion. Topographic organization within the ganglion is not apparent, with respect to the distribution of labelled cells as related to distance of injected papilla on anterior tongue from the lingual tip. Sizes of fungiform papillae appear to be independent of location within this restricted (3 mm) tongue tip region (anteriormost 1.5 mm: range = 21 600–42 100 μm^2; posteriormost: range = 26 200–49 400 μm^2. However, there is a slight tendency (r^2 = 0.423) for more ganglion cells to innervate the larger papillae.

Analysis of anterogradely labelled fibers in the tongue demonstrates that 2–8 taste buds, located within a 2 mm radius of the injected papilla, share collateral innervation with the injected bud. Therefore, widely dispersed sensory ganglion cells converge to innervate a localized set of fungiform taste buds and their perigemmal surround. Transganglionic fluorescence of the tracer was absent in the nucleus of the solitary tract.

Supported by NIH grant DC0191.

63. Neuron–neuron and neuron–glia interactions during honeybee olfactory system development

J. Gascuel, L. Kopysova and C. Masson

Laboratoire de Neurobiologie Expérimentale et Théorie des Systémes complexes, CNRS, UPR9081, INA, 16, rue C. Bernard, F-75005 Paris and Centre Européen des Sciences du Goút CNRS, 15, rue H. Picardet, F-21000 Dijon, France

The adult and developing bee olfactory system presents interesting features that brings up crucial questions in terms of (i) selective fasciculation of receptors cell axons, (ii) axonal guidance and (iii) neuron–neuron and neuron–glia interactions.

By using hybridoma technology we generated monoclonal antibodies specific to neuron categories and/or to molecules putatively involved in cell–cell interactions. Emphasis will be put on A2B7, an antibody which stains specifically brain areas where axon fasciculation occurs, such as mushroom body and antennal nerve, and recognizes an extracellular glycoprotein of 91 kDa. This antigen is a good candidate for playing a role in cell interactions.

Besides, a co-culture system of the different cell categories involved in the interaction has been developed. Thus (i) the characterization of the morphology of the neurons when grown in different micro environmental conditions and (ii) the study of the dynamic of the cell–cell interactions between neurons or/and between neuron and glia, is in progress. Moreover, such culture system, using monoclonals, will enable us to carry out blocking experiments in order to show the function of the corresponding antigens.

64. The influence of odour plume structure on the upwind flight of female *Aedes aegypti* (Diptera: Culicidae)

Martin Geier, Oliver J. Bosch and Jürgen Boeckh

Institut für Zoologie, Universität Regensburg, D-93040 Regensburg, Germany

The effects of fine-scale plume structure and the concentration of host odours on the upwind flight of female mosquitoes *Aedes aegypti* (L.) (Diptera: Culicidae) was investigated in a wind tunnel. The experimental design allowed to seperate activating and attractant properties of the test stimuli. Carbon dioxide, L-(+)-lactic acid and human skin odour were tested in homogeneous and nonhomogeneous odour plumes. The latter odour plumes were separated into two categories: (a) turbulent and (b) filamentous.

Under control conditions, only few mosquitoes (~6%) flew upwind. L-(+)-Lactic acid caused a slight increase in the number of animals being attracted (~20%). No difference was observed with regard to different plume structures. In contrast, the upwind flight response towards CO_2 depended on the structure of the plume. In a homogeneous plume, an initial enhancement of flight activity (up to 77%) was observed, but the percentage of mosquitoes flying upwind was low (up to 26%). In the filamentous plume, activation was similar (up to 90%), but attraction was much higher (up to 68%). The opposite effect was found with skin odour: in the homogeneous plume, the majority of mosquitoes (up to 92%) flew upwind. In the nonhomogeneous ones, only few animals (up to 33%) were attracted. In all sorts of plumes, the percentage of upwind flying mosquitoes increased with higher concentrations.

These findings suggest that mosquitoes also orient upwind under non-disruptive odour stimulation and that attractiveness of different host odour components depend upon different plume structure.

65. Up-regulated cell cycle protein expression associated with increased basal cell proliferation in the olfactory epithelium of TGF-α transgenic mice

T.V. Getchell[1,2] and M.L. Getchell[1,3]

[1]Sanders-Brown Center on Aging & Departments of [2]Physiology and [3]Otolaryngology/Surgery, University of Kentucky College of Medicine, Lexington, KY 40536, USA. e-mail: mgetchell@aging.coa.uky.edu

The olfactory epithelium (OE) retains the capacity for cell proliferation leading to neurogenesis for the replacement of olfactory receptor neurons following cell death throughout the life span. Considerable evidence suggests that growth factors stimulate quiescent pre-S phase olfactory progenitor cells to enter the cell cycle and proliferate. However, little is known about the signaling mechanisms that initiate and regulate the proliferative process *in vivo*. The cell cycle regulatory molecule cyclin D1 has been characterized as a 'growth factor sensor' whose expression is up-regulated as quiescent cells are stimulated to enter the G1 phase of the cell cycle. We have begun to examine its expression in transgenic mice (K14 TGF-α T) in which the keratin-14 (K14) promoter drives the expression of the gene for the growth factor TGF-α in the basal cells of the OE.

^{125}I-Radioimmunoassays showed that there was 73% greater expression of TGF-α protein in the OE of 6-week-old transgenic (T) mice than in their nontransgenic (NT) littermate controls, confirming that the transgene is expressed in the OE. Counts of BrdU-labeled cells indicated a 39% increase in the number of proliferative basal cells in the OE of T compared with NT mice, establishing that up-regulated TGF-α levels are associated with increased basal cell proliferation. Western blot analysis demonstrated that there was 167% greater expression of cyclin D1 in T compared with NT mice, documenting that cyclin D1 protein is associated with growth-factor-stimulated proliferation in the OE. These results (i) confirm and extend our earlier results on the expression of TGF-α and cyclin D1 mRNAs using RT-PCR and *in situ* RT-PCR; (ii) demonstrate the up-regulation of a key cell cycle regulatory protein in the presence of increased basal cell proliferation; and (iii) suggest that cyclin D1, acting as a 'growth factor sensor', will be useful in detecting the molecular events and signaling pathways that regulate the transition from quiescent to proliferative basal cells in the OE.

Supported by National Institute on Aging, National Institutes of Health.

66. Mitral cell temporal activity evoked by binary mixtures of odorants: recording and modeling

Pascale Giraudet, Michel Chaput and Frédéric Berthommier[1]

Neurosciences et Systèmes Sensoriels, CNRS-ESA 5020, UCB Lyon 1, 43, bd du 11 novembre 1918, F-69622 Villeurbanne Cedex and [1]Institut de la Communication Parlée, INPG, 46, avenue Félix Viallet, F-38031 Grenoble Cedex, France. e-mail: giraudet,chaput@olfac.univ-lyon1.fr; giraudet,bertho@icp.inpg.fr

The olfactory system, as other sensory systems, has generally to

deal with complex sensory stimuli. It has to extract information from mixtures of several components. To analyze this processing at the olfactory bulb level, we studied mitral cell responses to binary mixtures in freely breathing rats. We focused on their temporal distribution of activity, and combined electrophysiological recordings and modeling.

In a first step, the single-unit activities of 63 mitral cells (main output of the olfactory bulb) were recorded in 14 freely breathing anesthetized rats during 10 s odor presentations of 10 pure odors and their various two-component mixtures delivered at the same concentration. The aim of this work was to test if mitral cell responses to binary mixtures resembled the response induced by one of their component alone or if they were a combination of the responses to the two odorants. A main assumption is that odor is encoded in a spatio-temporal discharge pattern driven by the odor input flow time-locked on respiratory cycle and carrying spatial features depending on sensitivity and distribution of olfactory receptors. Since we have already shown that, at a single neuron level, a large part of olfactory information is carried by the distribution of the neural activity along the respiratory cycle (Chaput *et al.*, 1992, Eur. J. Neurosci., 4: 813–822), we utilized this temporal distribution to characterize and compare response patterns of randomly chosen single-units across the ventral mitral layer of the olfactory bulb. Hence, the comparison criterion includes distribution of activity among temporal dimension and not only the mean discharge rate.

Response patterns to binary mixtures were often found to be similar to patterns induced by one of their components. Therefore, when response patterns to the two components presented in isolation were not identical, one of the responses was often able to assert itself over the other and to mask it in the mixture. This raises the question of what determines which of the two responses dominates in the mixture. Three variables were tested: the nature of the odorants, their ability to evoke a response, and the mean frequency and shape of the odor-evoked response pattern. We concluded that, at a single neuron level, some odors are stronger than others to impose their patterns, and that odors inducing a response are more likely to dominate than other odors, especially if the response pattern has a good synchronization with the respiratory cycle. Studies are yet in progress to determine how this effect is distributed across the olfactory bulb mitral cell layer.

In a second step, these physiological results and previous experimental results obtained in our group [Berthommier *et al.*, 1995, in Hermes (ed.), Le Neuromimétisme: Epistémologie, pp. 107–123] were used to build a physiological and temporal model of the olfactory bulb. We focus on which intra- and extra-bulbar connections are able to account for our experimental results, and the dominance effect observed with mixtures.

67. The compositional content of olfactory epithelium surface structures in some vertebrates

Olga Gladysheva

Institute of Educational Development, Nizhny Novgorod, Russia

The compositional content of olfactory epithelium surface and olfactory mucus was studied by an original preparatory method. The soluble proteins in this surface preparation (olfactory mucus + cilia) and the nature of their glycoproteins were determined. The localization of the glycoproteins in olfactory mucus secreting structures and in mucus was recognized in some subjects of different vertebrates (fish, amphibians and mammals). A series of ectophosphatases were founded among studied proteins in olfactory mucus, near olfactory ciliary membranes and on external part ciliary membranes.

ATP level and its changes in surface structures of olfactory mucosa was studied in special experiments. These changes may be dependent on the activity of ectoenzymes during the different stages of olfactory transduction: transport of odorants through olfactory mucus, phosphorylation of olfactory receptor proteins on ciliary membranes, generating receptor potential, etc.

68. The properties of ecto-ATPase on surface frog olfactory epithelium

Olga Gladysheva

Institute of Educational Development, Nizhny Novgorod, Russia

The ATPase activity was determined on non-destructive frog olfactory epithelium surface using biochemical and electron cytochemical methods.

The properties of this enzyme are different from ATPases which take part in energy transduction and ion transport processes. This ATPase was defined as having a wide substrate specificity and its activity being dependent on Mg^{2+} and Ca^{2+} ions. EDTA and EGTA had a strong inhibitory effect and affected the concentration of ATP too. The enzyme activity was insensitive to action of the mitochondrial inhibitor oligomycin and Na,K-ATPase inhibitor ouabain, but was dependent on some SH-reagents, NaF and La^{3+} ions.

The functions of this ecto-ATPase in olfactory processes are discussed.

69. Responses of goldfish olfactory bulb relay neurons during epithelial application of pre-ovulatory and ovulatory pheromones

Marion Gloger, Samer Nasser and H. Peter Zippel

Physiol. Inst. der Universität, Humboldtallee 23, D-37073 Göttingen, Germany

Single unit activity from both types of relay neurons was simultaneously recorded with tungsten microelectrodes (for details see Zippel and Wilcke, this vol.). Using an electro-olfactogram (EOG), highly effective and less effective preovulatory steroidal pheromones (Sorensen *et al.*, 1990, J. Comp. Physiol. A, 166: 373–383) were investigated: highly effective: 17,20β-dihydroxyprogesterone (10^{-9}–10^{-11} M) and 17,20β,21-trihydroxyprogesterone (10^{-9}–10^{-11} M); and less effective: 4-pregnen-20α-ol-3-one (10^{-7}–10^{-9} M), 4-pregnen-20β-ol-3-one (10^{-7}–10^{-9} M), 17α,20α-dihydroxyprogesterol (10^{-7}–10^{-9} M) and androstenedione (10^{-7}–10^{-9} M). Furthermore, the effectiveness of two ovulatory pheromones—prostaglandin $F_{2\alpha}$ (10^{-7}–10^{-11} M) and 15-keto-prostaglandin $F_{2\alpha}$, 10^{-7}–10^{-11} M)—was investigated in comparison to two amino acids (Arg 10^{-7} M, Pro 10^{-7} M) that are representative of important food stimuli. Olfactory bulb relay

neurons frequently respond to a comparatively great number of olfactory stimuli: amino acids, preovulatory and ovulatory stimuli. Contrasting interactions between MC and RC frequently were recorded during stimulus application. In contrast to EOG recording (Sorensen *et al.*, 1990), application of highly effective and lesser effective pheromone stimuli resulted in less contrasting responses in olfactory bulb relay neuron. The EOG is a slow (DC) potential change recorded in teleosts in response to chemical stimulation and is suggested to be the population average to receptor potentials responsible for the initiation of neural impulses. From the present recordings from olfactory bulb relay neurons, however, the EOG obviously is not an excellent indicator of olfactory organ sensitivity and specificity to odorants in fishes.

Supported by DFG, Zi 112/7-1.

70. Comparative study of 19 synthetic aromas of lemon, orange and mandarin and their respective mixtures: from chemical compositions to linguistic representations

N. Godinot, S. David, D. Dubois, E. Bouchet-Rallet and C. Rouby

Laboratoire de physiologie neurosensorielle, neurosciences & systèmes sensoriels, CNRS UPRESA 5020, Université Claude Bernard Lyon 1, F-69622 Villeurbanne Cedex, France. e-mail: godinot@olfac.univ-lyon1.fr

Among the wide variety of odour notes, citrus is one of those most commonly shared by academic classifications and by public representation of odours. Much of previous works conducted on this olfactory note focused on chemical composition and perception of synthetic recompositions or extracts of diverse citrus fruit. In the present work, we compared several levels of knowledge on the variations of this particular note: chemical composition of synthetic aromas, analytical perception, holistic perception, description and naming.

We designed a set of 19 odour samples, each being synthetic aromas already explored in the literature, and containing up to 12 distinct molecules in diverse concentrations. We selected three 'lemon', three 'orange', four 'mandarin' and nine samples of their respective mixtures 'orange + lemon', 'orange + mandarin', 'lemon + mandarin' and 'orange + lemon + mandarin'.

The perceptual and cognitive similarities of these 19 samples were tested in three experiments. In the first, 40 assessors (20 male, 20 female) were asked to describe each of the 19 samples and to rate their respective notes on four 10-point-scales ('Lemon', 'Orange', 'Mandarin', 'Other' respectively). A second free-sorting task experiment was conducted on these samples with another set of subjects ($n = 40$) using a procedure in which assessors could sort the samples in as many groups as they considered relevant. The third experiment ($n = 30$) was a replicate of the second one but the sorting task was cued by three suggested category names ('Lemon', 'Orange' and 'Mandarin'). After each of these sorting tasks was completed, assessors were asked to describe each sample individually, using their own word.

Linguistic analysis, descriptive and multidimensional statistical procedures were used to compute and represent the data. Our results focus on the mapping as well on the discrepancies between the different images of the olfactory space given at each level of knowledge (chemical, perceptual, cognitive and linguistic). The discussion is concerned with the properties of the 'lemon' versus 'orange' note as a candidate prototype along which the olfactive space for citrus may be structured.

71. Lack of masking or counteracting effects of cyclohexyl methyl pentanone on the sweaty odorant isolvaleric acid: contrast with A. Schleppnik's previous works

N. Godinot, T. Thomas-Danguin and G. Sicard

Laboratoire de physiologie neurosensorielle, neurosciences & systèmes sensoriels, CNRS UPRESA 5020, Université Claude Bernard Lyon 1, F-69622 Villeurbanne Cedex, France. e-mail: godinot@olfac.univ-lyon1.fr

In order to study molecular interaction at the peripheral level of odor processing mechanisms, we were looking for experimental models of odor counteraction and masking. Among all the patents and scientific publications reviewed, the most attractive were those few published by Schleppnik in the late seventies. Following the presentation of his enzymatic model of olfaction (Schleppnik, 1975, Cosm. Perfum., 90: 60–66), his company (MONSANTO) took on a patent (Schleppnik and Vanata, 1977, US Patent No. 4,009.253, February 22, 1977) on a molecule found to be particularly efficient as a counteractant for malodors: 4-cyclohexyl-4-methylpentan-2-one (CMP). A few weeks later, he illustrated the neutralization effect of this molecule in a poster presented at the Third ECRO Congress (Schleppnik, 1978, Specific Peripheral Regulatory Interaction of Odorivectors, Third Congress of the European Chemoreception Research Organisation, Pavia, Italy) in Pavi. In another publication [Schleppnik, in Moskowitz and Warren (eds), Odor Quality and Chemical Structure, American Chemical Society, Washington, DC, pp. 162–175], the same author claimed for even stronger counteracting effects on the isovaleric acid than the one previously obtained, but without any clear experimental data and procedure supplied. Since that time, no experiments related to this molecule have been published either reproducing its effects or invalidating them.

As a preliminary experiment to electrophysiological and biological studies, we wanted to confirm the counteracting effects of the CMP. The synthesis of CMP was conducted as described in the patent, and chemical purity was checked by gas chromatography. Using a dynamic olfactometer we presented to naïve assessors several mixtures of isovaleric acid at a vapour concentration eliciting an average intensity rating, and CMP at various concentrations (50, 10, 5 and 0.5% of the saturated vapour at 20°C). Assessors had to evaluate the intensity and the pleasantness of the stimuli and record their rating by a two-way anchored continuous rating scale (very unpleasant/very pleasant, no odor/very intense).

In contradiction with Schleppnik results, we do not show any counteracting effect of the CMP on the sweaty odor of isovaleric acid at the concentration levels used, either in dichorhinal or in monorhinal mixture presentation. We still cannot explain the discrepancy of these results. We may argue that the experimental conditions were not exactly identical, demonstrating that if the CMP has any effects, it has to be in some really specific conditions.

72. Determination of odourant compounds in bitumen vapours: GC/MS/AED analysis and sensory evaluation using two GC/sniffing procedure

N. Godinot, H. Casabianca, M.C. Bertrand and G. Sicard

Laboratoire de physiologie neurosensorielle, neurosciences & systèmes sensoriels, CNRS UPRESA 5020, Université Claude Bernard Lyon 1, F-69622 Villeurbanne Cedex, France. e-mail: godinot@olfac.univ-lyon1.fr

Storage, haulage and use of heated bitumen are sources of olfactory annoyances. In an attempt to fight efficiently against the unpleasant effect of such products, we first decided to develop and use analytical procedures to identify the origin of the unpleasant odor of bitumen.

Volatile compounds were isolated from heated bitumen (VIATOTAL®, from TOTAL Raffinage, heated at 185 ± 5°C) by cryo-condensation of the vapors. Chemical analysis was performed using gas chromatography (GC) coupled with mass spectrometry (MS) and atomic emission detection (AED).

Sensory evaluation was performed by two separate GC/sniffing methods. For each one, assessors had to stay on the sniffing port, to breeze freely, and to report any olfactory sensation (recording the beginning, the end, and a description of their sensations). Charm Analysis (Acree *et al.*, 1984, Food. Chem., 14: 273–286) of the bitumen extract was conducted with three assessors trained on a Charm Analysis of a synthetic mixture, and selected among five assessors considering their respective results and consistencies. Since the principle of Charm Analysis is questionable, we developed another method, called multiple olfactory profiles (POM), which is similar to the SNIF method recently proposed by Pollien and collaborators (1997, J. Agric Food Chem., 45: 2630–2637). With POM analysis, results are based on odor detection frequencies by a panel rather than detection thresholds measured on successive dilutions of the bitumen extract. POM analysis was performed with eight naïve assessors who received only minimal training to the sniffing procedure.

Each procedure gave similar results. The relationship between chemical composition and aromagram is difficult to establish because of the large chemical complexity of the extract. Still, one may find that the main contributors to the bitumen odor are thiophen, butanethiol, diethyl disulfide, benzothiophen (+ methylated isomers) and indane (+ methylated isomers). However, some synthetic reconstructions with some of these compounds did not evoke clearly the bitumen odor.

This work was realized with the financial support of TOTAL Raffinage.

73. Lateralization of olfactory performance—a study with olfactory evoked potentials

Birgit Gottwald, Christine Daniels, Bernfried Sojka[1], Bettina M. Pause[1], Roman Ferstl[1] and Hubertus M. Mehdorn

Department of Neurosurgery, University of Kiel, Weimarer Straße 8, D-24106 Kiel and [1]Institute of Psychology, University of Kiel, Germany

During the last years there has been a growth of research interest in olfactory processing. In this context the dominance of either the right or left cerebal hemisphere and their interaction was discussed controversial.

To investigate the contribution of the two cerebral hemispheres to olfactory processing we compared a 31-year-old woman with agenesis of the corpus callosum with a control group (n = 9). An olfactometer (method according to Kobal) was used to present three different odors to either the right, left or both nostrils.

First the subject and controls were asked to describe the three odors verbally.

During the experiment their task was to show different reactions (registrated fingermovements) to the odors to prove that they were able to recognize and distinguish them. At the same time olfactory evoked potentials (OEP) were measured from scalp locations Fz, Cz, Pz, F3, F4, P3, P4.

Concerning the verbal descriptions of the odors the subject chose divergent odor qualities compared with the controls. Furthermore, her ability to recognize and distinguish the odors was significant lower than that of the controls. While the controls showed best performance when stimulation was right or bilateral the subjects performance was better when the stimulation was right.

The olfactory evoked potentials showed a prolonged N1 latency when the left nostril was stimulated. This effect was remarkable for the subject. In general the largest responses were evoked when stimulation was right or bilateral.

These findings indicate a dominance of the right hemisphere which is proven by behavioural data as well as by olfactory event related potentials concerning olfactory information processing. The results also stress the importance of interaction between the two cerebral hemispheres to distinguish and name odors correctly.

74. Early development and intrinsic organization of the mammalian glomerulus

Charles A. Greer and Helen Treloar

Department of Neurosurgery and Section of Neurobiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA

In the adult olfactory bulb glomerulus there is a complex interdigitation of axonal and dendritic processes that interact via both axo- and dendrodendritic synapses. Although the rules governing the development of this synaptic neuropil remain largely unknown, some principles are beginning to emerge. Recently, we demonstrated that, in contrast with the visual system, the projection of olfactory receptor cell (ORC) axons to target glomeruli appears to be activity independent. In Golgi preparations of the developing olfactory bulb, ORC axons innervated single glomeruli and matured to an adult-like phenotype without evidence of the intra- or interglomerular hypertrophy evident in other sensory systems. To learn more about these early events we are using immunocytochemistry to examine the interactions between ORC axons and dendritic processes in the olfactory bulb during early development. By embryonic day 17 (E17) both dendritic processes and ORC axons are present in the olfactory bulb but remain largely segregated into two distinct laminae with the exception of a few axons penetrating deep into the olfactory bulb. At E18 more ORC axons have penetrated the outermost dendritic layer of the olfactory bulb but without evidence of glomerular formation. Beginning at E19, axons within the dendritic layer show evidence of coalescing into glomerular-like knots (protoglomeruli) while dendritic processes remain uniformly distributed throughout this layer without evidence of a glomerular-like organization. The density of the protoglomeruli increases by E20 but dendritic processes remain uniformly distributed without evidence of a glomerular organization. By E21

and continuing on through P0 the protoglomeruli become increasingly well delineated because the dendritic processes between protoglomeruli diminish as they become restricted to their protoglomerular neuropil. Interestingly, astrocytes did not contribute to the protoglomerular phenotypes until E21, after the ORC axons and dendritic processes began to form a neuropil. These data are consistent with the notion that the ORC axons play an inductive role in glomerular formation. While the specific molecular mechanisms that promote the initial coalescence of the ORC axons into protoglomeruli remains to be established, one plausible hypothesis, requiring further testing, is the expression of ORC specific odor receptors.

75. Odor-stimulated expression of the activity-regulated cytoskeletal-associated protein (Arc) in olfactory bulb

Kathleen Guthrie, Jessica Rayhanabad and Christine Gall

University of California, Irvine, CA 92697, USA

Recent studies have focused on the role of activity-regulated immediate early genes (IEGs) in neuronal plasticity. These genes can be separated into two groups: (i) those encoding transcription factors and (ii) those encoding gene products that directly influence cell function. The immediate early gene, *Arc* (activity-regulated cytoskeleton-associated protein) encodes a protein that exhibits homology to α-spectrin and complexes with the cytoskeletal protein actin, leading to the hypothesis that *Arc* may mediate long-lasting activity-dependent neuroanatomical changes by interacting with or modifying functions of structural proteins.

Both *Arc* mRNA and protein levels are increased in brain by types of stimulation known to produce synaptic plasticity. We examined the effect of specific odor stimulation on *Arc* mRNA and protein levels in rat olfactory bulb. Adjacent sections were processed for *in situ* hybridization using ^{35}S-labeled riboprobes for *Arc* or the IEG *c-fos*. Odor stimulation increased *Arc* mRNA levels in activated bulb regions as identified by elevated *c-fos* expression. Within activated fields, *Arc* cRNA densely labeled periglomerular and tufted cells surrounding individual glomeruli, as well as underlying mitral and granule cells. Quantitative densitometry revealed significant increases in *Arc* mRNA levels in odor-activated bulb regions compared with unactivated regions (~6-fold increase in the glomerular layer; ~1.5-fold increase in the granule cell layer), and compared with levels measured in control littermates maintained in clean air. All rats also exhibited grain densities greater than background in the external plexiform layer, suggestive of dendritic *Arc* mRNA transport. Additional rats were exposed to odor and the olfactory bulbs processed for *Arc* immunohistochemistry. *Arc*-immunoreactivity was distributed throughout the bulb and localized primarily to dendritic processes and terminals. Tufted/mitral cell dendrites within glomeruli were well labeled, and the intensity of this labeling varied for individual glomeruli. Granule cells and their dendrites were also labeled. These data suggest that odor induction of *Arc* expression may play a role in activity-dependent anatomical plasticity in the olfactory system.

76. Morphology and plant odour perception of sensilla auricillica in the moth, *Scoliopteryx libatrix* (Lepidoptera: Noctuidae)

E. Hallberg, P. Anderson[1] and M. Subchev[2]

Department of Zoology, Helgonavägen 3, S-223 62 Lund, [1]Swedish University of Agricultural Sciences, Department of Plant Protection Sciences, Box 44, S-230 53 Alnarp, Sweden and [2]Institute of Zoology, Blvd. Tzar Osvoboditel 1, 1000 Sofia, Bulgaria

The olfactory sensilla in moths belong to the trichoid, basiconic, coeloconic and auricillic types. The functional characteristics of the auricillic sensilla are less well established. There are only very few examples where sensilla auricillica have been shown to respond to plant odours. In the moth *Spodoptera exigua* the s. auricillica were found to respond to a flower odour, phenylethyl alcohol (Mochizuki *et al.*, 1992, Appl. Entomol. Zool., 27: 547). In another moth, *Cydia pomonella*, responses to potential sex pheromone compounds have been recorded from s. auricillica (Ebbinghaus *et al.*, 1998, J. Insect Physiol., 44: 49).

In the moth *Scoliopteryx libatrix* there are single auricillic sensilla, as well as groups of s. auricillica located in cavities on the antennae. The sensillar hairs are innervated by two sensory cells, one larger and one considerably smaller. The diameter of the smaller dendritic segment is roughly half of the larger one. The larger dendritic outer segment branches profusely in the hair, whereas the smaller exhibits few branches. Within the hair the branches of the outer dendritic segment may display turns, indicating that they are not aligned with the longitudinal axis of the sensillar hair.

An electrophysiological single-sensillum technique was used to record the responses from s. auricillica. In all, contact was established with 45 neurons in the cavity. Of these 12 were found to respond to at least one of the compounds tested, while the remaining 33 neurons did not respond to any of these compounds.

Neurons responding to Δ-3-carene, linalool, α-pinene and green leaf volatiles (GLV-compounds) were found. No difference was found in the sensitivity between males and females. In two contacts responses from two neurons could be recorded simultaneously. Both neurons responded to GLV, but the difference in sensitivity between the two neurons was 2–3 orders of magnitude.

The occurrence of the sensilla auricillica in both pits and on the antennal surface is puzzling. The location of sensilla in cavities below the surface must imply that they are not in an optimal position for receiving olfactory stimulus as regards the sensitivity. Probable explanations for this are: temporal integration of stimulus, and/or the ability to determine concentration levels if the same substances are perceived by differently located sensilla.

77. Neuroethology of garter snake prey detection

Mimi Halpern

SUNY Health Science Center at Brooklyn, 450 Clarkson Avenue, Brooklyn, NY 11203, USA. e-mail: mhalpern@netmail.hscbklyn.edu

Garter snakes require a functional vomeronasal system to detect and respond appropriately to chemicals derived from prey. The snake tongue delivers chemicals to the vomeronasal ducts in the roof of the mouth and they are transported from there to the microvilli of the receptor cells in the sensory epithelium. We have purified a 20 kDa chemoattractant, ES20, from earthworm electric

shock secretions which binds specifically to vomeronasal sensory epithelial membranes and generates activity in mitral cells of the accessory olfactory bulb. The gene for this chemoattractant has been cloned and the fusion protein is active in our bioassay. ES20 binds to a G-protein-coupled receptor and this binding results in an increase in intracellular IP_3 and a decrease in cAMP. Whole cell patch clamp recordings reveal that intracellular injection of IP_3 results in a large inward, depolarizing, current, as does extracellular stimulation with the chemoattractant, ES20. Intracellular injection of cAMP has no effect on the electrical activity of the cell.

78. The complex inner life of a glomerulus in the antennal lobe of *Drosophila*

Stephan Halter, Pierre Philippe Laissue and Reinhard F. Stocker

Institute of Zoology and Program in Neuroscience, University of Fribourg, CH-1700 Fribourg, Switzerland. e-mail: stephan.halter@unifr.ch; reinhard.stocker@unifr.ch

Throughout the animal kingdom, the first order olfactory brain centers are organized in modular units, called glomeruli. In insect brains, glomeruli of the antennal lobe (AL) comprise the synaptic sites between afferent terminals and their targets, the dendritic arbors of projection neurons (PNs) and local interneurons. Glomeruli are believed to be the focal units of the chemotopic code. Recent calcium imaging of odorant-evoked activation patterns in the olfactory bulb of the zebrafish indicated smaller spots than glomeruli, which were described as glomerular modules (Friedrich and Korsching, 1997, Neuron, 18: 737).

Here we present a possible morphological base for modules in the glomeruli of *Drosophila melanogaster*. Based on the staining pattern of a neuropile-specific monoclonal antibody, our new 3-D model of the AL (Laissue *et al.*, 1997, Proceedings of the 25th Göttingen Neurobiology Confernce, p. 414) proposes distinct subcompartments in several glomeruli. As shown by a PN specific P[Gal4] line, subcompartments are innervated by independent dendritic arbors of PNs. Loss of a specific cluster of PNs by chemical ablation of an AL neuroblast leaves some of the subcompartmented glomeruli with only one subcompartment, suggesting that at least some of them are innervated by PNs from distinct cell clusters. Such units may therefore constitute separate coding entities.

Afferents show an even higher level of modular organization within glomeruli. They invade glomeruli from the surface of the AL and terminate in the rind of each glomerulus. In certain cases this rind separates the glomerulus into large subcompartment-like units, but in most cases distinct clusters of afferent terminals extend into the center of the glomerulus, forming smaller modules. A similar degree of mini-compartmentalization is also shown by small dendritic side branches of PNs protruding into neighboring glomeruli. This modular organization of afferent terminals and PN dendrites may be analogous to the glomerular modules seen in zebrafish calcium imaging. If these substructures represent functional units, they may considerably increase the chemotopic combinatorial potential of the 43 glomeruli of *D. melanogaster*.

79. Temporal coding in the central olfactory system of the male moth *Agrotis segetum*

Bill S. Hansson and Hong Lei

Department of Ecology, Lund University, S-223 62 Lund, Sweden

In the mate-finding process, a male moth needs to detect the pheromone molecules emitted by conspecific females, lock onto the pheromone plume and follow the plume to the female. In the natural situation, due to air turbulence, a plume is not homogeneous. Instead, it has a filamentous structure, i.e. packages of pheromone molecules are intermixed with clean air. Wind tunnel observations have proved that such a filamentous structure is of crucial importance for a male moth to proceed towards the pheromone source. If the pheromone cloud is homogeneous, the male engages in casting, i.e. across-wind flight without upwind progress, similar to when the male loses contact with the plume.

In the present study, we used intracellular recording methods to observe the response pattern of antennal lobe and protocerebral neurons in male *Agrotis segetum* to pulsed stimulation with various frequencies (1–10 Hz). Ten nanograms of single pheromone components (Z5–10:OAc, Z5–12:OAc, Z7–12:OAc and Z9–14:OAc) as well as their blend at the ratio of 10:1:50:25 were used as stimuli.

In the antennal lobe, neurons mostly responded in a biphasic manner to olfactory stimulation. An initial excitatory phase was followed by an inhibitory phase. The large majority of the neurons investigated could resove a pulse frequency of 3 Hz. The pulse-following capability of a neuron was strongly correlated with the biphasic response pattern observed. The stronger and faster the inhibitory phase, the higher frequency followed. If the inhibitory phase was absent, not even 1 Hz pulses could be followed.

No effect of the full pheromone blend, as compared with single components, was observed. If a neuron could follow a certain frequency at single component stimulation, it could perform the same task at blend stimulation, and vice versa.

Protocerebral neurons showed much more diverse response characteristics as compared with antennal lobe neurons. Some displayed a biphasic response pattern, while others were characterized by a short, phasic burst of action potentials. Among both these groups, neurons following 3 Hz pulses were found.

In summary, the results from this study show that in *A. segetum*, the spatiotemporal feature of a pheromone plume can be encoded by most antennal lobe neurons and by some protocerebral neurons in a way of firing discrete bursts of action potentials corresponding to the stimulus pulses. In antennal lobe neurons, the biphasic response pattern is likely to be the key to stimulus pulse resolution, while blend interactions are not important in temporal coding. In protocerebral neurons, the mechanism behind pulse coding remains elusive.

80. The development of taste buds in the zebrafish, *Danio rerio*

Anne Hansen, Eckart Zeiske and Klaus Reutter[1]

Zoological Institute, University of Hamburg, D-20146 Hamburg and [1]Department of Anatomy, University of Tübingen, D-72074 Tübingen, Germany

Except for a few studies on fish taste buds (TBs) at the light microscopical level and a preliminary note on the ultrastructure of

turbot TBs, little is known about the development of the gustatory system of teleosts. The aim of this study was to investigate the development of the taste buds (TBs) in the zebrafish, a member of the widespread cyprinid family. Embryos and larvae from our own breeding colony were kept at a temperature of 26.5°C. Transmission (TEM) and scanning (SEM) electron microscopy were used to visualize the ultrastructural features of the developing taste buds.

Immunocytochemistry was used on whole mounts and sections to detect the differentiation of TB cells and the early formation of the nerve fiber plexus. As seen in TEM and calretinin-like immunoreactivity, the first few receptor areas (corresponding to the taste pores in mammals) appear on the lips and in the oropharyngeal cavity of larvae 4–5 days after fertilization. The appearance of open receptor areas coincides with the onset of feeding. On the lips, TBs are distributed randomly. Within the oropharyngeal cavity, a receptor area is located on the tip of every gill raker primordium, additional randomly distributed TBs develop slightly later. Some TBs sit in an epidermal hillock. Their receptor areas appear first between two epithelial cells. One of these two cells grows around the receptor area covering the TB. Later in development additional epithelial cells contribute to the cover of the hillock of the TB. Other receptor areas appear in the nook of three epithelial cells. These TBs usually do not protrude over the epithelium. Even during development dying cells are evident in the TBs.

As TEM revealed, mature TBs in zebrafish have cells with three different types of apical microvillar structures. During the formation of a TB, the cells with several small villi (dark cells) appear first, followed by cells with one stout villus (light cells). The third cell type with a brush-like apical ending is the last type to appear. During development, nerve fibers are seen throughout the TBs, even close to the upper portion of a TB cell.

81. Olfactory discrimination in the moth *Spodoptera littoralis*: effect of odour similarity

Elke Hartlieb[1] and Bill S. Hansson

Department of Ecology, Lund University, S-22362 Lund, Sweden

[1]*Present address: Max-Planck Institut fuer Verhaltensphysiologie, D-82319 Seewiesen, Germany*

In previous experiments we showed that moths are able to associate odours with different behavioural meaning, such as flower odours and individual pheromone components, with a sucrose reward. In the present study we investigated the sensitivity of the moth olfactory system in discriminating between two odours, using odour pairs with different chemical similarity.

Moths were differentially conditioned to two odours, using a 16-trial training sequence; that is, every 5 min each moth was presented with either one rewarded odour (CS^+) or the other unrewarded odour (CS^-). The two odours were presented in a pseudo-randomized sequence ($CS^+CS^-CS^-CS^+CS^-CS^+CS^+CS^-$, repeated two times). The odour pairs used were geraniol and nerolidol (a mono- and a sesquiterpene) and the two aldehydes phenylacetaldehyde and benzaldehyde. Conditioned proboscis extension reflex was observed during training and 15 and 120 min after the last conditioning trial.

The results showed that moths can discriminate between two odours even if they are chemically very similar such as phenylacetaldehyde (PAA) and benzaldehyde. The experiments revealed that PAA is a more salient compound than benzaldehyde, as strong generalization to PAA occurred in the 120 min test after discrimination conditioning using benzaldehyde as rewarded stimulus, but not vice versa.

In addition to the behavioural experiments first results from electrophysiological recordings from brain neurons during one trial conditioning in intact moths will be presented.

Supported by a TMR grant from the European Commission to E.H.

82. Modulation of $GABA_A$ receptors in the rat olfactory bulb by protein kinase C

H. Hatt, I. Brunig and J. Bormann

Ruhr-Universitat Bochum, Department of Cell Physiology, Universitatsstraße, 150, D-44780 Bochum, Germany

Gamma-aminobutyric acid (GABA), a major inhibitory neurotransmitter in the mammalian central nervous system, binds to bicuculline-sensitive $GABA_A$ receptors and causes the opening of chloride-permeable membrane channels. The intracellular loop of the α-subunit contains consensus sites for phoshporylation by protein kinase C (PKC), suggesting a role for PKC in the modulation of $GABA_A$ receptor function.

We have used the patch-clamp technique to examine the effects of PKC on $GABA_A$ receptors in the rat olfactory bulb. GABA (5 μM)-induced whole-cell currents were measured in dissociation cultures obtained from animals at embryonic day 19. The GABA respones remained stable for at least 12 min after commencement of the recording. Stimulation of PKC by inclusion of phorbol ester (PMA) in the patch pipette revealed a time-dependent increase of GABA responses to 149% of control after 12 min of recording. By contrast, a decrease to 60% of control was observed following intracellular application of tamoxifen, an inhibitor of PKC. These results indicate that phosphorylation by PKC enhances the function of $GABA_A$ receptors in olfactory bulb neurons. Interestingly, phosphatase also increased $GABA_A$ receptor activity (178% of control after 12 min). This suggests further involvement of another protein kinase, with functional consequences opposite to that of PKC phosphorylation.

83. Patch-clamp recording of the responses to three bitter stimuli in mouse taste

Yukako Hayashi, Eri Shigeta and Tomohiko Mori

Research Institute for Food Science, Kyoto University, Uji, Kyoto 611-0011, Japan

Various studies have been made on the bitter taste transduction mechanisms and have proposed the existence of at least three mechanisms. The first of these involves a block of K channel. The second involves receptor, G proteins and the generation of IP_3 which could increase the concentration of intracellular calcium. The third involves receptor, G protein (Gustducin) and the decreasing the concentration of intracellular cyclic nucleotides. But these mechanisms have not been fully understood yet. In the present study, the electrophysiological responses to three types of bitter taste stimuli were recorded in order to investigate the differences of the concerned mechanisms between the bitter substances with mouse isolated taste cells. Taste cells were isolated from 8-week-old female mice (C57BL/6J) by enzyme treatment.

The electrophysiological responses were recorded by whole-cell patch clamp technique under voltage or current clamp configuration. Using the ramp voltage commands, the stimuli-induced changes of current–voltage relationships were observed. Three bitter taste stimulants, quinine, denatonium and naringin, were applied to the taste cells by pressure ejection from a capillary glass. Ten millimolar quinine depolarized the taste cells and induced the inward current responses under the voltage clamp mode (holding potential: –80 mV). One millimolar denatonium depolarized the taste cells, too, but it induced outward current responses under the voltage clamp mode. Stimulation of 1 mM naringin induced no responses, so it seems that naringin-induced responses are not detectable ones in this condition of measurements. To examine the difference between quinine-induced responses and denatonium-induced responses under the voltage clamp mode, cyclic nucleotide analogue and inhibitors of effectors were added to the pipette solution.

The results obtained here suggest that mouse taste cells have several taste transduction mechanisms for bitter taste. The mechanism involved depends on the type of bitter substance, and one bitter substance may be perceptible by using one or more transduction mechanisms in mouse taste cells.

84. What transgenic *Drosophila* lines can tell us about the function and development of the chemosensory system

G. Heimbeck, S. Halter, V. Bugnon, C. Häberlin, N. Gendre and R.F. Stocker

Institute of Zoology & Program in Neuroscience, University of Fribourg, CH-1700 Fribourg, Switzerland. e-mail: gertrud.heimbeck@unifr.ch; reinhard.stocker@unifr.ch

P[Gal4] enhancer trap lines in *Drosophila* provide excellent cellular markers for studying neuronal anatomy, function and development. We have isolated a number of lines that label elements in the chemosensory system, as shown by Gal4-driven expression of lacZ, tau, GFP or tetanus toxin (TNT) reporters. In addition, TNT expression blocks synaptic activity and thus allows us to assess *in vivo* the role of neurons in chemosensation, by testing odor or gustatory preferences.

To dissect chemosensory function, we made use of the simple larval system which consists of only ~20 olfactory receptor neurons (ORN). We tested olfactory preference in the P[Gal4] line GH86 that labels subsets of putative larval ORN. Control larvae were attracted by butanol, ethylacetate, propionic acid, *p*-cresol and cyclohexanone (CH) and repelled by *n*-octylacetate. In contrast, larvae in which synaptic activity was selectively blocked in these ORN became indifferent to the undiluted odors, except CH which lost its attractiveness only at lower concentration. Line GH86 also labels neurons in larval contact chemoreceptors. In gustatory preference tests, TNT expression did not significantly alter attractive responses toward fructose and sucrose, but completely abolished the avoidance response to 1 M NaCl. These two assays show that the inactivated neurons play crucial roles in larval chemosensation and suggest that the olfactory and gustatory neurons are functionally non-homogeneous cell populations.

P[Gal4] lines are also ideal neuronal markers to study effects of olfactory afferents and interneurons on the structure of the antennal lobe (AL). Genetic ablation of basiconic sensilla was previously shown to delete a known basiconic target glomerulus (Stocker and Gendre, 1988, Devl Biol., 127: 12). The role of interneurons was studied by chemically ablating AL neuroblasts in P[Gal4] lines (Stocker *et al.*, 1997, J. Neurobiol., 32: 443). Thanks to the improved resolution of confocal imaging, it is now possible to analyse the effects of these ablations in the recently created 3-D model of the AL. A drastic loss of afferents or projection neurons (PN) leads to massive changes, ranging from misplaced to reduced or missing glomeruli, and to aberrant projections of afferents in the AL or the dorsal brain. Afferents and PN are thus equally crucial for glomerulus formation and neuronal pathfinding in the AL.

85. Fragrance compounds and their influence on human attentional processing

E. Heuberger, J. Ilmberger[1] and G. Buchbauer

Institute of Pharmaceutical Chemistry, Center of Pharmacy, University of Vienna, Althanstraße 14, A-1090 Vienna, Austria and [1]Department Physical Medicine, Klinikum Großhadern, Ludwig-Maximilians-Universität, Marchioninistraße 15, D-81377 Munich, Germany

The use of essential oils as remedies in aromatherapeutical applications is very popular. However, apart from a few exceptions (e.g. microbiocide effects), so far little is known about the therapeutic properties of essential oils and possible underlying mechanisms. In particular, the stimulating and sedative effects which are attributed to many of these substances remain scientifically unproven, at least in the human domain. The aim of the present investigation was to examine whether fragrance compounds administered by inhalation affect human attentional performance on an activating–sedating dimension.

Two components of essential oils, i.e. 1,8-cineol and linalool, and a placebo substance (pure air) were applied to the subjects by an olfactometer for 20 min. Afterwards subjects had to perform a visual vigilance task for 30 min. In addition, subjects were asked to rate the inhaled substances as well as their mental and emotional conditions at the beginning and at the end of inhalation. This procedure was repeated twice. In the first inhalation period the placebo substance was administered to all subjects, in the second either one of the fragrances or the placebo substance were applied.

Between-group comparison showed no effects of inhalation of the fragrances on vigilance performance. Analysis of the subjective ratings revealed that subjects having inhaled linalool felt more energetic, more cheerful and less tired than subjects in the control group. This rather surprising result may be explained by the fact that most of the subjects associated lemon or melissa with the scent of linalool. No such effects were observed in the cineol group although activating effects would have been expected on the basis of animal research. Intra-group analysis revealed multi-dimensional interactions between vigilance performance and individual ratings in both the fragrance groups and the control group. These results indicate that fragrances when applied by inhalation do not possess any specific effects on human vigilance performance, but that subjective evaluation of these substances influences their effects on cognitive behavior.

86. Odor-active compounds in tobacco sidestream smoke

Nobukazu Higashi, Kazuhiro Hayakawa, Masaomi Fukamachi and Hiroki Shikata

Tobacco Science Research Laboratory, Japan Tobacco Inc., 6-2, Umegaoka, Aoba-ku, Yokohama, 227 Japan

Although there have been many studies of tobacco smoke odor, they have been limited to the studies of simple sensory evaluation or quantitative analysis of main components. Only a few studies focused on the odor characteristic and quantitative yield of odor-active compounds in tobacco smoke. In this study we investigated the components which contribute to the odor of tobacco sidestream smoke. To accomplish this purpose, we introduced a new analytical system which could retrieve the all tobacco smoke components efficiently and quantitatively. Experimental tobacco was burnt in a cylindrical chamber. Semivolatile and nonvolatile components were collected by a glass fiber filter that was placed at the upper part of a chamber, and gas and vapor phase components that passed through the filter were collected into a gas sampling bag. Semivolatile components were led to GC/FID and GC/MS analysis after extraction and concentration. The characteristic of odor was evaluated by sniffing the eluted components directly, while the strength of odor was evaluated by FD value (Flavor Dilution value) based on the sample dilution ratio. Gas and vapor phase components that were collected in the gas sampling bag were led to GC/FID and GC/MS analysis after concentration using cryo-trap preconcentrator. Odor characteristic and strength of odor was evaluated in the same way as the case of semivolatile components, except the sample was diluted by nitrogen gas instead of solvent. To identify odor-active compounds among many smoke components, we applied off-lined multi-column gas chromatography technique. At first, target components that eluted from the analytical column were collected into the gas sampling bag repeatedly, and then the collected gas containing the target compounds was analyzed by GC/FID and GC/MS via a preconcentrator, with another analytical column which had different polarity. With these analytical approaches we made aromagrams of tobacco sidestream smoke which showed the contribution of each components to its odor. In the gas and vapor phase components, high contribution was observed for the components having pungent, popcorn-like, or potato-like odor characteristics. While in semivolatile components, odor-active components having odor characteristic of sweet, smoky, spicy, or medicinal showed a high contribution to tobacco smoke odor. Several odor-active compounds were identified, which were considered to be responsible for the odor of tobacco sidestream smoke.

87. Exploration of a model olfactory system

John G. Hildebrand

ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721, USA

Experimentally favorable invertebrate models offer opportunities for discovery and testing of principles of organization, function, and development of neural systems, including those of human beings. In that spirit, my coworkers and I have explored the olfactory systems of moths extensively by means of behavioral, electrophysiological, molecular, morphological and neurochemical methods. We have focused mainly on the sexually dimorphic olfactory subsystem responsible for the male moth's detection and perception of the female's sex pheromone. Much has been learned about the functional organization of the pheromone-processing pathway, from olfactory receptors in the antennae through the primary olfactory centers in the brain (the antennal lobes, ALs), to higher-order information-processing centers in the CNS.

An important challenge in olfactory research is to understand the functional significance of the synaptic modules called glomeruli that are typically found in the first-order olfactory centers vertebrate and invertebrate animals alike. Investigators have long sought to understand both the mapping of olfactory primary afferents onto the glomeruli and the nature of the sensory coding accomplished in those synaptic modules. A leading hypothesis is that the glomeruli are organized odotopically, with each glomerulus processing information about specific chemical features of odor molecules. This theory has gained support from our work on the ALs, which offer the advantages of anatomical simplicity, identifiable glomeruli, accessible receptor cells and central neurons, and chemically identified, behaviorally relevant odors. Evidence has come from studies of certain glomeruli in both the male-specific, pheromone-processing subsystem and a recently discovered, female-specific, plant-odor-processing subsystem.

This presentation will review some of our findings about the neural processing of odor information in, and the postembryonic development of, sexually dimorphic glomeruli in the ALs of moths.

Supported by NIH grants AI-23253, DC-02751 and NS-28495.

88. Localization of IP_3 receptor types, including a novel isoform, in olfactory epithelium of rat and catfish

S. Hilmi, N. Rawson and D.L. Kalinoski

Monell Chemical Senses Center, Philadelphia, PA 10104-3308, USA

Odorant molecules interact with specific receptors on cilia of olfactory receptor neurons leading to rapid and transient elevations in the intracellular second messengers cyclic AMP (cAMP) and inositol 1,4,5-triphosphate (IP_3). These elevations in second messenger lead to activation of specifically gated calcium channels, and an increase in calcium influx in olfactory cilia resulting in membrane depolarization. To further our understanding of how IP_3 responses are processed in the olfactory neuron subsequent to the elevation of IP_3, we have utilized RT-PCR to clone cDNA fragments from olfactory tissue corresponding to differents types of IP_3 receptors (IP_3R). *In situ* hybridization was performed to determine the expression patterns of these IP_3R types within the olfactory epithelium, and to determine whether IP_3R types are expressed in regenerative basal cells or whether IP_3R expression is restricted to mature olfactory neurons. The results from *in situ* hybridization using non-radioactively labeled receptor probes in olfactory epithelium of both rat and catfish demonstrate that both IP_3R type 1 and type 3 are found in this tissue. The reaction product for IP_3R type 1 and type 3 appears to be more prominently expressed in catfish olfactory epithelium than rat, and is found in the cell body of a subset of mature olfactory receptor neurons and in the basal cells of both rat and catfish olfactory epithelia. The distribution of the

reaction product for IP_3R type 1 and type 3 is quite similar in catfish. In rat olfactory epithelium, the expression of IP_3R type 3 is regionally localized to a subset of receptors neurons and basal cells, while the reaction product for IP_3R type 1 is visible in the cell bodies of individual neurons scattered throughout the epithelium. *In situ* hybridization studies with a probe representing a novel splice variant of the IP_3R that was identified from rat olfactory epithelium cDNA (Hilmi *et al.*, Neurosci. Abstr., 1997) showed a staining pattern that was regionally localized to only a subset of mature receptor neurons in rat olfactory epithelium.

89. Short-term memory for odors and electrophysiological responses to odors in epileptic patients

J.L. Hudry, J.P. Royet, P. Ryvlin[1], F. Mauguière[1], J. Isnard[1] and R. Gervais[2]

Neurosciences et Systèmes Sensoriels, University Claude Bernard Lyon 1, F-69622 Villeurbanne, [1]Neurologie et d'Epileptologie fonctionnelle, Hôpital Neurologique, F-69003 Lyon and [2]Institut des sciences cognitives, boulevard Pinel, F-69675 Bron, France

This study investigated short-term memory for odors in 14 epileptic patients undergoing depth recording prior to surgical treatment for relief of intractable seizures. The behavioral performances were compared with those of 14 normal control subjects matched in gender and age, and the electrophysiological responses evoked by the odors were simultaneously recorded in various structures such as the amygdala or the orbito-frontal cortex. Stimuli were 24 unfamiliar odors. Each subject received eight trials with rests of 2 min between trials. A trial consisted of three stages: an acquisition stage (one odorant is presented), a 30 s retention interval and a recognition test (another odorant is presented) in which the subject had to decide whether the two odorants of the trial are identical or different. Recognition performances were assessed by computing hit and false alarm rates, and also discrimination measure and response bias scores. Compared with the performance of the 14 control subjects, the 14 patients showed a normal hit rate, but a significantly higher false alarm rate. Thus, they often recognized different odors as being identical. Discrimination scores were lower in epileptic patients than in control subjects, and response bias indicated clearly negative values in patients. It was concluded, on one hand, that patients recognized odors less correctly after 30 s, and on the other hand, that they were more liberal than controls. Electrophysiological responses were often observed without any averaging in several areas of the amygdala and hippocampus when presenting odorants for the memory task. Our purpose will be to distinguish the electrophysiological responses as a function of the odors' characteristics, of the task (encoding versus retrieval), and of the brain areas.

90. Pre-operative study of olfactory function in epileptic patients

J.L. Hudry, J.P. Royet, P. Ryvlin[1], J. Isnard[1] and F. Mauguière[1]

Neurosciences et Systèmes Sensoriels, University Claude Bernard Lyon 1, F-69622 Villeurbanne and [1]S Neurologie et d'Epileptologie Fonctionnelle, Hôpital Neurologique, F-69003 Lyon, France

Epileptic seizures often results from paroxystic disturbances of cerebral structures involved in olfaction, including the medial temporal and orbito-frontal cortices, the amygdala, the hippocampus and the thalamus. In an attempt to determine precisely the differential effect of such disordered structures on the olfactory function, we investigated several tasks requiring the processing of the olfactory information at different levels (from the most sensory to the deepest semantic levels). The experimental procedure included two phases. In the first phase, the subjects were asked to judge successively the intensity, the hedonicity, the familiarity and the comestibility of 12 common odors. These four judgements, which forced the subjects to process the odors in a bottom-up way, were made using evaluation scales graduated from 1 to 10. In the second phase, the same 12 odors were presented again, and subjects had to identify them. The procedure of identification was forced-choice, and five alternative names were provided for each odor that was read after the subject smelt the odor. Compared with the 35 normal control subjects, the 16 epileptic patients tested showed a significant disruption of the olfactory function at each of these processing levels. The odorants were perceived as less strong in intensity, less familiar and more neutral in hedonicity. Scores of comestibility were less contrasted than in the control subjects. Finally, they identified most of the odorants less well than the control subjects. Comparing the results of the five tasks, it was noted that more the processing level was high, more the olfactory deficit was pronounced. It was hypothetized that, using a greater number of patients, we could evidence an olfactory deficit specific to a given processing level and to a determined cerebral brain structure.

91. Gustatory event-related potentials: investigations in healthy controls and patients with hypogeusia or ageusia

Thomas Hummel, Alexandra Genow, Hans Kroger, Ritu Bagla and Douglas C. Bigelow

Smell & Taste, University of Pennsylvania, Philadelphia, USA, University of Erlangen Medical School and Department of ORL, University of Dresden, Germany. e-mail: thummel@rcs.urz.tu-dresden.de

Gustatory event-related potentials (ERPs) can be recorded in response to stimulation of the tongue with the vapor phase of acetic acid. This study investigated these responses regarding gender-related differences, their relation to stimulus intensity and psychophysical measures of taste function, and the test–retest reliability. Sixteen healthy volunteers (9 female, 7 male, mean age 24 years) participated in two sessions separated by at least 1 day. As established by a pipette test of each tongue quadrant, all subjects had normal gustatory function. To record ERPs, vapor phase acetic acid was brought onto the left or right anterior portion of the tongue; stimuli were embedded in a constantly flowing airstream (70 and 100% v/v acetic acid; flow 8 l/min; stimulus duration 250 ms, interval 24 s). Amplitudes and latencies of ERP peaks P1, N1, P2 and P3 were measured. Subjects rated the stimulus intensity using visual analogue scales. Lateralized sour thresholds (citric acid; ascending limits; anterior third of tongue) were established by means of a double forced-choice filter-paper method.

Amplitudes P1 and N1 were largest at fronto-central recording sites while amplitudes P2 and P3 had a more parietal distribution. Women had significantly larger P1N1 amplitudes and shorter latencies P1, N1 and P2 than men [$F(1,10) > 5.31$, $P < 0.045$]. Intensity-related differences were found for amplitudes P3 and

N1P2, and for latencies P1 and N1 [$F(1,10) > 5.17$, $P < 0.047$]. The subjects' ratings also differentiated between intensities; other than with ERPs, no gender-related differences were found. None of the measures demonstrated differences between the left and right sides of the tongue. Test–retest reliability was highest for the higher stimulus concentration and, as a rule, highest coefficients of correlation were found for latencies of ERP peaks P1 and N1 ($0.62 < r < 0.75$). Preliminary investigations in patients with hypogeusia or ageusia indicated the potential usefulness of gustatory ERPs in the diagnostic process, especially with regard to medico-legal cases.

Supported by NIDCD grant POI 00161, NIH, and the University of Pennsylvania Research Foundation.

92. Interfacial properties, water mobility and the sweet taste mechanism

F. Hutteau and M. Mathlouthi

Laboratoire de Chimie Physique Industrielle, Centre Europol'Agro, Université de Reims Champagne-Ardenne, BP 1039, F-51687 Reims Cedex 2, France

Surface tensions, contact angle and adhesion forces on a hydrophobic surface were determined for sugars, polyols, intense sweeteners and their mixtures. Sugars and polyols do not modify appreciably surface properties of pure water, contrarily to artificial sweeteners which are characterized by surface activity and hydrophobic properties. Adhesion force is correlated to the relative sweetness for sugars and polyols as well as for intense sweeteners. Interfacial properties of bulk/intense sweeteners mixtures depend on the intrinsic hydrophobic characteristics of artificial sweeteners, which could be at the origin of synergy or suppression. From the interpretation of Raman spectra, an intensification of sweetness intensity is related to an increase in water mobility.

Adhesion force seems to be an important concept in the understanding of the sweet taste mechanism and can be extended to the adhesion of the sweet solution onto the proteinic receptor. Interfacial properties can be used as a predictive tool of control of sweetness potency and synergy in the mixtures. Raman spectroscopy informs about the water mobility and helps in interpreting the behaviour of sweeteners in solution.

93. Central nervous processing of pheromones in the desert locust, *Schistocerca gregaria*

Rickard Ignell, Sylvia Anton and Bill S. Hansson

Department of Ecology, Lund University, Lund, Sweden. e-mail: rickard.ignell@zooekol.lu.se

Desert locusts aggregate in response to two different aggregation pheromone systems: adult locusts aggregate only in response to male produced volatiles, whereas nymphal volatiles only elicit a behavioural response in the nymphs. We have shown that the integration of aggregation pheromones in the antennal lobe, the primary olfactory centre, is stage-dependent. Projection neurons in different developmental stages of the desert locust exhibit stage-dependent response-spectra highly correlated with the preferential behavioural attraction to the different aggregation pheromone systems. These changes are probably due to a differential increase in the number of specific receptor neurons, at each consecutive moult.

Desert locusts exhibit density-dependent phase polymorphism, i.e. they are able to transform between two extreme phases, solitaria and gregaria. Gregarious and solitary locusts show phase-dependent and phase-independent behaviours in response to aggregation pheromones. Gregarious locusts interact socially and are more active compared with isolated solitary locusts, which are initially repelled by aggregation pheromones and show behavioural responses more consistent with a cryptic lifestyle. Aggregation pheromones have, however, been suggested to play a major role in the arrestment and subsequent recruitment of solitary individuals into gregarious groups, i.e. elicit phase-independent responses. In our study we have shown some phase-dependent differences in the response spectra and in the sensitivity of antennal lobe interneurons, whereas responses to certain odours are phase-independent. Phase-dependent differences may be explained by differences in the afferent input between the two phases.

Antennal lobe neurons in both phases and in all developmental stages showed similar physiological characteristics; excitatory, inhibitory, combined excitatory and inhibitory and delayed responses were found. In addition, two neurons showing an initial inhibition followed by an excitation and inhibition response were found.

94. Nectar feeding and sweet taste reception mechanism of the swallowtail butterfly *Papilil xuthus*

Inoue A. Takashi, Asaoka Kitoshi, Seta Kazuaki[1], Niki Sawako[1] and Ozaki Mamiko[2]

NISES Neurophysiology, Ohwashi 1-2, Tsukuba, 305-8634 Ibaraki, [1]Biopark of Adachi, Hogima, Adachi, 121-0064 Tokyo and [2]Department of Science, University of Osaka, Machikaneyama, Toyonaka, 560 Osaka, Japan

We found contact chemosensilla on the inside wall of the proboscis in the Asian swallowtail butterfly, *Papilil xuthus*. These sensilla possess taste receptor cells sensitive to sugars such as sucrose. In the presence of starch, however, the sensitivity of these sensilla to sucrose was reduced. This suggested that starch could competitively bind a sugar receptor molecule with sucrose. Based on this idea, we detected a putative sugar taste receptor protein in the proboscis extract, applying an affinity electrophoresis with starch. We did not find sugar sensitivity in the tarsal chemosensilla of this butterfly and did not detect any putative molecules for the sugar receptor protein either. Thus, we consider that the newly found sensilla inside of the proboscis contribute to the nectar feeding in swallowtail butterflies.

95. The olfactory information pathway in the *Drosophila* brain and the role of the mushroom bodies in the courtship behaviour

Kei Ito

National Institute for Basic Biology, 444-8585 Okazaki-shi, Myodaiji-cho, Japan. e-mail: itokei@nibb.ac.jp

Olfactory information that reached insect antennal lobes is transferred to the mushroom bodies and the lateral horns. Among the two target neuropils, the former has been studied much more intensively, partly because of its beautiful array structure of Kenyon cells. Screening of the *Drosophila* GAL4 enhancer-trap strains revealed three morphological classes of Kenyon cells: those

that bifurcate and contribute to alpha and beta lobes (class 1, most abundant), those that bifurcate to form alpha prime and gamma (class 2), and those that do not bifurcate and contribute only to gamma (class 3). Extrinsic neurons connect the lobes with the surrounding neuropils, suggesting that the mushroom bodies function in close collaboration with these neuropils.

Previous studies by Ferveur *et al.* and O'Dell *et al.* showed that feminization of Kenyon cells may make the flies bisexual but does not suppress male-specific courtship behaviour. Our strains also showed similar phenotype. These results raise a possibility that ectopic expression of the transformer (*tra*) gene is not enough for feminizing male brain cells completely. To test this, we observed the behaviour of male flies in which all the neurons express *tra* (with elav-GAL4). These males did not court female. Thus, *tra* expression in mature neurons is enough and sufficient for suppressing male behaviour.

This gave us hope that male behaviour should be suppressed, if appropriate GAL4 enhancer-trap strains are used. To find such strains, we abandoned any assumption to pre-select strains that drive the *tra* gene in particular brain structures. Instead, we crossed all the available homozygous viable GAL4 strains with UAS-tra. Among 446 strains tested, only two strains were found to show near-total suppression of male-specific courtship behaviour, regardless of the sex of the targets (SAP and CI < 0.01, $n = 20–33$). Both strains, however, expressed GAL4 in virtually all the brain cells.

The results can be explained by assuming the following: (i) the courtship behaviour is controlled by distributed brain regions with varieties of biochemical cell types; and (ii) the male behaviour would manifest unless all the relevant neurons are female. These cells may not share common enhancer activity. Then, only GAL4 strains with near-ubiquitous expression pattern would be able to feminize all of these cells simultaneously.

96. Molecular cloning of proteins involved in pheromone/odour binding and discrimination in the cabbage armyworm *Mamestra brassicae* (Lepidoptera: Noctuidae)

Emmanuelle Jacquin-Joly, Marie-Christine François, Martine Maïbèche-Coisné[1], Jonathan Bohbot and Patricia Nagnan-Le Meillour

Unité de Phytopharmacie et des Médiateurs Chimiques, INRA, Route de St-Cyr, F-78026 Versailles Cedex and [1]ENS, CNRS EP-119, Signaux et Régulations Endocrines, 46 rue d'Ulm, 75230 Paris Cedex 05, France

Odour reception in insects is mediated by specific olfactory receptor neurons (ORNs) housed in different types of antennal sensilla. The transfer of the airborne hydrophobic odorant molecules through the fluid-filled lumen to receptor proteins in the dendritic membranes of ORNs is ensured by small soluble proteins named odorant binding proteins (OBP). These small proteins are synthesized by accessory cells and accumulated to high concentrations in the sensillar lymph. In Lepidoptera, three groups were defined, the pheromone binding proteins (PBP) and the general odorant binding proteins (GOBP), which were further divided into two groups—GOBP1 and GOBP2—according to their N-terminal amino acid sequences.

In the cabbage armyworm *Mamestra brassicae*, several PBPs and OBPs have been purified by RP-HPLC in both sexes and identified by their N-terminal sequences (Nagnan-Le Meillour *et al.*, 1996, Insect Biochem. Mol. Biol., 26: 59–67). Two male PBPs (named Mbra-PBP1 and Mbra-PBP2) were used in binding experiments with a tritiated analogue of the major pheromone component, the Z11–16:OAc, and were shown to have opposite binding affinities (Maïbèche-Coisne *et al.*, 1997, Insect Biochem. Mol. Biol., 27: 213–221). In order to relate the primary structure of the *M. brassicae* OBPs to their binding affinitites, we isolated and sequenced the cDNAs encoding two PBPs and one GOBP using RT- and RACE-PCR (Maïbèche-Coisne *et al.*, 1998, Insect Biochem. Mol. Biol., in press). Then, the differential distribution of OBP expression in different types of sensilla has been studied through *in situ* hybridization to rely the electrophysiological and molecular coding data.

97. The food-related odor environment of French newborns: human and formula milk odors compared by adult nose

T. Jiang, B. Schaal[1], L. Marlier[2] and R. Soussignan[3]

Lab. Neurobiologie de la Prise Alimentaire, Inserm U308, Université de Nancy, 38 rue Lionnois, F-54000 Nancy, [1]Lab. Comportement, CNRS Ura 1291, Inra, Nouzilly, [2]Lab. Psychophysique Sensorielle, CNRS Ep 618, Université Louis Pasteur, Strasbourg and [3]Lab. Psychobiologie du Développement, CNRS Ep 1595, Ephe, Paris, France

In France, 50% newborn infants are fed by formula milk in the first postnatal week (1975 statistics), and this percentage is rapidly increasing with advancing age. It is puzzling that infantile hedonic response to the chemosensory properties of these milks has rarely been systematically examined. The impetus came from recent data showing that both breast- and bottle-fed infants display a clear preference for the odour of human milk when exposed to a paired-odour test opposing unfamiliar human and formula milks of similar intensity (Marlier *et al.*, in preparation). As neonatal testing is difficult and time-consuming, the rationale of the present study was to first characterize the sensory basis of this early odour differentiation with a panel of adults.

Human milk (HM) samples ($n = 7$) from seven different non-smoking women and locally used formula milks (FM; six for regular formulas, RF, and two for hypoallergenic formulas, HF) were assessed for: (i) perceived odour intensity, (ii) odour quality description and (iii) hedonic evaluation. Sensory evaluation was conducted individually by 21 trained panelists in a standard sensory test room (Nancy University).

The results showed that: (i) all FMs have a relatively homogeneous perceived intensity, except one of the HF, which is significantly more intense. (ii) The odour quality description also gives contrasted frequencies of descriptor used for both categories of milks. 'Milk' and 'caramel' descriptors predominate in formula milks, while 'fruity', 'sour' and 'earthly' predominate in HM. (iii) Regarding hedonic evaluation, the average scores of milks of both kinds is situated on the negative side of the scale. Nevertheless, HM samples are significantly closer to neutral than the formula brands. Within the formulas, one of the HF was particularly disliked.

These sensory evaluation data clearly indicate that breast- and bottle-fed infants are exposed to blatantly different flavour environments from the first feeds onwards. Bottle-fed infants experience strong and invariable odour intensity and qualities in their formulas, whereas breast-fed newborns ingest milks that are

more variable in both intensity and quality. The relationships between the lactating mother's food variability, early flavour experience in milk, and chemosensory processing abilities will be discussed.

Supported by MAPA/DGAL grant R96/07.

98. Spatial representation of odorant chemistry in the rat olfactory bulb

Brett Johnson, Cynthia Woo and Michael Leon

University of California, Irvine, CA 92697-4550, USA

Our previous findings when we presented a series of dissimilar aliphatic esters to rats in the presence of [^{14}C]2-deoxyglucose indicated that: different odorants generate distinct patterns of glomerular activation, uptake patterns are similar across rats exposed to the same odorant, bulb activity patterns correspond to features of molecules, odorants with what appear to be more recognized features activate increased numbers of glomeruli, both lateral and medial 2-DG foci are observed for each molecular feature, and odorants can activate as few as one or two lateral and medial glomeruli per 2-DG focus.

In this study, we determined the response patterns of similar odorants in the glomerular layer. We were particularly interested in whether related odorants have similar spatial maps. Therefore, we exposed rats to a series of closely related aliphatic acids, presented at the same vapor phase concentration (7.2 parts/million). We then constructed maps of [^{14}C]2-DG uptake in bulb glomeruli. These similar odorants evoke a multi-dimensional response in the glomerular layer; these dimensions include differing spatial patterns, uptake densities and sizes of 2-DG foci. In addition, spatial activity patterns for similar odorants are similar, with all tested acids displaying clustered, albeit distinct, foci. These acids also activate pairs of focal medial and lateral focal glomerular regions, a finding consistent with the projection patterns of specific olfactory receptor neurons. Increasing carbon number or increasing odorant concentration increases both 2-DG uptake density and the size of 2-DG foci. Complete high-resolution mapping of the bulb reveals that previous lesion studies have not eliminated all primary focal regions of odorant-evoked activity. Finally, we found that superficial granule cell activity mirrors glomerular activity, supporting the notion that there are spatially specific mitral cell responses in the bulb evoked by different odorants.

99. Immunogold ultrastructural localization of clusterin/ApoJ in the olfactory mucosa of adult mouse following olfactory bulb ablation

A.K. Julliard and F. Jourdan

Neurosciences et Systèmes Sensoriels, CNRS-Université Claude Bernard, F-69622 Villeurbanne Cedex, France

Clusterin is a secreted glycoprotein which is highly induced in neurodegenerative diseases, in close relationship with apoptotic cell death. However, the accurate function(s) of clusterin in damaged nervous system remains to be elucidated. In the olfactory system, a previous study (Michel *et al.*, 1997, J. Cell Sci., 110: 1635–1645) has shown that clusterin mRNA is strongly expressed in the olfactory mucosa from 16 to 72 h following olfactory bulb ablation, in synchrony with the onset of olfactory neurons apoptosis and the initiation of the regenerative process. This previous study also demonstrated the clear-cut mismatch between clusterin mRNA expression, which occurred exclusively in the lamina propria, and protein accumulation which was mainly observed in the olfactory epithelium.

The aim of the present work was to identify the cell type(s) accumulating clusterin in the olfactory mucosa following olfactory bulb ablation. Three-month-old mice of the C57BL/6J strain were sacrificed 72 h following bilateral olfactory bulbectomy, and their olfactory mucosa was processed for clusterin immunocytochemistry at the ultrastructural level, using 15 nm gold particles. Our results show that clusterin accumulates in the olfactory epithelium, in both supporting cells and maturing olfactory receptor cells. Gold particles are associated with lipidic heterogeneous inclusions or phagosomes, sometimes dispaying a myelin-like appearance. Our data are in accordance with previous ones obtained in light microscopy and bring new informations about the nature of cells accumulating clusterin. They suggest that clusterin could be involved in both lipid recycling in supporting cells and lipid storage in neuroreceptor cells engaged in the regenerative process.

This work was supported by the 'Région Rhône-Alpes', Programme 'Vieillissement'.

100. Introduction: from receptors to central processing

Karl-Ernst Kaissling

Max-Planck-Institut für Verhaltensphysiologie Seewiesen, D-82319 Starnberg, Germany

The olfactory pathway from the periphery to the central nervous system starts with the so-called perireceptor events. These include various interactions of the odorant with extracellular sensory structures: the adsorption of odorants on the surface of the olfactory organ, their diffusion along the sensory structures, their interaction with odorant binding proteins, their rapid deactivation or removal, and finally their enzymatic degradation. In principle, all of these reactions can contribute to the chemical specificity of the receptor cell response. This may be especially important for the insect nose where few receptor cells have their 'private' perireceptor compartments. These are tube-like olfactory hairs of various morphological constructions with unknown surface chemistry, and with specific composition of the 'sensillum lymph', differing at least with respect to binding proteins and odorant degrading enzymes. There is evidence that perireceptor events in insect olfactory organs govern the temporal characteristics of the receptor potential, the first electrical response of a receptor cell to an odor stimulus. One argument is based on the observation in several species of moths that the time course of the receptor potential of a given type of pheromone receptor cell characteristically depends on the chemical structure of the stimulus compound. For example, certain derivatives of the pheromone may produce a receptor potential with more rapid transients at stimulus onset and offset, compared with the receptor potential elicited by the pheromone. Thus, the derivative may appear as a less effective stimulus because it is more quickly deactivated. Conversely, a compound may stimulate more effectively if it

cannot be enzymatically degraded. In the bombykol receptor cell of the silkmoth *Bombyx mori*, the bombykol derivative (*E*,*Z*)-4,6-hexadecadiene produces a very much prolonged after-response, possible because this compound cannot be metabolized by a bombykol-degrading dehydrogenase of the olfactory hair. In conclusion, perireceptor events have to be considered in studies of structure–activity relationships where the chemical structures of odorants are related to their physiological effectiveness. A quantitative kinetic model of perireceptor and receptor events has been developed in collaboration with J. Thorson (Oxford) which simulates the time course of the receptor potential and its dependence on stimulus duration and intensity.

101. A quantitative kinetic model of the perireceptor and receptor events in insect pheromone receptors

Karl-Ernst Kaissling

Max-Planck-Institut für Verhaltensphysiologie Seewiesen, D-82319 Starnberg, Germany

A mathematical model of perireceptor and receptor events has been developed for olfactory hairs of moth antennae (in collaboration with J. Thorson, Oxford). It includes the adsorptive uptake of pheromone molecules by the olfactory hairs, the formation of a complex of pheromone (F) and the reduced form of the pheromone binding protein (Bred), the interaction of this complex with receptor molecules of the plasma membrane, the deactivation of the pheromone, and finally its enzymatic degradation. The model is consistent with morphometrical, radiometrical, electrophysiological and biochemical data reported from several authors. It allows us to simulate the time course of the receptor potential if the dose-dependent relationship between receptor occupation and potential amplitude during transients is the same as determined for the equilibrium. The model includes the following hypotheses: diffusion of pheromone is not time-limiting for the receptor potential, only the complex pheromone-PBPred activates the receptor molecules, the duration of the elementary receptor potentials elicited by single pheromone molecules reflects the lifetime of the ternary complex pheromone-PBPred-receptor molecule, the membrane area is maximally covered with receptor molecules, the receptor molecules catalyze the observed redox shift of the PBPred, this shift deactivates the pheromone molecules bound to PBP, and only the free pheromone molecules are enzymatically degraded. These assumptions allow us to determine tentative rate constants of all reactions involved in the model. The simulation of the receptor potential kinetics suggests that the interaction of the stimulatory complex FBred and the receptor including the proposed deactivation mechanism is responsible for the primary decline of the receptor potential after end of stimulation whereas the enzymatic degradation is not. Its function is to slowly reduce the tailing level of the response. The study suggests that perireceptor and receptor events are slower than intracellular signalling processes and, therefore, time-limiting for the receptor potential. Consequently, perireceptor events are responsible for characteristic differences in the kinetics of receptor potentials observed with certain pheromone derivatives.

102. The effects of addition of ethanol on the taste of non-alcoholic beer

Hideaki Kajiura, Kumiko Shimamoto and Yoshiko Yokomukai

Central Laboratory for Key Technology, Kirin Brewery Co., Ltd, Yokohama 236-0004 Japan

We previously investigated the effects on taste intensity when ethanol was added to solutions of isohumulones which are the main bitter components of beer, and reported that bitterness intensity increased as ethanol concentration was increased and the average rating for sweetness intensity increased slightly, though not all subjects perceived sweetness. In the present study, to understand the effects of ethanol on the taste of a more complicated solution, beer, we examined the changes of taste intensity when ethanol was added to non-alcoholic beer. Two brands of non-alcoholic beers with added water or ethanol (2, 4, 6 or 8% after addition) were prepared. Samples were served to subjects at 8°C. In a completely randomized design, bitterness, sweetness and sourness intensity were rated by 30 subjects using a 13-point category scale. Each subject rated the samples twice. In the evaluations of both brands of non-alcoholic beers, increasing the ethanol concentration from 0 to 6 or 8% produced significant increases in bitterness ratings ($P < 0.05$). Sweetness intensity also increased slightly by addition of ethanol. On the other hand, sourness intensity decreased as ethanol concentration was increased. These data suggest that ethanol may contribute to the taste of beer. For all taste intensities, the degree of the effect of ethanol on taste was different between the two brands, which suggests that the differences come from the difference of the components of the non-alcoholic beers.

103. Urinary pheromone reception in rat vomeronasal sensory neurons

Makoto Kashiwayanagi, Kouhei Inamura, Kazuyo Sasaki and Kenzo Kurihara

Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan

The vomeronasal organ exists in many vertebrates for receiving pheromones related to sexual and social behaviours. Regulation of gonadal functions by urine has been well established in the rodent vomeronasal organ. In female rats, pheromones in urine excreted from males and females induce various changes in gonadal functions such as reflex ovulation in the absence of coitus and mounting, reduction in the oestrous cycle of female rats from 5 to 4 days, and oestrous synchrony among females that are living together. These results suggest that the vomeronasal organ receives multiple kinds of urinary pheromones. In the present study, we measured responses of vomeronasal sensory neurons in epithelium slices of the female Wistar rat to urine preparations excreted from male and female Wistar rats and male Donryu rats by the on-cell patch clamp method. We also measured accumulation of IP_3 and cAMP in response to these three urine preparations by biochemical methods. Introduction of the urine preparations obtained from the male Wistar rat, the female Wistar rat and the male Donryu rat increased impulse frequency in the female

vomeronasal sensory neurons. Thirty-three neurons among 34 neurons examined responded only to one of three urine preparations. Application of these urine preparations induced IP_3 accumulation in the vomeronasal membrane preparation while these urine preparations did not change the cAMP level. These results suggest that pheromonal responses to various urine are generated via the IP_3-dependent pathway. The sensory neurons which responded to the male Wistar urine were localized at the upper part of the epithelium where $G_{i2\alpha}$ was selectively expressed. The neurons at the lower layer expressing $G_{o\alpha}$ responded to the female Wistar urine and the male Donryu urine. Thus, the present study demonstrated that sensory neurons responsive to different urinary pheromones are localized in a segregated layer in the rat vomeronasal sensory epithelium.

104. Effects of background odour on the sensitivity of olfactory receptors in antennae of *Musca domestica* L.

Frits J. Kelling and Cornelis J. den Otter

Group Sensory Organs and Behaviour, Department of Animal Physiology, University of Groningen, PO Box 14, 9750 AA Haren, the Netherlands

Houseflies can be vectors of pathogens for cattle. In order to control housefly populations in stables, traps with attractant odours may be used. The luring chemicals should be distinguished by the flies from the ambient odour in the stable. This research was done to investigate the effect of background odour on the sensitivity of the olfactory system of houseflies to odours.

The sensitivity of the olfactory system of houseflies was measured using electroantennography. 1-Octen-3-ol yielded clear EAGs and was chosen as the background odour in this study. A continuous flow of air (3 ml/s) was passed through a clean bottle or a bottle with filter paper onto which 1 ml of pure 1-octen-3-ol had been pipetted. This airstream was combined with a flow of clean charcoal-filtered air (10 ml/s) directed towards the antenna of a fly. Stimulus pulses were given by blowing air for 0.2 s through a Pasteur pipette, containing an odour source, into this airstream. The charcoal-filtered air was simultaneously switched off, to keep total airflow constant. Dose–response curves of 1-octen-3-ol, R-limonene and 2-pentanone were made before, during and after application of background 1-octen-3-ol. During application of background 1-octen-3-ol, the baseline of the EAGs became less stable, indicating an increased activity of the olfactory cells.

Dose–response curves showed a decrease of sensitivity to the 1-octen-3-ol pulses added on top of the background 1-octen-3-ol, the EAGs being smaller than when presented in clean air. After switching back to clean air, the EAGs returned to the pre-background odour levels. During background 1-octen-3-ol, responses to R-limonene and 2-pentanone may also decrease, indicating an overall decreased sensitivity to new pulses of odour in the background odour. However, in some experiments, no change in sensitivity of the olfactory system to these odours was measured during application of the background odour. Further experiments will be done to investigate this phenomenon.

105. Signal-induced selection among spontaneous oscillatory patterns in a model of the bee olfactory glomeruli

Michel Kerszberg[1,2] and Claudine Masson[1]

[1]*Laboratoire de Neurobiologie Expérimentale et Théorie des Systemes Complexes, CNRS, INA-PG, 16, rue Claude Bernard, F-75005 Paris and* [2]*Institut Pasteur, Neurobiologie Moléculaire, 25 rue du Dr Roux, F-75724 Paris, France*

Do the oscillations which have been observed in many neural assemblies have a cognitive significance? We investigate this question by mathematical modelling of the honeybee's olfactory glomeruli, which are a subsystem of the antennal lobe nervous network, involved in food odor recognition during foraging behavior. Our computations reveal spontaneous oscillations. In those units where they manifest themselves, however, application of input signals modulate only slightly the autonomous activity: thus, an intense, synchronized oscillatory background tends to hinder odor discrimination. In contrast, where and when spontaneous oscillations are repressed, due to low excitability, different input signals will re-excite selectively distinct subsets of spontaneous oscillatory modes. These observations agree well with experimental findings and suggest new, quantitative experiments. Because they rely on very general properties of nonlinear systems, our results further indicate a possible role for the modulation and differential activation of endogenous oscillations in odor identification and possibly in other cognitive activities subserved e.g. by the mammalian cortex.

106. Immunohistochemical analysis of synaptic proteins in vallate rat taste buds of the rat

John C. Kinnamon, Michael E. Rock, Rui-Biao Yang, Hildegard H. Crowley and Douglas Orndorf

Department of Biological Sciences, University of Denver, Denver, CO 80208, USA. e-mail: merock@du.edu

We are currently using immunohistochemical techniques to determine the presence and distribution of synaptic proteins (syntaxin, synaptobrevin, synaptotagmin, synaptogyrin, amphiphysin and SNAP-25) and calcium binding proteins (calbindin and calretinin) in taste cells and nerves of the rat circumvallate papillae (CVP).

Syntaxin-1 and SNAP-25 are membrane proteins associated with the presynaptic terminal and are possible target proteins for synaptic vesicle (SV) docking (t-SNARES). Synaptobrevin-2, a vesicle-associated membrane protein [VAMP-2], is a membrane protein associated with the synaptic vesicle. These three proteins make up a part of the core complex, necessary for vesicle docking and fusion. Synaptotagmin is a glycoprotein which interacts with syntaxin in a Ca^{2+} dependent manner, possibly a 'trigger' (or 'clamp') mechanism regulating neurotransmitter release from SVs. Calbindin is a 28 kDa calcium binding protein that occurs in a subset of neurons and other tissues where it participate in the buffering of calcium ions. Calretinin is a calcium binding protein that regulates the calcium concentration inside certain cells.

Subsets of taste cells express immunoreactivity to syntaxin, synaptobrevin-2 [VAMP-2], synaptotagmin, SNAP-25, calbindin, calretinin, synaptogyrin and amphiphysin. Intragemmal nerve processes display immunoreactivity (IR) to synaptobrevin-2

[VAMP-2], synaptotagmin, SNAP-25, calretinin, synaptogyrin and amphiphysin. These results represent the first demonstration of the presence of these synaptic proteins in taste buds. The distribution of the IR, however, raises new questions concerning the putative role of those synaptic proteins in synaptic function in taste buds.

Supported in part by NIH grants DC 00285 and DC 00244.

107. Electrophysiology of sweet taste in rodents

Sue C. Kinnamon and Brian Varkevisser

Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523 and the Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, Denver, CO 80262, USA

Recent studies have shown that sugars and synthetic sweeteners activate different second messenger pathways in taste cells. Synthetic sweeteners stimulate production of IP_3 and diacylglycerol, while sugars elicit increases in cAMP (Bernhardt *et al.*, 1996, J. Physiol., 490: 325–336). These two second messenger pathways apparently block the same resting K^+ conductance to depolarize taste cells (Cummings *et al.*, 1996, J. Neurophysiol., 75: 1256–1263); however, the detailed mechanisms have not been investigated. We utilized a loose patch technique for recording from taste buds *in situ* to investigate the role of phosphorylation in sweet taste transduction. Hamster fungiform taste buds were tested for their response to sucrose (200 mM) and the synthetic sweetener NC-00274-01 (NC01; 200 μM) in the presence and absence of cell-permeable protein kinase inhibitors. In the absence of inhibitors, trains of action potentials were elicited in response to both sucrose and NC01 in sweet-responsive taste buds. In the presence of the protein kinase C (PKC) inhibitor bisindolylmaleimide I (5 μM), responses to NC01 were inhibited ~75%, while responses to sucrose were unaffected. In contrast, in the presence of the protein kinase A (PKA) inhibitor H-89 (19 μM), responses to both NC01 and sucrose were enhanced ~2-fold over control responses. These data, taken together, suggest that while PKC phosphorylation is involved directly in the transduction of synthetic sweeteners, PKA phosphorylation may play a different role in the transduction of sugars, such as adaptation. Finally, cAMP can be produced either by activation of adenylyl cylase (AC) or by inhibition of phosphodiesterase (PDE). To determine whether sugars activate AC or inhibit PDE, we examined responses to sucrose and NC01 in the presence of the AC inhibitors MDL-12,330A (250 μM) and 2′,5′-dideoxyadenosine (150 μM). In the presence of either inhibitor, responses to both sucrose and NC01 were significantly enhanced, presumably by decreased PKA activity. These data provide evidence that sugars increase cAMP in taste cells by inhibiting PDE.

Supported by NIH grant DC00244.

108. Perfumes and malodour control

Rick Kolodziej and John Ramsbotham

IFF, Europe

Perfumes and many malodours are complex blends of organic chemicals which interact with receptors in the nose. The olfactory nervous system exhibit various characteristics, which can be exploited when creating perfumes to mask malodours. Moreover, some malodours chemicals can actually be blended or incorporated in perfumes. Principles such as these have led IFF to develop their proprietary Deodiff and Neutriff technologies for malodour-masking perfumes.

109. Spatial representation of physiological and not so physiological odors in the zebrafish olfactory bulb

Sigrun I. Korsching, Rainer W. Friedrich[1], Stefan Fuss and Franco Weth[1]

Institut für Genetik, Universität zu Köln, Zuelpicherstraße 47, D-50674 Köln and [1]Max-Planck-Institut für Entwicklungsbiologie, Spemannstraße 35/I, D-72076 Tübingen, Germany. e-mail: Sigrun.Korsching@uni-koeln.de

How features are extracted from chemical stimuli by the sense of smell still is not clear. Presumably many more odors can be distinguished than there are receptors in the albeit large family of odorant receptor molecules. Thus, perception of odorants seems to require a combinatorial coding strategy. We have used the zebrafish, *Danio rerio*, as a model system to analyse the neural representation of odorants. Zebrafish possess a well-developed sense of smell which governs a variety of behaviors. The family of odorant receptor genes and the number of modules in the olfactory bulb (glomeruli) are about an order of magnitude smaller than those of mammals. We observe spatial patterning of odorant receptor gene expression, of glomeruli and of odorant-induced responses in the olfactory bulb, all of which are reproducible between individuals. Response to odorants, as inferred from the odorant receptor gene expression, is broadly distributed within the sensory surface. In contrast, representation of odorants is much more localized at the input level of the olfactory bulb. Classes and subclasses of odorants are represented chemotopically. Both combinatorial and non-combinatorial coding is observed. Systematic variation of odorant chemical structure allows us to infer some odorant receptor properties.

110. Design of macrocyclic musks and bicyclic ionones. Insight into structure–odor relationships

Philip Kraft

Givaudan Roure Research Ltd, CH-8600 Dübendorf, Switzerland. e-mail philip.kraft@roche.com

Why is the threshold of nitro musks ~20–30 times lower than that of the natural lead *R*(–)-muscone? Are there additional binding sites for polar groups? Can more powerful macrocycles be designed by introducing additional structural features? To find some answers, a series of thio- and thia-macrolides was synthesized, the latter via a new synthetic sequence starting from simple lactones (Kraft and Cadalbert, 1997, Synlett, 600–602). Probably because of different binding angles as a hydrogen-bond acceptor and lower electronegativity, sulfur was found to be unable to replace either or both oxygen atoms in 15-pentadecanolide. However, sulfur could replace the double bond of ambrettolide, which indicates that the function of double bonds in macrocycles is not restricted to conformational effects, but is also of electronic importance. The best position of sulfur was found to be in 1,7-distance to a carbonyl group in even-membered rings, and in

1,6-position in odd-membered rings. Using the data of 13 musk-smelling thia-macrolides, a CATALYST™ hypothesis was generated that could calculate the threshold values with a correlation of 0.96. The features of this hypothesis were useful in the design and synthesis of a methylated oxamacrolide that resembles Musk Ambrette very closely in odor, and even possesses a lower threshold (Kraft and Cadalbert, 1998, *Synthesis*, in press). A conformational study might explain the reason why. Molecular modeling calculations on ionones led to the design and synthesis of less flexible bicyclo[6,4,0]dodecenyl ketones with very similar olfactory properties to their parent compounds. In these studies, the global energy minimum for β-ionone and local energy minima of the molecular targets were used. Since the resulting bicyclic ionones are relatively rigid, they provide good insight into the structural requirements for the characteristic fruity–woody–floral ionone odor. For a review on fragrance chemistry and structure–odor relationships see Fráter *et al.* (1998, Tetrahedron, 54: 7633–7703).

111. Cloning of a large number of putative murine olfactory receptors, and functional expression in HEK 293 cells

Dietmar Krautwurst[1], King-Wai Yau[2] and Randall R. Reed[1,2]

Departments of [1]Molecular Biology & Genetics and [2]Neuroscience, Howard Hughes Medical Institute, Johns Hopkins University, School of Medicine, Baltimore, MD 21205, USA

The recognition of ligands by individual olfactory receptors underlies the remarkable ability of mammals to detect >10 000 different odorants. In spite of the identification of a complex gene family that appears to mediate this response, little is known regarding the molecular mechanisms that underlie this recognition phenomenon. To address this question, we have heterologously expressed ~100 out of perhaps 500 cloned murine olfactory receptor chimeras and screened them for responses to individual odorants. Receptor chimeras were constructed from mouse olfactory epithelium cDNA by PCR using degenerate primers to conserved sequences in transmembrane regions II and VII of olfactory receptors. PCR fragments were cloned into a CMV promotor driven expression vector cassette between the N terminus/TM region I and the C terminus of a known mouse olfactory receptor (M4). The chimeric constructs were tagged with an N-terminal 20-amino-acid peptide fragment of rhodopsin (rho-tag) and transiently transfected into HEK 293 cells. Using confocal microscopy, we observed immunofluorescence at, or near, the plasma membrane after staining with a monoclonal antibody against the rho-tag epitope. In control experiments, the beta2 adrenergic or the rat I7 receptor chimera in the same construct or as full-length protein were coexpressed with Ga15,16. These G proteins promiscuously couple 7TM receptors to phosphoinositide metabolism. Ratiometric Ca^{2+} imaging with FURA2 has shown a transient increase in intracellular Ca^{2+} when the transfected cells were exposed to 10^{-5} M isoproterenol (beta2) or octanal (I7). In addition, we have found that the expression of the mouse homolog of the I7 receptor, either in the above chimeric construct or as a full length protein, was sufficient to mediate Ca^{2+} transients specifically induced by heptanal (10 mm). A single amino acid difference appears to contribute to this species difference.

Using the same co-expression strategy combined with Ca^{2+} imaging, we screened 10 pools, each with eight olfactory receptor chimeras, against 25 individual odorants. Responsive pools were subdivided. Screening was repeated with single receptor chimeras; three of which responded specifically to 10 mM (–)citronellal, carvone or limonene. Further screening and functional expression will allow the identification of additional receptors and their odorants.

112. Central nervous representation of odor discriminability

Kerstin Krauel, Bernfried Sojka, Roman Ferstl and Bettina M. Pause

Institute of Psychology, Kiel, Germany

Event-related potentials (ERPs), recorded from the intact human scalp, provide the opportunity to objectively assess central information processing. Amplitudes and latencies of the ERP are sensitive not only to the individual features of a stimulus, e.g. intensity, but also to the relation between sequentially presented stimuli. ERPs can therefore be used in attending and non-attending subjects to determine whether stimuli are discriminated. As the determinants of chemosensory event-related potentials (CSERP) have only been partly unravelled, the present study intended to investigate whether non-attending subjects show evidence of pre-attentive odor discrimination.

Twelve subjects (aged 20–32 years; six women) were exposed to the odors phenyl ethyl alcohol (PEA/*rose*), linalool (*lavender*) and allylcaproate (*apple*) in two sessions. Based on the results of a pilot study (n = 20) we found that subjects could discriminate allylcaproate more easily from PEA than linalool. One session included five runs with six blocks of 10 trials. Within a block the interstimulus interval (ISI) was always 8 s; between blocks the interval varied between 30–60 s. PEA was delivered with a probability of 80% whereas linalool and allylcaproate were interspersed as rare deviant events with a probability of 10% each. The odors were presented birhinally over a computer-controlled constant flow olfactometer non-synchronously to breathing. The EEG was recorded from seven electrode positions in reference to linked mastoids. In the first session subjects were asked to ignore the odors and to focus their attention on an auditory task (ignore cognition). In the second session subjects were instructed to concentrate on the odors and to react with a motor response everytime they detected linalool and allylcaproate (attend condition).

The performance data obtained in the attend condition revealed that only about half of the subjects could discriminate allylcaproate deviants more easily. Subjects were therefore separated into groups of linalool sensitives and allylcaproate sensitives. The statistical analyses showed that both groups responded already under the ignore condition with a significantly larger P300 to the subjectively easier deviant odor. The results strongly suggest that the subjective discriminability of odors can be already objectively measured at a pre-attentive level.

The study was supported by the Olfactory Research Fund.

113. Differential expression of RNA and protein of the three pore-forming subunits of the amiloride-sensitive epithelial sodium channel in taste buds of the rat

Oliver Kretz[1], Pascal Barbry[2], Rudolf Bock[3] and Bernd Lindemann[3]

Departments of [1]Anatomy and [3]Physiology, Saar University, D-66421 Homburg, Germany and [2]Institut de Pharmacologie Moléculaire et Cellulaire, CNRS, Sophia Antipolis, Valbonne, France

Salt taste signals from the rat anterior tongue are probably transduced via epithelial sodium channels (ENaCs) residing in the apical cellular pole of taste cells. The signals are blocked by mucosal amiloride in low micromolar concentrations. In contrast, the rat vallate papilla does not contribute to amiloride-blockable salt taste. Two approaches were used to probe for the three subunits of ENaC in the anterior and posterior tongue of the rat: (i) immunohistochemistry with antibodies against ENaC subunits and against amiloride binding sites. In the anterior tongue, reactivity for α, β and γ subunits was present in taste buds and lingual epithelium. In the posterior tongue vallate papilla, reactivity for the α subunit and for amiloride binding sites was easily demonstrable, while that for the β and especially for the γ subunits was weaker than in the anterior tongue. (ii) RT-PCR techniques were used to probe for the presence of ENaC subunit mRNA. In isolated taste buds of the anterior tongue, mRNA of all three subunits was found, while in isolated taste buds of the vallate papilla only mRNA of the α subunit was easily detectable. That of the β and γ subunits was much less abundant. All three subunits of ENaC were abundant only in taste buds of the anterior tongue. Thus subsets of elongated taste cells do express ENaC, but regional differences exist in the transcription and expression of subunits. Functional consequences of these differences may be expected.

114. Molecular elements of olfactory signal transduction in insect antennae

Jürgen Krieger, Jürgen Strobel, Ingrid Boekhoff, Wolfgang Hanke and Heinz Breer

University Stuttgart-Hohenheim, Institute of Physiology, Garbenstraße 30, D-70593 Stuttgart, Germany

Insects have evolved chemodetectors with an outstanding sensitivity and selectivity; this is accomplished by their antennal chemosensory neurons, which recognize and discriminate odorants and pheromones and convert the chemical signals into electrical responses. After entering the olfactory sensilla hairs through cuticle pores, odorous molecules have to cross the aqueous sensillum lymph to reach the perceptive membrane of the antennal receptor cell. This process is supposed to be mediated by specific binding proteins for pheromones and general odors. Antennal binding proteins from various insect species have been cloned and sequenced. They can be categorized into at least three different classes. The heterogeneity of these proteins and their expression in sensilla of different functional specificity have led to the concept that each class of binding protein may be fine-tuned to interact with a certain group of odorants and suggest a role in peripheral odor discrimination.

After reaching the dendritic membrane, pheromones and odorants are supposed to activate yet unidentified olfactory receptor proteins and trigger G-protein mediated second messenger cascades. Biochemical studies using antennal preparations from various insect species revealed that, short pulses of low odor doses elicit rapid and transient changes in second messenger concentrations. The response to pheromones appeared to be tissue-, species- and sex-specific. Previous studies have shown that in insect antennae pheromone and most odorants are processed via the phospholipase C/IP_3-cascade; however, some general odorants appear to activate the cAMP pathway. Using immunological and molecular cloning aproaches, elements of the signal transduction cascades (G-proteins and cyclic nucleotide gated ion channels) have been studied.

115. Cluster organization and chromosomal localization of avian olfactory receptor genes

Kafia Ladjali-Mohammedi, Michel Leibovici[1] and Christiane S. Ayer-Le Lievre[2]

UPR 9064 CNRS, Nogent sur Marne, [1]URA 1968 CNRS, Institut Pasteur, Paris and [2]ER 1794 CNRS, Faculté de Médecine, Limoges, France

Interactions between odorants and olfactory receptor molecules initiate the elaboration of a sensory signal in the olfactory neuron. Fifteen olfactory receptor genes (COR) belonging to three subfamilies have been cloned in the chick where they would represent >10% of the olfactory gene repertoire in this species. Clusters of olfactory receptor genes including genes of the same or different subfamilies have been evidenced and the intergenic fragment completely or partially sequenced in two cases. Representatives of two COR subfamilies have been found in quail and duck genomes as well.

Chromosomal localization of these COR genes has been performed using a combination of fluorescent *in situ* hybridization (FISH) and cytogenetic techniques on chick but also quail and duck genomes. The cluster organization of the COR genes on several macrochromosomes is thus confirmed.

These observations have important implications for the identification of mechanisms possibly involved in the exclusive expression of one subfamily of COR (or one COR) by each olfactory neuron.

116. Olfactory neuron differentiation: are centro-peripheral interactions involved in the choice of an olfactory receptor gene expression?

Fabrice Lalloue and Christiane S. Ayer Le Lievre

ER 1794 CNRS, Faculté de Médecine, Limoges, France

Olfactory receptors are seven transmembrane domain molecules present on the ciliary dendrites of the sensory neurons. They belong to a large family of receptors.

When they differentiate, each neuron expresses only one receptor or receptors belonging to only one subfamily of olfactory receptors, which accounts for their specificity of interaction with odorant molecules.

To try to understand how this choice is made we have used an avian embryo model using the fact that several olfactory receptor genes belonging to three subfamilies have been cloned in the chick but that only two of them were found in quail and duck. Chick/quail chimeras were obtained experimentally by orthotopic, orthochronic transplantations of olfactory placode, the embryonic primordium from which the olfactory epithelium develops. The

olfactory systems of the chimeras have been analyzed for their olfactory neuron differentiation and the levels of expression of the three subfamilies of olfactory receptors.

The results are discussed in comparison with the olfactory receptor expression at different critical stages of development of chick and quail embryos.

117. Genome dynamics in the olfactory receptor subgenome: deciphering the evolution of a large gene superfamily

D. Lancet[1,2], G. Glusman[2], S. Horn-Saban[2], D. Sharon[2], Y. Pilpel[2], M. Lapidot[2] and E. Ben-Asher[1,2]

[1]The Genome Center and [2]Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot 76100, Israel

The human 'olfactory subgenome' represents several hundred olfactory receptor (OR) genes in a dozen or more clusters on several chromosomes. We have launched a concerted effort to study the olfactory subgenome by genome analysis methods. One of the OR gene clusters, on human chromosome 17, has been characterized by us in detail. Based on large scale DNA sequence analysis of the chromosome 17 OR cluster, we have identified events of gene duplication as well as the generation of pseudogenes. The latter instances of 'gene death' could underlie the widespread phenomenon of human odor-specific olfactory deficits (specific anosmias). Comparative analysis of the duplicated genes has revealed the intron–exon structure of OR genes, including a putative control region, which is potentially important for OR clonal exclusion. We have analyzed a total of 60 OR genes which are located in clusters on chromosomes 11, 17 and 19. Whereas chromosome 11 contains many small and highly variable clusters, the other two contain one or two clusters of 8–16 genes each, comprising only two families per chromosome. We performed a comparative analysis of the human chromosome 17 cluster, versus the orthologous clusters in several primates as well as in mouse. This allowed us to identify several processes that underlie the generation of olfactory receptor diversity, including gene duplication as well as gene conversion. The latter may constitute an important evolutionary force in the long-term generation of germline diversity in this intriguing chemosensory system. On the protein level we have identified, through sequence and molecular modeling analyses, the putative odorant-binding site of the OR molecule. A peculiar feature of this recognition site is that it displays hypervariability, akin to the immunoglobulin complementarity determining region, instrumental for the detection of an unlimited set of potential odor molecules.

118. Detection of sex pheromones and plant odours by specialist neurons in male and female *Anomala cuprea* (Coleoptera: Scarabaeidae)

Mattias C. Larsson, Walter. S. Leal[1] and Bill S. Hansson

Department of Ecology, Lund University, S-223 62, Lund, Sweden and [1]Laboratory of Chemical Prospecting, National Institute of Sericultural and Entomological Science, Tsukuba, Ibaraki 305-8634, Japan. e-mail: mattias.larsson@zooekol.lu.se; bill.hansson@zooekol.lu.se; leal@nises.affrc.go.jp

Many herbivorous scarab beetles are important agricultural pests, and have been subject to trapping attempts with pheromones as well as plant odours. Their large size, and the high number of active semiochemicals identified, make scarabs interesting models for studies of insect olfaction. We have characterized olfactory receptor neurons in several ruteline scarab species by means of the tungsten penetration method. In all these species the antennae are similar, with the olfactory sensilla situated on flat lamellae forming the typical antennal club. The cuprous chafer *Anomala cuprea* is the most extensively studied in terms of odour detection, including responses from both males and females to pheromones as well as plant odours. The pheromonal stimuli consist of the two female-produced, chiral sex pheromone components (*R*)-buibuilactone and (*R*)-japonilure and their respective (*S*)-enantiomers. We also tested extracts of headspace collections from male *A. cuprea*. The plant odours include selected flower odours attractive to scarab beetles, and the green leaf volatiles (*Z*)-3-hexenyl acetate, (*Z*)-3-hexenol, (*E*)-2-hexenal and (*E*)-2-hexenol.

Both sexes have high numbers of receptor neurons for single pheromone components and for plant odours, while we have found no receptor neurons for the (*S*)-enantiomers. The two types of pheromone receptor neurons are found in separate placoid sensilla. On each antennal lamella, the pheromone sensilla are almost completely spatially separated from other types of sensilla. The pheromone sensilla cover approximately half of the lamella, forming a smooth, continuous area, while most other sensilla form a more heterogeneous band.

Most neurons encountered were very sensitive and specific, responding to one single compound in spite of stimulus concentrations spanning over several orders of magnitudes. This includes a high enantiomeric specificity of the pheromone neurons, as well as a high specificity of the GLV and flower odour neurons. Female pheromone neurons were normally less sensitive than in the males, but otherwise with similar response spectra. We also found neurons responding to the mixture of collected volatiles from male beetles. These neurons were found in both the smooth and the heterogeneous areas.

119. Olfactory discrimination ability of human subjects for 10 pairs of enantiomers

Matthias Laska and Peter Teubner

Department of Medical Psychology, University of Munich Medical School, Goethestraße 31, D-80336 Munich, Germany. e-mail: laska@imp.med.uni-muenchen.de

Chiral recognition of substances is one of the most important and widespread principles of biological activity. With regard to olfactory discrimination of enantiomers, however, the situation is still unclear. Whereas a variety of optical antipodes has been described as having different odor qualities, only few studies so far have directly tested the discriminability of (+)- and (–)-forms of such odorants, and even fewer have assessed whether inter- or intraindividual variability rather than substance differences may account for the widely differing findings.

Therefore, we have tested the ability of human subjects to distinguish between 10 pairs of enantiomers. In a forced-choice triangular test procedure 20 subjects were presented with 10 stimulus pairs twice per session and asked to identify the bottle containing the odd stimulus. Testing was repeated in four more sessions each 1–3 days apart, enabling 10 judgements per stimulus pair and panelist to be collected.

We found (i) that as a group, the subjects were only able to

significantly discriminate the enantiomers of alpha-pinene, carvone, and limonene, whereas they failed to distinguish between the (+)- and (–)-forms of menthol, fenchone, rose oxide, camphor, alpha-terpineol, beta-citronellol and 2-butanol; (ii) marked interindividual differences in discrimination performance, ranging from subjects who were able to significantly discriminate between six of the 10 odor pairs to subjects who failed to do so with nine of the 10 tasks; (iii) that with none of the 10 enantiomeric odor pairs were the antipodes reported to differ significantly in subjective intensity when presented at equal concentrations; and (iv) that error rates were quite stable and did not differ significantly between sessions and thus no learning or training effects at the group level were found.

Additional tests of the degree of trigeminality and threshold measurements of the (+)- and (–)-forms of alpha-pinene, carvone, and limonene suggest that the discriminability of these three enantiomeric odor pairs is indeed due to differences in odor quality.

Further, our findings support the assumption that enantio-selective molecular odor receptors may only exist for some but not all volatile enantiomers and thus that chiral recognition of odorants may be restricted to some substances.

Supported by the Deutsche Forschungsgemeinschaft (La 635/6-1 and 6-2).

120. Spatio-temporal codes for odours in oscillating neural assemblies

Gilles Laurent

California Institute of Technology, Biology Division, Pasadena, CA 91125, USA

Stimulus evoked oscillatory synchronization of neural assemblies has been most clearly documented in the olfactory and visual systems of several vertebrates and invertebrates. Our recent results from the olfactory system of locusts show that information about odour identity is contained in the identity of the recruited neurons as well as the timing of their action potentials in an oscillatory population response. This suggests that brain oscillations may reflect a common reference for messages encoded in time, allowing combinatorial representations of odors in time as well as in space. Although stimulus-evoked oscillatory phenomena are reliable, their roles in sensation, perception, memory formation and pattern recognition remained to be demonstrated—a task requiring a behavioral paradigm. Using honeybees, we have recently demonstrated that odour encoding involves, as in locusts, the oscillatory synchronization of assemblies of projection neurons, and that this synchronization is, here also, selectively abolished by the $GABA_A$ receptor antagonist picrotoxin. We then showed, using a behavioral learning paradigm, that picrotoxin-induced de-synchronization impairs the discrimination of molecularly similar odorants, but not that of dissimilar odours. It appears, therefore, that oscillatory synchronization of neuronal assemblies is functionally relevant, and essential for fine sensory discrimination. We finally identified a population of neurons downstream from the antennal lobe, whose individual responses are impaired by PN desynchronization. These results suggest that oscillatory synchronization and the kind of temporal encoding it affords provide an additional dimension by which the brain can segment spatially overlapping stimulus representations. We thus propose that, in addition to classical rate codes, the brain can also use temporal codes for the representation of complex sensory stimuli, even in cases where time is not an intrinsic component of the sensory stimulus represented.

121. Olfactory threshold, odor identification and odor memory in patients with temporal lobe epilepsy (TLE)

J. Lehrner, I. Kryspin-Exner[1] and C. Baumgartner

Neurological Clinic and [1]Institute of Psychology, University of Vienna, Austria

Medial temporal lobe structures play a prominent part in olfactory information processing and lesions in this region have resulted in olfactory dysfunction. In our study the question was addressed to which extent TLE-patients show unilateral olfactory deficits and furthermore whether this postulated impairment is a primary sensory event (e.g. loss in absolute sensitivity) or a higher olfactory system malfunctioning (e.g. odor naming and odor memory impairment). Unilateral olfactory evaluation was performed in patients who were considered a candidate for epilepsy surgery. All patients had continous video-EEG monitoring, magnetic resonance imaging (MRI), single photon emission CT (SPECT) and neuropsychological testing. The patients also underwent a standard sodium amobarbital procedure (WADA-test) for lateralization of language and memory functions. Only patients with (i) left-sided speech dominance and (ii) normal MRI or hippocampal atrophy/sclerosis were included. 25 patients with left-sided TLE, 17 with right-sided TLE and 19 controls were tested. The olfactory test battery assessed monorhinically the following subfunctions: olfactory threshold, odor identification and odor memory. No difference concerning olfactory threshold was found indicating normal olfactory sensitivity. However, right-sided TLE patients showed significantly lower right-sided odor identification and right-sided odor memory scores. In addition. left-sided TLE patients were significantly impaired in left-sided odor naming. The potential contributions of quantitative olfactory testing in the preoperative evaluation of epilepsy surgery candidates are discussed.

This research was supported by the Fonds zur Förderung der Wissenschaftlichen Forschung Österreichs (projects P11952 MED and P12697 MED).

122. Transient impairment of lamb recognition memory following specific basal forebrain cholinergic lesion in sheep

F. Lévy, G. Ferreira, M. Meurisse, R. Gervais[1] and N. Ravel[1].

Department of Animal Behavior, INRA/CNRS URA 1291, F-37380 Nouzilly and [1]Cognitive Sciences Institut, CNRS UPR 9075, F-69675 Bron, France

Within 4 h after parturition, ewes recognize the odor of their offspring. To investigate the involvement of basal forebrain cholinergic nuclei in this olfactory learning, we have developed a cholinergic immunotoxin in sheep in which antibody against p75-NGF-receptor (p75-IgG) is conjugated to a ribosome-inactivated protein saporin. Using a rat monoclonal antibody raised against pig choline acetyltransferase (ChAT) and a mouse p75-IgG (raised against primate p75-NGF-receptor) we have established whether ChAT and p75-NGF-receptor positives neurons are co-localized. The double labeling procedure reveals

that 100% of ChAT positive neurons are also p75-NGF-receptor positive in the medial septal nucleus (corresponding to Ch1 in rats and monkeys), in the vertical and horizontal limb of diagonal band of Broca (Ch2 and Ch3) and 85% in the nucleus basalis magnocellularis (Ch4). Moreover, throughout the Ch1-Ch4 nuclei all the p75-NGF-receptor positive cells are ChAT positive. The second step was to verify whether immunotoxin, mouse p75-IgG-saporin, is effective in sheep. Three doses of immunotoxin (50 μg, $n = 1$; 100 μg, $n = 2$; 150 μg, $n = 2$) were injected into lateral ventricles of sheep brain. Whereas the lowest dose produced 50% loss of basal forebrain cholinergic neurons, the highest dose produced >75% loss. These results allow us the use of this immunotoxin in sheep. Ewes received, 6 weeks before parturition, saline or 150 μg of p75-IgG-saporin into lateral ventricles. Lamb recognition was tested at 2 and 4 h post-partum. A ewe was considered selective if she did not accept alien lamb at suckling and butted it. At 2 h post-partum, whereas 7/9 sham ewes were selective, only 2/8 lesioned ewes did so ($P = 0.057$). However, at 4 h the inter-group difference vanished (treated 7/8 versus sham 8/9). The treatment did not alter olfactory sensitivity since lesioned ewes, as control, showed clear repulsive behavior towards the odor of dog feces. In order to test whether this memory deficit was specific to this type of olfactory learning, ewes were also trained on a visual discrimination task. Again, both groups managed the task although treated ewes took significantly more trials to reach the 12/15 correct choice criterion (52 ± 8 versus 32 ± 3; $P < 0.05$). The immunotoxin injection induced a loss of choline acetyl-transferase positive neurons in the four nuclei (Ch1–Ch4) of the basal forebrain of ~75–85%. These results provide evidence that these cholinergic nuclei contribute to early phases of olfactory and visual learning with no detectable effect on attentional, motivational or perceptual processes.

Supported by INRA/AIP No. 924881.

123. Taste transduction in the light of modern techniques

Bernd Lindemann

Department of Physiology, Saar University, D-66421 Homburg, Germany. e-mail: phblin@med-rz.uni-sb.de

Vertebrate taste cells are small polarized cells organized in taste buds of rather complex morphology. Of the four or more types of cells present, at least two are involved in chemoreception. The transduction processes were investigated using methods such as nerve recording, patch clamping, fluorescence imaging and molecular cloning. The recent introduction of further techniques, including activation assays, stopped flow recording, multiple dye imaging and confocal microscopy, as well as refined mounting procedures for patch clamping and imaging, has opened exciting new possibilities. Results will be reviewed and discussed (emphasizing some of those not represented in the subsequent Symposium III on taste transduction).

124. Imaging of amiloride in taste buds and lingual epithelium of the rat

Bernd Lindemann

Department of Physiology, Saar University, D-66421 Homburg, Germany. e-mail: phblin@med-rz.uni-sb.de

Amiloride is often used as a reversible blocker of the epithelial Na^+ channel (ENaC) contained in lingual epithelium and taste buds. How is amiloride handled by these tissues? It turns out that amiloride itself can be used as a vital fluorescent stain which is bright enough, when excited with blue to ultraviolet light, to investigate this question. The anterior lingual epithelium was isolated enzymatically and mounted in an open chamber placed on an inverted microscope. The basal side of the nearly planar epithelium faced the microscope objective. Mucosal and basal compartments were perfused separately while fluorescence imaging of epithelial cells and lateral intercellular spaces (LIS) proceeded. Taste buds remained *in situ* in the fungiform papillae and their fluorescence was imaged together with that of the epithelium.

Amiloride fluorescence appeared within lingual epithelial cells when the drug was added to the basal compartment at 1–50 μM. The signal was especially strong within some epithelial cells of characteristic location and in some sections of the LIS. However, the taste buds within fungiform papillae displayed a weak fluorescence only, part of which delineated cellular borders and was possibly contained in the LIS of the buds. In contrast, after their isolation from the epithelium taste buds accumulated amiloride readily into their cytosol. The time course of uptake had linear and exponential components (time constants of minutes).

Thus amiloride not only acts as a blocker of sodium channels and other transporters, a function which requires access to the outer surface of the plasma membrane; in addition, the molecule enters cells and is accumulated in the cytosol, where it potentially affects a variety of cellular functions. The accumulation differs among cell types, suggesting differential expression of uptake and/or elimination pathways. Furthermore, amiloride appears to accumulate not only within cells but also in extracellular compartments of lingual epithelium and taste buds. Possibly, the molecule is first entering the cytosol by non-ionic diffusion and is accumulating as a Nernstian dye of positive charge. It is then transported into the LIS, where it can accumulate if the pathway of diffusion between these spaces and the bulk bathing solution is constricted, e.g. by tight junctions and basal cells. In consequence, the concentration of amiloride, at significant parts of the outer membrane surface, may become higher than in the bulk solution. These effects should be taken into account when interpreting dose–response curves obtained with long-lasting exposure to amiloride.

125. Effects of amiloride and W-7 on the chemoreception of NaCl, KCl and LiCl in blowflies: implications for the salt and bitter qualities

A. Liscia, S. Linguini, P. Solari, M. Marras, E. Pisano and R. Crnjar

Dip. Biologia Sperimentale, Sez. Fisiologia Generale, Università di Cagliari, Viale Poetto 1, I-09126 Cagliari, Italy

In insects, unlike the case of vertebrates, salt reception is not mediated by any amiloride-sensitive cation conductance mechanism, although amiloride itself seems to have a stimulating effect. However, no information is available on the influence of amiloride on the stimulation by salts other than NaCl. We have thus investigated, in the labellar chemosensilla of the blowfly *Protophormia terraenovae*, the effects of amiloride (that reversibly blocks voltage-insensitive sodium channels in epithelial cells) or W-7 (a calmodulin antagonist) on stimulation with KCl and LiCl as

compared with NaCl. Besides, since amiloride is known to have a bitter taste in other organisms, we also compared response to amiloride with that of quinine. Spike discharges evoked by stimulation with the various salts were processed by spike waveform analysis in order to identify the activity of the different sensory cells. The results show that: (i) three sensory cells respond to the salts tested (except for KCl 50 mM), corresponding to the 'salt', 'water' and 'fifth' cells previously described in blowflies; (ii) W-7 depresses the spike activity of the 'salt' and 'fifth' cells in response to all the tested salts; (iii) In the case of KCl, unlike that of NaCl, the 'salt' cell is inhibited by amiloride at all but the lowest concentration; (iv) the 'fifth' cell responds to pure LiCl, amiloride and quinine more than the 'salt' cell; and (v) addition of amiloride does not affect the response at the lower LiCl concentration, but at the higher one (150 mM) it decreases the 'salt' cell response and increases that of the 'fifth' cell. In conclusion, for the 'salt' cell, more than one mechanism appears to be involved in the transduction process depending on the nature of the salt. In fact, while the response to NaCl is not affected by amiloride, both those to KCl and LiCl are decreased, even though to a different extent. The strong inhibition exerted by W-7 (that supposedly interferes with the Ca^{2+} cascade) on salt stimulation also supports this suggestion. On the other hand, a role as bitter detector is proposed for the 'fifth' cell in the blowfly, that is stimulated in a similar fashion by amiloride, LiCl and quinine, that reputedly taste bitter for other species, including humans.

126. Structural and functional characterization of odorant binding proteins

Dietrich Löbel, Silvana Marchese[1], Jürgen Krieger, Isabella Andreini, Paolo Pelosi[1] and Heinz Breer

University of Stuttgart-Hohenheim, Institute of Physiology, 70593 Stuttgart, Germany and [1]University of Pisa, Italy

Recognition and discrimination of thousands of odorous compounds is mediated by olfactory sensory neurons. In mammals, the chemosensory cells are surrounded by a protective aqueous milieu, the nasal mucus. The volatile, primarily lipophilic odorous molecules have to cross the hydrophilic barrier before reaching olfactory receptors in the chemosensory membrane. Odorant binding proteins (OBPs) are found at rather high concentration in the mucus layer of the vertebrate nose. Their ability to bind odorous compounds has led to the assumption that they may serve as carrier to concentrate odorant molecules in the aqueous phase and transfer the hydrophobic compounds towards the chemosensory neurons (Pelosi, 1994, Crit. Rev. Biochem. Mol. Biol., 29: 199–228). The OBPs belong to a large group of small extracellular proteins, the lipocalins. Two subtypes of these proteins from rat have been cloned and their primary structure deciphered. *In situ* hybridization and immuncytochemical approaches allowed to localize the glands where the OBPs are generated. To evaluate their binding properties two distinct OBP subtypes of the rat were expressed as N-terminal–His-tagged fusion proteins in *Escherichia coli*, thus allowing an efficient purification. Based on gel chromatography and CD spectroscopy analysis the recombinant OBP subtypes seem to share general structural features with other members of the lipocalin family. In addition, ligand binding studies as well as some new approaches using fluorescence spectroscopy and surface plasmon resonance (SPR) technology were employed to explore the biospecific interaction of these globular proteins with odorous compounds (Löbel *et al.*, 1998, Eur. J. Biochem., 254: 318–324). The results indicate that rat OBPs have distinct ligand specificity. This type of analysis is supposed to give some new insight into the structure/function relation of odorant binding proteins.

127. Olfactory deafferentation alters tyrosine hydroxylase but not calbindin or calretinin immunoreactivity

G. Maffiodo, M. Bonino, P. Bovolin, I. Perroteau and A. Fasolo

Department Human and Animal Biology, University of Turin, Italy. e-mail: fasolo@dm.unito.it

Several calcium binding proteins (CaBP) have been found in high concentrations in the central nervous system. In the present work we studied by immunocytochemistry the localization of two CaBPs, Calbindin-D 28k (CL) and Calretinin (CR), in the olfactory bulb (OB) of adult mice. CL-IR was observed in periglomerular (PG) cells and in a few perikarya in the external plexiform layer. CR-IR was present in PG cells as well as in some tufted and granule cells. Double immunostaining indicated that CL and CR are expressed by two separate subsets of PG cells. Moreover, almost none of these neurons was immunoreactive for tyrosine hydroxylase (TH). Semi-quantitative analysis were performed by counting the percentage of immunoreactive profiles out of the total Hoechst 333342-stained nuclei. CL+, CR+ and TH+ cells were respectively 7.88 ± 0.06, 8.35 ± 0.11 and 21.56 ± 0.27% of the total periglomerular population. It has already been established that the TH phenotype, in the glomerular layer, strictly depends on afferent olfactory innervation. Therefore, we investigated whether the same is true for CL and CR by performing $ZnSO_4$ irrigation of the olfactory epithelium. As expected, 21 days after the lesion the percentage of immunoreactivity for TH (4.97 ± 0.12) was reduced in deafferentated OBs. On the contrary, the CaBP phenotype (CL = 8.00 ± 0.32%; CR = 23.6 ± 1.05%) was unaffected by the lesion. Baker *et al.* (1988) showed that olfactory deafferentation does not alter GABA immunoreactivity in postsynaptic elements. On the other hand, in our laboratory it has been demonstrated recently that olfactory nerve lesion deafferentation down-regulates the expression of both mRNA and protein of the metabotropic glutamate receptor mGluR1a in mitral cells and of the neuregulin receptor erb-4 in both PG and mitral cells. On the whole these results would suggest that olfactory afferent innervation has an important role in phenotypic maintenance of some, but not all, postsynaptic elements. The mechanisms underlying these differential effects are poorly known. According to recent studies, CL-IR and possibly CR-IR neurons receive almost no synaptic contacts from olfactory nerve fibres. Therefore, our results could be explained by the lack of olfactory synaptic contacts on CL-IR and CR-IR periglomerular populations.

Supported by Italian MURST.

128. A novel family of highly conserved small putative carrier proteins (SCPs) in insects

R. Maleszka, N. Swasdipan, P. Ebert and G. Stange

Visual Sciences, Research School of Biological Sciences, The Australian National University, Canberra, ACT 0200 and Departments of Biochemistry and Entomology, University of Queensland, Brisbane, QLD 4072, Australia

Recent molecular, biochemical and bioinformatics studies have led to the delineation of a distinct family of highly conserved small putative carrier proteins (SCPs) that appears to be unique to insects. These proteins share a unique cysteine motif, with the consensus CX6CX18CX2C, that detects exclusively all known members of this protein family currently deposited in GenBank. The family shares some general features with odorant-binding proteins and pheromone -binding proteins, such as the presence of signal peptides and a cysteine motif. Members of this family have been localized on the antennae (*Drosophila* and *Apis*), as well as labial palps (*Cactoblastis*; Pikielny *et al.*, 1994, Neuron, 12: 35–49; Maleszka and Stange, 1997, Gene, 202: 39–43). Their strong expression in those appendages suggests that they are involved in some olfactory functions such as odorant-binding and/or CO_2 sensing (Maleszka, and Stange, 1997, Gene, 202: 39–43). As the primary structure indicates that the mature proteins are secreted, they may function either as passive carriers and solubilizers, or may be involved in codifying and recognition of odor message (Pelosi, 1996, J. Neurobiol., 30: 3–19). However, members of the family also occur in the ejaculatory bulb (*Drosophila*) and legs (*Periplaneta*; Nomura *et al.*, 1992, Int. J. Devl Biol., 36: 391–398), indicating that their function is not constrained to the olfactory context though, here too, they may serve a chemosensory function.

129. Mushroom body development and function in the honeybee

Dagmar Malun and Bertram Gerber

Institut für Neurobiologie, Freie Universität Berlin, D-14195 Berlin, Germany

The mushroom bodies of insects are thought to play a major role in processing and storage of chemosensory information because the main input to the mushroom bodies comes from the antennal olfactory lobes, the first central station in the olfactory pathway. The mushroom bodies are prominent neuropil structures arranged as pairs in the dorsal protocerebrum. In the honeybee, *Apis mellifera*, each mushroom body is composed of a medial and a lateral subunit. In this study, the proliferation pattern of mushroom body intrinsic cells, the Kenyon cells, was examined during larval and pupal development by monitoring bromodeoxyuridine (BrdU) incorporation and by chemical ablation. At the beginning of larval stage 3, precursors of Kenyon cells are arranged in two distinct cell clusters in each hemisphere indicating that proliferation of Kenyon cell neuroblasts starts during yet earlier larval stages. BrdU incorporation into newly synthesized DNA and its immunohistochemical detection revealed high mitotic activity in these proliferation clusters that lasts until mid-pupal stages. The uniform diameter of cells and the homogeneous distribution of BrdU-labeled nuclei indicate symmetrical cell divisions of Kenyon cell precursors. Each of these proliferation clusters produces Kenyon cells of the respective mushroom body subunit.

Hydroxyurea fed to newly hatched larvae causes the selective deletion of Kenyon precursor cells and thus mushroom body ablation in adults. Hydroxyurea-induced defects range from ablation of one to all four mushroom body subunits. This deletion of complete subunits suggests that each mushroom body subunit originates from a single stem cell. These findings also show that in honeybees, like in *Drosophila*, chemical ablation is a feasible method to selectively delete mushroom bodies.

To address the hypothesis that mushroom bodies are involved in learning and memory, a group of adult hydroxyurea-treated honeybees that had one medial mushroom body subunit missing but exhibited an otherwise intact brain, and control animals were tested in non-associative (sensitization and habituation) and associative (discriminative conditioning) behavioral paradigms. Preleminary results indicate that honeybees with mushroom lesions show no deficit in a classical discriminative conditioning paradigm that tests for associations of odor cues with sucrose reward. However, in a non-associative learning paradigm, habituation to the sucrose reward was slower in honeybees with mushroom body lesions. Thus, our approach will allow us to dissociate the mechanistic basis of associative and non-associative learning.

130. Binding and permeation of amphipathic tastants through liposomal membranes; possible implications to taste cells

Hanna Mamrud-Brains[1,3], Shlomo Nir[2,3], Irena Peri[1,3], Yechiel Shai[4] and Michael Naim[1,3]

[1]*Institute of Biochemistry, Food Science and Nutrition,* [2]*Seagram Center, Department of Soil and Water Sciences,* [3]*The Hebrew University of Jerusalem and* [4]*Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel*

Some amphipathic tastants induce changes in membrane diffusion potential in liposomes, activate G-protein directly *in vitro* and initiate physiological responses in cells not related to taste. Experiments employing the Valinomycin diffusion potential assay with small unilamellar liposomes (SUV) composed of Azolectin indicated that applications of the bitter tastants quinine and the dipeptide cyclo-(Leu–Trp) induced immediate increase in fluorescence in a dose-concentration manner, suggesting significant interactions with SUV (>70% fluorescence recovery compared with melittin). The bitter tastant naringin did not affect the fluorescence although its related compound, the sweetener neohesperidin dihydrochalcone, interacted strongly with SUV, suggesting specificity in such interactions. Thirty millimolar sucrose and 30 mM NaCl did not affect the diffusion potential of SUV. Next, we focused on translocation experiments using multilamellar liposomes (MLV) (phosphatidylcholine:cholesterol, 10:1). The assay employed was based on fluorescence quenching of quinine and cyclo-(Leu–Trp) by KI (non-permeant) added externally to the suspension containing MLV and tastant molecules. Results indicated that fractions of quinine (50 μM) and cyclo-(Leu–Trp) (0.5 mM) were protected from quenching by KI during experiments with MLV (18 mM lipid), suggesting a process of tastant permeation through MLV (18 mM lipid). Protection values (%) were as follows: quinine, 30.5 ± 3.1, 36.5 ± 0.9 and 58.8 ± 1.6 during 5, 15 and 30 min time periods, respectively; cyclo-(Leu–Trp), 34.8 ± 1.1, 41.5 ± 1.2 and 61.4 ± 0.5 during 5, 15 and 30 min time periods, respectively. Furthermore, such processes were very rapid. Protection values (%) were 14.3 ± 0.2, 23.3 ± 0.2,

23.5 ± 0.2 and 28.7 ± 0.98 for quinine, and 0, 6.6 ± 1.1, 9.6 ± 1.1 and 14.7 ± 1.1 for cyclo-(Leu–Trp) during 5, 10, 15 and 20 s time periods, respectively. Preliminary experiments employing rat taste bud sheets, isolated from the circumvallate papilla by collagenase treatment, indicated that the bitter tastants quinine and cyclo-(Leu–Trp) penetrate through the membranes of taste cells.

Supported by Grant I-2518-95 from BARD, The US-Israel Binational Agricultural Research and Development Fund.

131. Cloning of soluble chemosensory proteins in phasmids

S. Marchese, F. Ceron, S. Angeli, M. Mazza[1], J.F. Picimbon[2], W.S. Leal[2] and P. Pelosi

Dip. Chimica e Biotecnologie Agrarie, via S- Michele, 4, I-56124 Pisa, [1]Dip. Scienze Uomo Ambiente, Pisa, Italy and [2]NISES, Tsukuba, Japan. e-mail: ppelosi@agr.unipi.it

Based on our previously reported N-terminal sequences, we have amplified by PCR, cloned and sequenced genes encoding soluble proteins in chemosensory organs of two species of phasmids, *Eurycantha calcarata* and *Carausius morosus*. Several related sequences have been amplified in both species. Here we report the complete amino acid sequences of three proteins expressed in the antennae of *E. calcarata* and one in the tarsi of *C. morosus*. They consist of polypeptides of 100–117 residues, bearing four conserved cysteines. All exhibit significant similarity with a recently discovered class of chemosensory proteins, including *Drosophila melanogaster* OS-D, *Cactoblastis cactorum* CLP-1 and five members of *Schistocerca gregaria* (Angeli *et al.*, 1998, ECRO XIII).

One of the *E. calcarata* sequences is identical in its first 33 residues with that determined by direct Edman degradation on the purified protein, while in *C. morosus* the sequence deduced from the cDNA is markedly different from the N-terminal information obtained on the isolated protein. A comparison between proteins of this class across different orders shows that they are well conserved, when compared with OBPs.

Polyclonal antibodies prepared against purified proteins from both species, as well as against a similar protein of *S. gregaria*, indicated no cross-reactivity, despite the similarity between the three proteins. Each antiserum, however, stained bands of similar molecular weight in different chemosensory organs of the same species.

To test their hypothesized role in carbon dioxide sensing, we performed binding experiments with radioactive bicarbonate, but results were negative in the conditions employed.

Being the proteins of this class probably involved in chemoreception, as suggested by their tissue localization, we propose the general name of CSP (ChemoSensory Protein).

132. Chemical communication in the bank voles: vomeronasal system and behaviour

Anna Marchlewska-Koj and Malgorzata Kruczek

Institute of Environmental Sciences, Jagiellonian University, Krakow, Poland

The bank vole is a common, solitary living and promiscous rodent and behavioural interaction between conspecifics is mostly limited to sexual activity.The behaviour of female and male bank vole pairs was analysed during 180 min encounters.

Sexual experienced (EXP) but not naive (NONEXP) males discriminated between receptive and non-receptive females ($P < 0.05$) during the first 5 min of encounters. Higher behavioural activity of EXP males in the presence of receptive females was abolished by vomeronasalectomy ($H = 12.04$, $P = 0.03$), but copulation was recorded during the next 30–120 min. Mounting behaviour was inhibited by bulbectomy in 5/6 EXP males.

Bulbectomy of females did not influence reproductive activity. Bulbectomized females copulated, gave birth and exhibited typical maternal behaviour. However, females discriminated between the odour of sexually active and castrated males ($P < 0.01$) and between dominant and subordinate males ($P < 0.01$). Vomeronasalectomized females were not able to identify hormonal status of males.

In bank voles only males emit ultrasounds on the 20 kHz frequency. They vocalized in the presence of anaesthetized female or female bedding but vomeronasalectomy increased the latency of first call ($P < 0.01$) and decreased the number of ultrasounds ($P < 0.05$).

The results indicate that in female and male bank voles the vomeronasal system serves to select the sexual partner.

133. Patterns of glomerular innervation are reconstituted after ORN lesion: studies with transgenic mice

Frank L. Margolis and Diana M. Cummings

Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore MD, USA

The vertebrate olfactory neuroepithelium (OE) is unique in its ability to regenerate and re-innervate the olfactory bulb after deafferentation. It is assumed, but not proven, that the pattern of re-innervation replicates the original pattern of axonal innervation. To address this question we examined the pattern of olfactory receptor neuron (ORN) distribution and axon re-innervation of the deafferented bulb of H-OMP-LacZ-6 transgenic mice. In this strain, LacZ expression is limited to a subset of ORNs that are bilaterally distributed in the OE. These ORNs are concentrated in endoturbinates IIb and III in a subregion of olfactory receptor expression zone 2 and project to a few glomeruli in the ventromedial region olfactory bulb (Treloar *et al.*, 1996, J. Comp. Neurol.).

To address the question of whether ORN axons preserve their topographic organization when they re-innervate the bulb, we lesioned the OE in young adult H-OMP-LacZ-6 mice by bilateral intranasal irrigation with Triton X-100. We examined the distribution of beta-galactosidase immunoreactive (b-gal-ir) and X-gal-stained ORNs and processes after 10 days and 6–8 weeks. Our prior studies demonstrated that the maximum reduction of bulbar gene expression following this treatment occurs at ~10 days and that re-innervation is essentially normal at 6–8 weeks. Ten days after treatment, X-gal staining for beta-galactosidase in the olfactory mucosa and immunostaining for OMP and b-gal in the bulb were dramatically reduced. After 6–8 weeks, the pattern of b-gal-ir and X-gal staining in bulb and OE was very similar to that seen in untreated young animals. Interestingly, the expression of LacZ is virtually absent in intact older H-OMP-LacZ-6 mice. However, when the epithelia of these mice are lesioned and allowed to regenerate, the pattern of b-gal-ir and X-gal staining mimics that found in younger animals. These data demonstrate that the

pattern of *lacZ* gene expression in reconstituted OE and in re-innervated olfactory bulb accurately reflects that seen in young mice. Thus, the distribution of ORN gene expression and the pattern of innervation of bulbar targets following lesion recreates the initial pattern generated during ontogeny. The mechanisms underlying this recapitulation of axonal targeting are under investigation.

Supported in part by NIH grant DC-03112.

134. Structure/perception studies of androstenone analogues

Christian Margot and Christelle Porcherot

Firmenich SA. 1, Route des jeunes, CH-1211 Geneve, Switzerland

Since their initial discovery by Prelog and Ruzicka more than half a century ago, androsten-3-one and the corresponding axial alcohol androsten-3α-ol have been studied extensively by numerous laboratories. Perceptual studies of the ketone and chemical preparation of structural analogues have shown that non-steroidal molecules may possess a similar smell and some rules for the structural requirements of the androstenone odor have been proposed, especially by Amoore, Beets, Theimer, Ohloff and Zinkevich. We have prepared some new, nonsteroidal analogues of androstenone and measured their perception threshold distributions. It appears that the androstenone odor is essentially determined by two key molecular elements, the carbonyl group and a highly substituted lipophilic element. The molecular distance separating these two elements is surprisingly variable within the range 6.4–7.5 Å. Threshold measurements show a high degree of correlation between androstenone smelling molecules. Some odorants bear a different smell although they satisfy the structural requirement for androstenone odor. This sharp tuning of odor is difficult to understand if one considers only the olfactory receptor molecular recognition. However, it fits well with the reports of contrast enhancement performed by the olfactory bulb.

135. Human fetus encode the odor of their amniotic environment

L. Marlier, B. Schaal[1] and R. Soussignan[2]

Lab. Psychophysique Sensorielle, ULP-CNRS Ep 618, F-67000 Strasbourg, [1]Lab. Comportement Animal, CNRS Ura 1291 Inra, Nouzilly and [2]Lab. Psychobiologie du Développement, CNRS Ep 1595, EPHE, Paris, France

Several mammalian newborns have been shown to exhibit selective responses to odors they were exposed to *in utero*. Newly born rats and lambs are more attracted by the odor of their own amniotic fluid (AF) presented simultaneously with AF odor collected from an unrelated female. As yet, no stringent evidence of fetal acquisition of odor information is at hand in humans. To examine this point, we analyzed the responsiveness of 4-day-old breast-fed (Brf) and bottle-fed (Bof) newborns to the odors of own and unfamiliar AF (i.e. from an unrelated infant) presented simultaneously in a two-choice test. The dependent measure was the relative duration of head orientation to either stimulus. Both Brf and Bof infants exhibited significantly longer orientation to the familiar than to the unfamiliar AF odor. As Brf infants may have been exposed to AF-like cues potentially carried in their mother's milk, they could have acquired the preference for their own AF odor through postnatal experience. Nevertheless, the response pattern of Bof infants who were never re-exposed to such cues indicate that the preference for own AF develops independently of postnatal experience. These results show that human fetuses can acquire olfactory learning in their normal amniotic environment and that this prenatal memory trace can remain active for at least 4 days after birth.

136. Olfactory perception by the European bee *Apis mellifera mellifera* of the parasite *Varroa jacobsoni*

C. Martin, E. Provost[1], Y. Leconte and J.L. Clément[1]

INRA, Unité de Zoologie, Laboratoire de Biologie de l'Abeille, Domaine St Paul, Site Agroparc, F-84914 Avignon Cedex 9 and [1]CNRS, Laboratoire de Neurobiologie, Equipe Communication Chimique, 31 Chemin J. Aiguier, F-13402 Marseille Cedex 20, France

The European bee *Apis mellifera mellifera* is susceptible to parasitization by the mite *Varroa jacobsoni*. Parasitization can kill the colony within 2–3 years. This study carried out on colonies in southern France using both behavioral and electrophysiologic techniques was designed to assess susceptibility to parasitization by *Varroa*.

Behavioral tests were performed in the laboratory. In each experiment, five worker bees were placed in a Petri dish with one mite attached using wax. Results showed that under our experimental conditions there were two categories of workers in natural colonies. The first category included individuals that recognized and attacked *Varroa*. Most workers were in the second category that did not attack *Varroa*.

Olfactory perception of *Varroa* by the two categories of workers was studied by electroantennography. Antenna were stimulated using cuticular extracts obtained using hexane from *Varroa* taken either from adult bees or from the brood inside operculated cells. There was no significant difference in antennal response between bees that did or did not attack *Varroa*. Nor was any difference observed according to whether *Verroa* were taken from adult bees or the brood, even when only bees showing aggressive behavior were considered.

Based on these findings, we conclude that the difference in behavior towards *Varroa* results from a difference in signal integration at the level of the central nervous system rather than to a difference in perception at the peripheral level.

137. Isolation of putative olfactory receptor sequences from pignasal epithelium

Valery Matarazzo, Alain Tirard, Marielle Renucci, Anne Belaich [1] and Jean-Luc Clement

CNRS-UPR 9024, Laboratoire de Neurobiologie and [1]CNRS-UPR 9036 Bioenergetique et ingenierie des proteines, 31 Chemin Joseph Aiguier, F-13402 Marseille, France

The initial step in the recognition and discrimination of an odorant molecule or mixture is binding to odorant receptor proteins present in nasal epithelium. These receptors constitute one of the most important, although poorly known, families of neuronal receptors. In this study we used degenerated oligonucleotides and a RT-PCR approach to selectively amplify olfactory receptors in the nasal epithelium of the domestic pig *Sus scrofa*. Several combinations of oligonucleotide were tested and enabled the isolation of 11 different partial sequences belonging to the seven transmembrane olfactory receptor family. These

receptors formed a separate family within the seven transmembrane receptor superfamily in pigs. Using the criteria of Ben Arie *et al.* (1994, Hum. Mol. Genet., 3: 229–235), the 11 receptors described here can be classified into three known families and seven subfamilies (one known and six new).

138. Ultrastructural localization of $G_{i\alpha 2}$ and $G_{o\alpha}$ to microvilli of rat vomeronasal receptor cells

Bert Ph. M. Menco and Maya P. Yankova

Department of Neurobiology and Physiology, O.T. Hogan Hall, Room 5-120, 2153 North Campus Drive, Northwestern University, Evanston, IL 60208-3520, USA. e-mail: bmenco@casbah.acns.nwu.edu

In the vomeronasal organ (VNO), odor transduction is thought to occur in the microvilli of the sensory neurons. Light microscopic studies from several groups of investigators (review: Bargmann, 1997, Cell, 90: 585–587) have suggested that there are at least two different odor signal transducing pathways in the VNO. Each pathway has a different type of odor receptor and a different signal transducing G-protein, $G_{i\alpha 2}$ or $G_{o\alpha}$, and molecules of each pathway localize in different VNO receptor cells that coexist side by side. To examine the distribution of these different G-proteins in the receptor cell microvilli at the ultrastructural level we used post-embedding ultrastructural immunocytochemistry; primary antibodies and gold conjugated secondary antibodies were applied to sectioned mildly fixed and cryoprotected rat VNO specimens that were freeze-substituted and embedded in Lowicryl K11M. Results showed that expression of both $G_{i\alpha 2}$ (using a polyclonal antibody to a $G_{i\alpha 2}$ peptide, Calbiochem) and $G_{o\alpha}$ (also using a polyclonal anti-peptide antibody, Santa Cruz) is readily apparent in the receptor cell microvilli, and that the level of expression is considerably higher in these microvilli than in other cellular regions, including axons. Dendrites showed hardly any labeling. Labeling was also absent from cilia and microvilli of the non-sensory epithelium, located on the opposite surface of the VNO sensory epithelium, and from microvilli in the transition zone between both epithelia. Although individual cells with microvilli, immunopositive for either $G_{i\alpha 2}$ or $G_{o\alpha}$, could be identified along the sensory epithelial surface in serial sections, the ultrastructural morphology of apices and microvilli of the two cell types labeled with antibodies to $G_{i\alpha 2}$ or $G_{o\alpha}$ was indistinguishable. Finally, there also seems to be a diffuse zonal topography in the VNO epithelial surface: some areas, especially near the transition zone, had considerably more cells with $G_{i\alpha 2}$-positive microvilli than cells that with $G_{o\alpha}$-positive microvilli, while other areas, especially more centrally, showed about equal numbers of cells with $G_{i\alpha 2}$- or $G_{o\alpha}$-positive microvilli. Areas showing the opposite situation, with noticeably more cells with $G_{o\alpha}$-positive than $G_{i\alpha 2}$-positive microvilli, were not seen. These data thus provide ultrastructural evidence of a distinct microvillar localization of signal transducing G-proteins as well as evidence of zonal topography in the VNO epithelial surface.

Supported by NIH-NIDCD Grant RO1 DC02491.

139. Effects of cigarette smoking on the flavor of human milk

Julia A. Mennella and Gary K. Beauchamp

Monell Chemical Senses Center, Philadelphia, PA 19104 USA. e-mail: mennella@monell.org

In view of the vast array of inherent and added flavors found in cigarettes, we hypothesized that the odor, and consequently the flavor, of human milk would be distinctively altered following smoking. To test this, we evaluated milk samples collected from five lactating women immediately prior to and at fixed intervals following cigarette smoking. After acclimatization to the testing room and personnel, each mother expressed a baseline sample of ~15 ml of foremilk by using an electric breast pump. The mother, shielded with a disposable lab coat and wearing gloves, then went into a 700 ft^3 stainless steel environmental chamber alone and smoked one or two cigarettes of her regular brand within 20 min. After removing the lab coat and gloves, she washed her hands with an unscented soap and returned to the testing room, where additional milk samples were obtained at 0.5, 1, 2, 3 and 4 h after smoking. A sensory panel of adults then evaluated the odor of the milk samples and the milk samples were analyzed for nicotine using gas chromatography with nitrogen phosphorous detection. The results were unambiguous. The sensory panelists were more likely to identify samples collected 0.5–1 h following smoking as smelling 'stronger' or 'more like cigarettes' than the other samples. This was statistically significant for four of the five donors (Friedman two-way analysis of variance: Ps < 0.02; for the fifth, $P < 0.10$) and highly significant for pooled data ($P < 0.001$). The changes in the sensory evaluation paralleled the changing concentrations of nicotine in the milk [Spearman rank correlation (5 df) = 0.94; $P < 0.01$]. Although the basis for the sensory change remains to be determined, the current data indicate that smoking by lactating women clearly alters the odor of their milk. Based on many animal model studies, as well as some suggestive human studies, we hypothesized that early exposure to the flavor of foods and beverages consumed by their mothers may influence long-term acceptance of those flavors in childhood and beyond, thus raising the possibility that early experiences with the flavor of tobacco in mother's milk may influence the likelihood that exposed children will find these flavors appealing later in life.

140. Sensory hyperreactivity—a possible mechanism underlying cough and asthma-like symptoms caused by chemical irritants

Eva Millqvist, Mats Bende[1] and Olle Löwhagen

Asthma and Allergy Centre, University of Göteborg, Sahlgrenska Hospital, Göteborg and [1]Department of Otorhinolaryngology, Central Hospital, Skövde, Sweden. e-mail: eva.millqvist@medfak.gu.se

Ten patients with asthma-like symptoms after exposure to non-specific irritating stimuli like strong scents, but without IgE-mediated allergy or demonstrable bronchial obstruction, were recruited for the study. In order to find a provocation model for recording the individuals' sensory airway reactivity, inhalation of capsaicin solutions in stepwise increasing concentrations were used. The patients reacted with coughing in a dose-dependent way. The number of coughs was significantly greater than in healthy controls and in a group of patients with verified bronchial asthma.

The latter two groups did not differ significantly. It is concluded that a capsaicin provocation test may be a valuable method for showing not only a higher cough sensitivity, but also for asthma-like symptoms. To date, no such method has been available. The pathophysiology underlying the symptoms may be related to increased sensitivity of free, overacting nerve endings in the respiratory mucosa. We therefore suggest that this overreaction in the lower airways should be called sensory hyperreactivity.

In another study 11 patients with sensory hyperreactivity were provoked with perfume in a single-blind, placebo-controlled randomized order either by the airways or by exposure of the eyes. During the inhalation of perfume, the eyes were carefully covered and during the eye provocations, the patients breathed fresh air. A special face-mask or a nasal clamp was used to exclude any smell of perfume.

Perfume caused symptoms by airway inhalation and by exposure of the eyes. Dypnoea was the most common symptom and eye provocation caused more symptoms than provocation of the airways.

Our conclusion of this study is that in patients with sensory hyperreactivity, asthma-like and other symptoms may originate not only by inhaling irritants but also after exposure of the eyes. This indicates involvement of the sensory nervous system in the eyes and airways.

141. Influence of the target tissue upon axonal outgrowth and glomerulus formation in rat explant co-cultures of olfactory epithelium and olfactory bulb

Vincent Mittoux, Diane Saucier1, Jean-Louis Andrieu and Liliane Astic

CNRS/UPRESA 5020, UMR 5578, UCB/Lyon1, F-69622 Villeurbanne Cedex, France and [1]Université de Moncton, Campus de Shippagan (NB), Canada

In mammals, the peripheral olfactory system is characterized by its complex epithelio-bulbar connectivity. It has been demonstrated that axons merging from different areas of the neuroepithelium end on a small group of neighbouring glomeruli in the olfactory bulb. It has also been shown that neurons expressing the same odorant receptor type send their axons onto one or only a few specific glomeruli whose location is similar from one animal to another. In addition, data from metabolic studies indicate that axons of olfactory neurons that are sensitive to a given odor tend to converge to the same glomeruli. Such a complex organization puts forward the question of the various factors that may favor during embryogenesis, the guidance of olfactory axons converging to a highly circumscribed region in the bulb. One of these factors might be the important process of axon defasciculation occurring in the olfactory nerve layer of the bulb, which may favor both the redistribution of axons and the formation of specific glomeruli. There is an indication that glomeruli could be formed even in absence of direct contact between olfactory axons and target tissue, but the whole epithelium should be required.

In order to identify some of the factors that could be involved in the process of glomerulus formation, we have designed a model of co-cultures of embryonic olfactory epithelium and olfactory bulb explants. Explants of the olfactory epithelium and of various telencephalic areas (olfactory bulb, peribulbar cortex or other cortical regions) were obtained from E15–E17 rat embryos. Tissue fragments were maintained on nitrocellulose confetti in emerged medium. The characterization of cellular components was achieved by immunocytochemical approach using neuronal and glial markers.

Preliminary results show that the olfactory bulb has a positive effect on the rate of axon sprouting. When olfactory bulb fragments were present, ~75% of the epithelial explants sent out axonal processes compared with a level of 50% in the other experimental conditions. Pseudo-glomerular structures were observed exclusively in the co-cultures of epithelium and bulb. Except in one case where a pseudo-glomerulus was observed in the olfactory bulb fragment, the other pseudo-glomeruli had an ectopic localization on the edge of epithelial explants. Data suggest that some diffusible factors coming from the olfactory bulb might somehow favor both the axon growing and the formation of the pseudo-glomeruli, at least by extending the neuron survival.

142. Targeting olfaction

Peter Mombaerts

The Rockefeller University, New York, NY, USA

The initial step in olfactory discrimination involves the interaction of odorous ligands with receptors on the surface of the dendrites of olfactory sensory neurons. A molecular basis for odor recognition was provided by the identification of a large family of genes (1000 in rodents), each of which encodes a seven-transmembrane protein, that are selectively expressed in olfactory sensory neurons. These genes most likely encode odorant receptors. A single olfactory sensory neuron expresses probably only one odorant receptor (OR) gene. Neurons expressing a given OR are scattered throughout one of four zones of the olfactory epithelium, yet their axons converge on a small number of glomeruli in the olfactory bulb. Thus, the bulb is topographically organized, with 1800 glomeruli representing 1000 OR genes and occupying fixed positions. This topographic organization poses a formidable wiring problem: axons of 1000 different populations of olfactory sensory neurons, which are distributed over a wide area of the olfactory epithelium, have to be connected to 1800 fixed glomerular targets in the olfactory bulb. We have developed a genetic approach to visualize individual axons of olfactory sensory neurons as they project from the epithelium to the bulb. This approach is based on gene targeting in mouse embryonic stem cells and exploits the histochemical axonal marker tau-lacZ. We have introduced into the germline of mice various mutations in an OR gene, termed P2. Our results suggest that the OR receptor itself may be a codeterminant in the guidance process that establishes the precise connections between the epithelium and the bulb. More recently, we have generated several strains of mice in which olfactory sensory neurons expressing a given OR also express a fusion of tau with the green fluorescent protein (GFP). We are now able to visualize neurons expressing a given receptor without having to kill them. This ability to examine live neurons opens new lines of investigation, such as isolation of nucleic acids from neurons, observing axons growing to their targets in the bulb in time-lapse microscopy, and electrophysiological recordings from neurons expressing the same OR.

143. Odours borne by mouse major urinary proteins act as male cues

Carla Mucignat-Caretta and Antonio Caretta[1]

Institute of Human Physiology, University of Padova, Via Marzolo 3, I-35131 Padova and [1]Institute of Human Physiology, University of Parma, Italy. e-mail: mucignat@ux1.unipd.it

Male mouse urine contains different androgen-dependent molecules, that can be used to convey informations about sex and social status of the emitter. The two most prominent volatile molecules are 3,4-dehydro-*exo*-brevicomin and 2-*sec*-butyl-4,5-dihydrothiazole. They are found in urine bound to the major urinary proteins (MUPs) that are produced under androgen control. MUPs share with other lipocalins the capacity of binding odorant molecules in their central hydrophobic cavity, and also have a role in chemical communication. MUPs-borne natural ligands can modulate the behaviour of mice in the same way as the whole adult male urine; they are avoided by adult males and are preferred by adult females. On the other hand, MUPs but not their ligands are involved in the acceleration of puberty onset.

We tested whether the chemical cues contained in adult male urine, and in particular MUPs-borne volatiles, induce the same behavioural modifications under different environmental conditions, by presenting the test substances in a box with a dark chamber and a brightly illuminated one.

In the control condition, with no chemical stimuli in the box, mice display a strong preference for the dark environment, by showing a short latency in moving from the illuminated towards the dark side and a very long latency in moving in the opposite way. When male urine is present in the illuminated chamber, adult male mice display a reduced latency in moving towards the illuminated chamber. This result is obtained also when male urine is substituted by purified MUPs, bearing the natural ligands. Since mice cannot contact the test substances before reaching the illuminated chamber, we conclude that only airborne molecules, noticeably MUPs ligands, can modify mice behaviour. The reduced latency in moving from the dark chamber can tentatively be related to the countermarking behaviour.

Data obtained from female mice, tested under the same conditions as males, are in agreement with the idea that MUPs ligands act as male cues.

144. Microdiversity of odorant-binding proteins from the sheep respiratory mucosa

Patricia Nagnan-Le Meillour, David Delanoë, Benoist Schaal[1] and Frédéric Lévy[1]

INRA, Unité de Phytopharmacie et des Médiateurs Chimiques, Route de Saint-Cyr, F-78026 Versailles Cedex and [1]INRA, Laboratoire de Comportement Animal, URA CNRS/INRA, F-37380 Nouzilly, France

In the ewe, attraction towards amniotic fluids is the first step in the development of the mother–young bond. This behaviour is mediated by still unidentified olfactory cues coming from the amniotic fluid.

According to immunoreactivity to specific antisera, N-terminal sequences and odour binding capacities, two distinct classes of odorant binding proteins were characterized in extracts of the ewe respiratory mucosa.

One protein cross-reacts with the anti-bovine OBP serum (provided by Prof. Pelosi, University of Pisa, Italy) and binds pyrazine, but also insect pheromone components such as Z11–16:Ac and Z11–16:OH. This protein possesses in its N-terminus the typical lipocalin motif G-X-W. These data are in accordance with the structural data obtained on the bovine OBP which allow a broad spectrum of binding and a weak specificity towards the ligands for this class of protein.

Several more acidic proteins in native-PAGE share poor N-terminal sequence similarities with the vertebrate OBPs (20–30%). However, they cross-react with the antisera raised against the PBP and the GOBP of the moth *Antheraea polyphemus* (provided by Dr Ziegelberger, MPI, Seewiesen, Germany). One of the proteins labelled by the anti-PBP serum shares 30% N-terminal sequence similarity with a new PBP purified from *Mamestra brassicae* (Lepidoptera: Noctuidae). None of these proteins show any binding with the tritiated analogues used in our study. Thus, they seem more selective towards the odorant ligands than the lipocalin described above.

145. Examining the roles of nitric oxide synthase and soluble guanylyl cyclase in the development and function of the olfactory system of *Manduca sexta*

Alan Nighorn, Nicholas J. Gibson, Monika Stengl and John G. Hildebrand

Arizona Research Laboratories Division of Neurobiology, University of Arizona, Tucson, AZ 85721, USA

Nitric oxide stimulation of soluble guanylyl cyclase has been suggested to play a role in mediating olfactory function and development in both insect and mammalian species (Breer and Shepherd, 1993, Trends Neurosci., 16: 5). In insects, this suggestion has been strengthened by the demonstration of a slow rise in cGMP levels in the antennae in response to pheromonal cues (Zeigelberger *et al.*, 1990, J. Neurosci., 10: 1217) and the demonstration of NADPH-diaphorase staining in the antennal lobes (Muller and Hildebrandt, 1995, Eur. J. Neurosci., 7: 2240; Elphick *et al.*, 1995, J. Exp. Biol., 198: 821) and antennae (Stengl and Zintl, 1996, J. Exp. Biol., 199: 1063). To better investigate this we have obtained full-length cDNA clones of nitric oxide synthase (MsNOS) and three isoforms of soluble guanylyl cyclase (MsGCα1, MsGCβ1 and MsGCβ3) from the *Manduca sexta* nervous system. In the adult olfactory system, we find, using Northern blot and in-situ hybridization analyses, that MsGCα1 and MsGCβ1 are enriched in the antennal lobes while MsNOS is enriched in the antennae. This suggests that olfactory receptor neurons from the antennae could express MsNOS in their axons and create NO within the glomeruli to communicate with antennal lobe projection and interneurons. Using NO-stimulated cGMP immunocytochemistry we find that projections neurons are indeed a likely target for NO produced in the antennal lobe.

In the antennae, we find that NOS is highly expressed in ORCs but that MsGCα1 and MsGCβ1 are not. We do find expression of both MsGCα1 and MsGCβ1 in a small number of non-olfactory neurons in each annulus. NO-stimulated cGMP immunohistochemistry confirms this pattern of expression suggesting that NO stimulation of cGMP does not play a role in the cell bodies of the ORCs.

We also investigated the role of this signaling system during development. Using Northern blot analyses we find that MsNOS

is expressed at a high level early in development of both the antennae and antennal lobe. Its highest expression occurs at the time at which the incoming sensory afferents from the antennae are entering the developing antennal lobe and beginning the formation of glomeruli. MsGCα1 and MsGCβ1 levels mirror each other with a slow steady increase in expression level throughout the development of both the antennae and the antennal lobes.

146. Examining the role of cGMP in the olfactory system of *Manduca sexta*

Alan J. Nighorn, Nicholas J. Gibson and John G. Hildebrand

Arizona Research Laboratories Division of Neurobiology, University of Arizona, Tucson, AZ 85721, USA

We are studying the role of the intracellular messenger, cGMP, in the *Manduca sexta* olfactory system by examining the nature, distribution, and biochemical activities of the enzymes that generate cGMP (guanylyl cyclases) and break it down (phosphodiesterases). Guanylyl cyclases are generally considered to be either, soluble, and activated by nitric oxide, or receptor, and activated by ligand binding. We have obtained full-length cDNA clones of six different *M. sexta* guanylyl cyclases including two soluble GCs, two receptor GCs and two that appear to have novel, intermediate properties. Northern blot analysis shows that all of these clones are highly expressed in the nervous system and are present in the adult or developing olfactory system of *M. sexta*. To get a clearer picture of cGMP regulation we have also cloned nitric oxide synthase and a phosphodiesterase that are expressed in the olfactory system.

Examining the expression patterns of these clones using in-situ hybridization and immunohistochemistry has provided us with some insights into the function of these various genes. In the antennae, we find that the NO-sensitive sGC is not expressed in ORCs but NOS message is present at high levels. This suggests that one of the other types of guanylyl cyclase mediate increases in cGMP that are known to occur in ORCs in response to odorants. In the antennal lobe, we find that projection neurons express high levels of NO-sensitive guanylyl cyclases while an NO-insensitive cyclase is present in the interneurons. A close examination of all of these genes will help to determine the function of this important intracellular messenger both during development and the adult functioning of the *M. sexta* olfactory system.

147. Analysis of gene expression in individual olfactory neurons

Johannes Noe, Jürgen Krieger, Mario Mezler and Heinz Breer

University of Stuttgart-Hohenheim, Institute of Physiology, D-70593 Stuttgart, Germany

Unravelling the molecular basis for the distinct chemospecificity of sensory cells is of fundamental importance not only for understanding the molecular principles of odor discrimination but also for exploring the mechanisms controlling the differential expression of olfactory specific genes. This problem has been approached by analysing individual olfactory sensory neurons combining analytical techniques, such as calcium imaging, single cell RT-PCR and *in situ* hybridization. The responsiveness of isolated olfactory sensory cells to stimulation with distinct odorants was monitored using calcium imaging techniques. Functionally characterized olfactory sensory neurons, i.e. cells responding selectively to certain odors, were subsequently analysed for differential gene expression using single cell RT-PCR approaches. This includes the isolation of mRNA from identified cells, the reverse transcription (RT) of RNA and an amplification of the cDNA, representing the complete RNA-spectrum, by a polymerase chain reaction (PCR). The resulting cDNA from a single cell was subsequently analysed for distinct transcripts by employing specific primers and PCR-approaches. Elements which may play an important role in olfactory signal transduction were preferentially analysed. Using several subtype specific primer pairs led to the identification of G_{olf} and G_o in several individual cells; in contrast G_q and G_i were not found. In addition, adenylylcyclase III, the main enzyme of the cAMP-pathway, was also detected in the cDNAs from various individual receptor neurons. For some cells coexpression of G_{olf} and adenylylcyclase III could be documented. For the amplification of olfactory receptor fragments degenerated primers targetting conserved regions within the transmembrane domain III and VII were employed. PCR products of the expected length were obtained from several isolated olfactory neurons. Analysis of the resulting clones support the notion that olfactory neurons express only one or a small number of receptor types.

148. Variations in sexual activity, electrophysiological responses and muscalure quantities between different strains of house flies

Nico Noorman, Cornelis J. Den Otter and Giovanni Biancaniello[1]

Group Sensory Organs and Behaviour, Department of Animal Physiology, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands and [1]Università degli Studi del Molise, Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Via F. Sanctis Loc. Vazzieri, 86100 Campobasso, Italy

A laboratory WHO Ij2 strain and two wild type strains from, respectively, a chicken farm (Van Diermen strain), and a cattle and pig farm (Pesse strain) in The Netherlands were investigated for sexual activity of males, quantity of muscalure present on the skin of females, and EAG responses to muscalure of both males and females. It is known from experiments on flies from laboratory strains that muscalure, (*Z*)-9-tricosene, present on the cuticle of females is important for inducing courtship behaviour in males, females with more muscalure being more sexually attractive to males. We found that females of the WHO laboratory strain have about twice as much muscalure on their body as females of the Pesse strain and ~10 times that of the Van Diermen strain. In accordance with this, males of all three strains were more attracted to WHO females than to females of the wild strains. Nevertheless, males of both wild strains are less attracted to WHO females than WHO males. This indicates that other factors may also play a role in inducing sexual behaviour in male flies.

EAGs recorded on stimulation with different concentrations of muscalure from males and females of both the WHO and wild type strains did not show differences in the ability to perceive muscalure between males and females and between different strains. The fact that EAG amplitudes did not differ between males and females suggests that muscalure may also affect female behaviour.

149. BDNF and other neurotrophic factors in taste bud development and innervation in rodents and humans

Christopher A. Nosrat

Department of Neuroscience, Karolinska Institute, S-171 77, Stockholm, Sweden

In embryonic rats, the timing of arrival of gustatory (e.g. chorda tympani) and somatosensory (e.g. trigeminal) nerves is matched and preceded by BDNF and NT3 mRNA expression respectively. BDNF mRNA is expressed in developing gustatory epithelium, NT3 mRNA in surrounding epithelium. This embryonic neurotrophin mRNA expression is not nerve-dependent and seems to be an inherent programming of the lingual epithelium based on organ culture studies.

Precedence of innervation by neurotrophin mRNA expression is an example of prespecialization of lingual epithelium based on the type of neurotrophin expressed. Neurotrophin mRNA expression persists into adulthood, BDNF mRNA in taste buds and NT3 mRNA in somatosensory-related areas. The same pattern of neurotrophin expression is also seen in humans. The broader and somewhat overlapping expression patterns of BDNF and NT3 mRNAs in humans suggests additional and possibly somewhat overlapping roles for BDNF and NT3 in the human tongue and indicates possible differences between species.

Conventional gene-knockouts allow studies of consequences of development in the absence of a given protein. BDNF knockouts show clear anatomical, histological and physiological deficits in their gustatory system. With NT3 knockouts, on the other hand, the gustatory apparatus appears intact while there is severe loss of lingual somatosensory innervation. Data from BDNF-overexpressing transgenic mice will also be discussed.

These novel findings may have clinical implications in rare human conditions such as familial dysautonomia and/or in more common cases of problems with loss of taste and sensation in the mouth such as those seen after injury to the nerves, either by accident or following oral/facial surgery. This knowledge might also become helpful in stimulating regeneration of injured nerves in patients.

Supported by the Dental Faculty at the Karolinska Institute and the Swedish Dental Association and conducted in collaboration with Lars Olson, Charlotte Mistretta, Patrik Ernfors and Carlos Ibáñez.

150. Investigation of the human taste from a viewpoint of the buffer action of saliva

Ichiro, Ohsawa

Institute of Psychosomatic Disease, Dental Research Centre, Nihon University School of Dentistry; Department of Oral Diagnosis, Nihon University Dental Hospital

It is well known that there is a close relationship between human taste and saliva. I have reported the investigation of the relation to saliva volume, pH, ion concentration in saliva, viscosity and index of refraction of saliva in a previous congress. From a viewpoint of saliva pH and the concentration of some ions, I supposed that human taste was affected by the buffer action of saliva.

For the measurement of the buffer action, it was enough that measured continuously the changing of saliva's pH under continuous application of an acid or base. Buffer capacity curves (–pH curves) were formulated for this purpose, because human saliva is a complex substance of weak electrolyte. –pH curves have the advantage that the distribution pattern of weak bases and acids can show that saliva has a buffering action; the definition was $x = B/\mathrm{pH}$ (where B is the concentration of a dropped strong alkali solution).

–pH curves showed the real variability of the buffering action of a solution at each pH. The plot of the solution as a function of pH is called the buffer action curve.

Subjects were 11 male students (23–25 years old) who understood and accepted the purpose of the experiments. Ten millilitres of mixed saliva was collected from these subjects who held a piece of paraffin wax in their mouths. Before the determination of the buffering action, the saliva was adjusted to pH 2 by the addition of 1 N HCl. –pH curves were recorded by dropping 0.5 N potassium hydroxide from a capillary at a rate of 1–2 µl/s into the stirred sample. Henderson–Hasselbalch's formula, $\mathrm{pH} = \mathrm{p}K_a + \log([\mathrm{base}]/[\mathrm{acid}]) = \mathrm{p}K_a + \log\{/(1-)\}$, was fundamental to the experiment.

The –pH curveand the results of the gustatory tests were compared. The results showed that there was the difference from pH 9 to 11 by the gustatory test. This pH is close to the$\mathrm{p}K_a$ values of aliphatic amines, phenolic acid and purine. I reached the conclusion that there were a close relationship between human taste and aliphatic amines, phenolic acid and purine in saliva.

151. A comparison of gender differences in brain olfactory responses using steady state visual evoked potential (SSVEP) topography

Caroline M. Owen[1,2,3], John Patterson[1,3] and Richard B. Silberstein[2]

[1]School of Biophysical Sciences and Electrical Engineering, [2]Brain Sciences Institute, Swinburne University of Technology and [3]CRC for International Food Manufacture and Packaging Science. e-mail: cowen@mind.scan.swin.edu.au

Psychophysiological and neurophysiological changes in response to undetected and low concentration odour stimuli and the variability of olfactory sensitivity responses associated with gender differences have been reported using electrophysiological techniques (Lorig *et al.*, 1993, Chem. Senses, 18: 379–387; Evans *et al.*, 1995, Electroencephalogr. Clin. Neurophysiol., 95: 293–301). In the current study, a visual probe stimulus was used during electrophysiological recording of steady state visual evoked potentials (SSVEP) and their modification during the presence of an odour administered during normal breathing. Gender differences in SSVEP topography were examined. During inspiration, 1 ml of either air or odour was delivered into a facemask, resulting in a series of air injections and a series of odour injections. Two groups of subjects (10 males, 10 females, right-handed, non-smoking, no obvious respiratory problems, mean age 25 years) attended two recording sessions during which either a low or high concentration of *n*-butanol (1 or 2.4 ppm in mask) was delivered. Behavioural detection responses ('yes' or 'no') were recorded during expiration. Electrophysiological data obtained from a 64-electrode helmet were analysed for all odour injections and all air injections, and topographic maps were produced. Following analysis of the behavioural detection responses, the subjects were assigned to either 'yes' or 'no' response subgroups, resulting in 11

'yes' responders (6 males, 5 females; ε 53% accurate detection; range 53–87%) and 9 'no' responders (4 males, 5 females; δ 6% detection; 5 subjects never detected the odour). Comparisons of the SSVEP results for the gender-response groups at both concentrations indicated differences in regional brain activation related to the ability to subjectively detect the odour or not, with activation changes associated with increased odour concentration. Although threshold testing demonstrated no significant difference between gender groups, there were group differences in SSVEP topography in response to concentrations of the *n*-butanol stimulus. These results are suggestive of differences in neural processing associated with the conscious perception of an odour stimulus, with concentration effects, and with gender differences, and indicate the potential for the technique to monitor objective responses and provide quantitative measures of olfactory experience.

152. Structural studies on pig odorant-binding protein-I by fluorescence spectroscopy

Sara Paolini, Carlo Fini and Paolo Pelosi[1]

Dipartimento di Biologia Cellulare e Molecolare, University of Perugia and [1]Dipartimento di Chimica e Biotecnologie Agrarie, University of Pisa, Italy

Odorant-binding proteins (OBPs), small soluble proteins highly expressed in the nasal mucus of vertebrates, are believed to mediate the perception of odors. Although several members of this class of proteins have been isolated and characterized, their role in the odor transduction mechanism is still unknown.

In order to obtain further information on the structure of these proteins, we investigated on OBP-I from pig nasal mucosa by:

- Intrinsic fluorescence spectroscopy. Positions of maximum emission wavelength (λ_{max}) shifts of the single tryptophan were monitored under different conditions: in the presence and in the absence of odorants, as a function of temperature and in the presence of quenchers.
- Extrinsic fluorescence spectroscopy. The fluorophore 1-aminoanthracene was introduced into OBP-I by simple binding and used as a reporter.

At 20°C, upon excitation at 295 nm, the λ_{max} of the OBP-I tryptophan is 337 nm and it did not change in the presence of good ligands, such as 3,7-dimethyl-1-octanol (DMO) and 2-isobutyl-3-methoxypyrazine (PYR). The binding of these odorants shifted the conformational change of the protein from 65 to 70°C.

In order to determine whether the tryptophan residue is localized on the surface of the protein or inside an internal cavity, fluorescence spectra were recorded in the presence of anionic (NaI), cationic (CsCl) and neutral (acrylamide) quenchers. At 20°C, we did not observe any significant quenching of tryptophan fluorescence intensity both in the absence and in the presence of the odorants.

The binding cavity of OBP-I was probed by the extrinsic fluor 1-aminoanthracene. When 2 μM free 1-aminoanthracene was excited at 256 nm, its λ_{max} was observed at 545 nm, with a fluorescence intensity of 25 arbitrary units. Protein addition shifted λ_{max} to 480 nm and fluorescence intensity increased to 750 arbitrary units. The excitation of free 1-aminoanthracene at 295 nm resulted in a very weak fluorescence emission with a λ_{max} of 537 nm. In the presence of OBP-I, the λ_{max} was shifted to 480 nm and the fluorescence intensity increased ~80 times. Similarly to DMO and PYR, the binding of this fluorophore did not affect both the λ_{max} and the fluorescence intensity of the protein tryptophan.

Competitive binding was assayed by determining fluorescence of mixtures containing fixed amonuts of OBP-I and 1-aminoanthracene in the presence of increasing amounts of the free ligands DMO, PYR and phenylethanol (PHE).

We conclude that:

- the binding of odorants to OBP-I does not affect the tryptophan environment up to 60°C and protects the protein structure against thermal denaturation.
- tryptophan is not involved in the binding of the odors;
- 1-aminoanthracene can be used as an extrinsic fluorescent probe to investigate the interactions between OBP-I and odorant molecules.

153. Can the solution behaviour of binary solute mixtures in water explain their taste characteristics?

Sneha A. Parke and Gordon G. Birch

Department of Food Science and Technology, University of Reading, Whiteknights, PO Box 226, Reading RG6 6AP, UK

This study attempts to explain the taste of binary mixtures of solutes in water in terms of their hydration behaviour. Hydrodynamic solution parameters such as apparent specific volumes and isentropic apparent compressibilities have been shown to reveal information about the state of hydration of solutes in an aqueous medium. This information can then be interpreted, in conjunction with the nature of the solution components, to explain the ease of accession of the sapid molecules to the appropriate receptor sites. Binary mixtures of solutes are very complex systems, yet solution data do provide relevant information as they would do for solutions of single solutes in water.

Different concentrations of three binary mixtures of a bulk sweetener (sucrose) and one intense sweetener (acesulfame K, aspartame and sodium cyclamate) in water have been studied, whose taste properties have previously been reported. Two other experiments have been carried out to substantiate the results of the above, by varying one sweetener and keeping the other constant. Apparent specific volumes give us information about the packing efficiency of the molecule in water. Isentropic compressibilities, however, illuminate smaller, yet important, differences which can be explained in terms of the solute–solute, solute–solvent or solvent–solvent interactions.

The sweetness synergy experienced for sucrose–sodium cyclamate mixtures, for instance, can be explained in terms of the collapsing effect of sodium cyclamate molecules on the other components of the solution, detectable by isentropic compressibility data. Sucrose–aspartame mixtures have extremely poor packing characteristics and show suppression. Sucrose–acesulfame K mixtures, which show additivity of sweetness, have intermediate values between the other two mixtures.

The especially well-marked synergy in sucrose–sodium cyclamate mixtures is both quantitative and qualitative. The latter

probably originates in the apparent specific volume of sodium cyclamate, which is identical to that of sucrose (0.61 cm^3/g). The former results from the markedly low apparent specific compressibility of sodium cyclamate (-4.3×10^{-5} $cm^3/g.bar$), which enhances receptor recruitment.

Supported by the European Community (EC-AIR PL-94-2107).

154. Evidence for chemorepulsive control of primary olfactory axon regeneration

R. Jeroen Pasterkamp, Fred De Winter, Anthony J.D.G. Holtmaat and Joost Verhaagen

Graduate School for Neurosciences, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

The stability and plasticity of neuronal networks is dependent on the relative balance between attractive and repulsive molecules. Most attention has so far focussed on cell adhesion molecules that promote axon extension and cell–cell recognition. We now show that the secreted chemorepulsive protein semaphorin III (sema III) and one of its receptors, neuropilin-1, display a coordinated pattern of expression in the intact adult and regenerating primary olfactory system of the rat suggesting a role for chemorepulsive signalling in the maintainance and regeneration of the primary olfactory pathway. In the intact adult olfactory epithelium neuropilin-1 mRNA and CRMP-2 (collapsin response mediator protein-2, an intrinsic protein involved intracellular semaphorin signalling) mRNA were expressed in immature (B-50/GAP-43 positive) and a subpopulation of mature (OMP positive) olfactory neurons. In the intact olfactory bulb primary olfactory axons expressing neuropilin-1 are present in close association with sema III-positive pial cells covering the cribriform plate and with sema III-positive second-order neurons in the bulb. Thus, sema III secreted from cells at the cribriform plate may instruct growing primary olfactory axons to enter the nerve layer of the olfactory bulb. As these fibers arrive into the glomeruli, sema III derived from target mitral, tufted and periglomerular cells may instruct them to cease growing and form synaptic contacts in their projection territory.

Unilateral bulbectomy and axotomy of the primary olfactory nerve resulted in the formation of new olfactory receptor neurons with increased neuropilin-1 and CRMP-2 mRNA expression. Bulbectomy resulted in the formation of a cellular scar in the bulbar cavity containing numerous sema III-expressing fibroblast-like cells. These sema III-positive cells are likely to be derived from the sema III-positive pia. At later post lesion timepoints (30 and 60 days after bulbectomy) the sema III-positive cells encapsulated bundles of regenerating neuropilin-1 positive primary olfactory fibers. Following axotomy (allowing regeneration and target reinnervation) regenerating olfactory fibers were meandering between strings of sema III-expressing cells. However, following axotomy the appearance of sema III-positive fibroblast-like cells at the lesion site was transient and the sema III-positive cells did not encapsulate regenerating primary olfactory axons. The differential arrangement of sema III-positive non-neuronal cells and their relation to regenerating olfactory fibers following bulbectomy, as compared with axotomy, could represent a new chemorepulsive mechanism underlying the failure of long distance axonal regeneration into the central nervous system.

155. Olfaction and affective state: is odor evaluation and perception altered in patients with major depression?

Bettina M. Pause, Alejandra Miranda, Ninja Raack, Bernfried Sojka, Robert Göder[1], Josef B. Aldenhoff[1] and Roman Ferstl

Institute of Psychology and [1]Institute of Psychiatry and Psychotherapy, University of Kiel, Germany

Some studies indicate that olfactory perception is altered in patients with affective disorders. Whereas a decreased olfactory sensitivity has been observed for patients with major depression (MD), an enlarged sensitivity has been found in patients after successful therapy and might thus be a trait marker. Considering the responsible mechanisms for this phenomenon the close link between olfactory and affective stimulus processing should be focused. The aim of the present study was to investigate olfactory and affective perception in patients with MD by means of threshold tests, evaluative ratings and ERP (event-related potential) analysis.

Two studies were conducted. In study I 20 patients with MD were compared with control subjects, matched by age and sex. All subjects were tested twice, the patients in the beginning and end of their therapy and the control subjects in a respective interval. Olfactory sensitivity was assessed for eugenol and phenylethyl-alcohol. Moreover, 10 odors had to be evaluated on an 18-color scale and their hedonic values to be rated according to the 'Self-Assessment-Manikin' (SAM). To control if the affective judgements were odor-specific, slides from the 'International Affective Picture System' (IAPS) were presented. In study II patients with MD and control subjects attended two EEG sessions, which included three different tasks. During the first part two odors (isobutyraldehyde and phenylethylalcohol) were administered within a constant flow olfactometer. In the second part two colors (yellow and red) and thereafter IAPS slides were presented by a slide projector. The subjects' task was to discriminate the stimuli either by their quality (odors and colors) or by their valence (IAPS slides). The EEG was recorded from 32 scalp locations.

First results show that the olfactory sensitivity was decreased during depressive episodes. Positive odors and slides activated patients less and negative odors and slides activated patients more than controls (SAM ratings). In the EEG study the processing speed for phenylethylalcohol was smaller in patients and during a first stimulus encoding phase (N1 lantency window) the voltage scalp distribution in patients was altered. Colors and emotional slides evoked smaller late positivities in patients than in control subjects. Further results considering the second measurement (after a successful treatment) will be discussed.

The study was supported by a DFG grant (Fe151/12-1).

156. Is olfactory performance related to the degree of state or trait emotionality?

Bettina M. Pause, Margrete Nysterud and Roman Ferstl

Institute of Psychology, University of Kiel, Germany

Studies comparing personality styles and olfactory performance have so far mainly focused on the introversion/extraversion dimension and did not reveal consistent results. However, recent data indicate that the degree of emotionality (neuroticism) might be a better predictor for olfactory performance than introversion

(Pause *et al.*, 1998, J. Res. Personal., in press). According to Eysenck a higher degree of emotionality is supposed to be associated with a higher activation of the limbic system. As the anatomical areas involved in emotional and odor processing partly overlap this higher activation could also explain a better olfactory performance. This theory found recent evidence by PET studies measuring odor and emotional stimulus processing. The aim of the present study was to investigate whether the degree of emotionality (trait or state) predicts olfactory sensitivity and the hedonics of odors.

Thirty subjects (15 female) were tested twice in a 2 month interval. The second measurement was carried out to assess the stability of the trait tests for emotionality. Olfactory sensitivity was examined for eugenol and phenylethylalcohol (two-alternative staircase detection threshold measurement). Moreover, 10 odors had to be evaluated on an 18-color scale and their hedonic values to be rated according to the 'Self-Assessment-Manikin' (SAM). Trait emotionality was determined by two personality inventories (Eysenck Personality Inventory, NEO Five Factors Inventory). Additionally, the Bem Sex Role Inventory was used to determine the degree of femininity and masculinity. State emotionality was measured by self descriptions (SAM) evoked through emotional pictures ('International Affective Picture System', IAPS). Subjects who reacted most differently to positive and negative slides were considered to be emotionally sensitive.

First results show that state emotionality might be a better predictor for olfactory sensitivity than trait emotionality. Subjects who described themselves as strongly activated by arousing slides did show a better olfactory sensitivity than subjects being less activated. The same subjects also showed more extreme hedonic ratings and tended to describe the odors as more intense. Further results considering the reliability of the personality inventories will be discussed.

157. Regulation of expression of SCG10 and stathmin proteins in the rat olfactory system during development and neuronal regeneration

Véronique Pellier-Monnin, Liliane Astic and Gabrielle Grenningloh[1]

CNRS/UPRESA 5020, UCB/Lyon1, Villeurbanne, France and [1]Institut de Biologie cellulaire et Morphologie, Lausanne, Switzerland

The SCG10 protein (*superior cervical ganglion*) belongs to the same gene family as stathmin. Stathmin is an ubiquitous protein whose phosphorylation is correlated with the action of various extracellular stimuli regulating cell proliferation and differentiation. While stathmin is expressed in a variety of cell types and tissues, SCG10 is only detected in neurons. The expression of both proteins is actively regulated during brain development and reaches a peak around birth. In the brain of adult animals, SCG10 and stathmin continue to be expressed in regions of synaptic plasticity. In PC12 cells, stathmin is required for neuronal differentiation in response to nerve growth factor whereas SCG10 overexpression favours the neurite outgrowth. The purpose of our study was to analyze in detail the role of SCG10 and stathmin in neurite outgrowth in *in vivo* conditions. By using immunocytochemistry, we have studied the spatio-temporal patterns of SCG10 and stathmin expression in the rat olfactory system during development. We have also verified if SCG10 is re-expressed during the process of axonal regeneration following unilateral bulbectomy in adult rats.

From E13–E14 to birth, a gradual increase in the number of strongly SCG10-positive neuroreceptors was observed in the olfactory epithelium. From P7, the SCG10 staining intensity of neurons was seen decreasing and no evident immunoreactivity was detected in the olfactory epithelium of adult rats. By contrast, the axonal processes exhibited a strong SCG10-immunoreactivity from E13 up to the adult stage. In the olfactory bulb of developing and adult rats, the olfactory nerve layer appeared strongly SCG10-labeled while no evident staining was detected in the glomeruli. The pattern of stathmin expression appeared relatively similar to that of SCG10 during embryogenesis and in early postnatal stages. Nevertheless, basal cells and immature neurons still expressed stathmin in adult rats. Otherwise, data show an upregulation of SCG10 expression in the olfactory epithelium during the process of axonal regeneration following unilateral bulbectomy in adults.

Our data clearly show that the expression of both SCG10 and stathmin proteins is developmentally regulated in the olfactory epithelium. The observation of a strong expression of SCG10 by the growing olfactory axons of newly formed neurons during development and following bulbectomy suggests that this protein plays a role in axonal outgrowth. This role might be directly linked to the function of SCG10 as key regulator of neurite extension through regulation of microtubule instability.

158. Expression of carnosine and tangential migration in the adult rat forebrain

P. Peretto, L. Bonfanti and A. Fasolo[1]

Dip. di Morfofisiologia Veterinaria and [1]Biologia Animale, Università di Torino, Italy

Introduction. Carnosine (β-alanyl-L-histidine) is an aminoacyl-histidine dipeptide present in the nervous system of adult mammals (Biffo *et al.*, 1990). Its neuronal localization is restricted to the olfactory receptor neurons, which undergo cell renewal during adulthood. Within the brain, carnosine is abundant in astrocytes and ependymal cells. We have recently shown that carnosine is also expressed in the subependymal layer (SEL) of adult rodents (Bonfanti *et al.*, 1995, Soc. Neurosci. Abstr., 1527), a site of persisting cell proliferation and migration in which the newly generated cells undergo tangential migration within astrocytic glial tubes (Peretto *et al.*, 1997, Brain Res. Bull., 42: 2). In the present study we investigated in greater detail the immunocytochemical distribution of carnosine in this area of the postnatal and adult forebrain.

Materials and methods. Brains were obtained from 16 young (P 2, 5, 9, 13, 17, 21, 25, 30) and six adult Wistar rats. Animals were anesthetized with sodium pentobarbital and perfused intracardially with 4% paraformaldehyde. Cryostat sections were treated immunocytochemically, in single and double labelling, with polyclonal and monoclonal antibodies raised against the dipeptide carnosine, the cytoskeletal proteins nestin and glial fibrillary acidic protein (GFAP), the cytosolic phosphoprotein stathmin and the highly sialylated form of the neural cell adhesion molecule (PSA-NCAM).

Results and discussion. The immunoreactivity for carnosine was associated to small, tightly packed cells, localized in the SEL of the lateral ventricle and its rostral extension toward the olfactory bulb.

These cells showed a cytoplasmic and, to a lesser extent, nuclear reaction. In GFAP/carnosine double labellings, carnosine-positive cells filled the compartments delimited by the glial tubes, whereas an overlapping of the two antigens was observed in the glial meshwork forming the tubes. In the olfactory bulb, an accumulation of carnosine-positive cells was also present in the SEL, although prevalently associated to the astrocytic cells. In carnosine/PSA-NCAM double labellings, a partial overlapping was detected all along the SEL, whereas in the olfactory bulb the coexistence of these antigens was not detectable outside the SEL. At this level, wherein PSA-NCAM is known to be expressed by radially oriented neuroblasts, carnosine was restricted to stellate, glial cells. Although carnosine immunoreactivity was consistently detected in the olfactory neurons since P2, in the forebrain it was firstly detected at P13–P17, as a faint reaction in some glial cells. At P21 virtually all the SEL area was filled by a great number of carnosine-positive cells, making difficult to distinguish between glial and migrating cells. Starting from P25, the distribution of carnosine-LI in the SEL was overlapping to that described in the adult. These results show that carnosine is associated to the tangentially oriented part of the migration pathway occurring within the SEL, being present both in chains of migrating cells and in the astrocytic glial tubes in which they are contained.

Supported by grants of Italian CNR and MURST.

159. Transregulation of erb-B/neuregulin expression in the olfactory bulb

I. Perroteau, M. Oberto, I. Soncin, P. Bovolin and A. Fasolo

Department Human and Animal Biology, University of Turin, Italy. e-mail: perroteau@dm.unito.it

We have shown that erbB4 localizes to the periglomerular and mitral/tufted cells of the mouse olfactory bulb (OB) and to the migratory stream of the subependimal layer, while erbB3 immunoreactivity is restricted to the ensheathing cells of the olfactory nerve, where the presence of neuregulins and erbB2 has already been demonstrated (Salehi-Ashtiani and Farbman, 1996). In order to study the response of erbB/neuregulin to the perturbation of the functional peripheral input, we deafferented the OB by intranasal irrigation with $ZnSO_4$. Upon total deafferentation, the overall erbB3 expression in the nerve layer is less abundant than control, reflecting the desappearence of many ensheathing cells following olfactory axon degeneration. ErbB4 expression decreases as well, but only in the cell types that normally make synaptic contacts with primary olfactory neurons in the glomeruli, i.e. periglomerular and mitral/tufted cells. In order to understand whether erbB4 down regulation arises at transcriptional level, we subjected OB RNA extracts, prepared at day 0, 2, 7, 14 and 28 after reversible $ZnSO_4$ lesion, to semi-quantitative RT-PCR analysis. We took advantage of the presence of OMP mRNA in olfactory receptor neuron terminals to follow axonal degeneration and regeneration. We show a minimum level of OMP and tyrosine hydroxylase (TH) mRNA expression at day 14 post-lesion followed by a return to control values by day 28. As for TH, erbB4 expression is shown to be reversibly down regulated at transcriptional level. However, the erbB4-mRNA level is minimum at day 7 post-lesion and has already returned to the control value at day 14. At this point, we have examined neuregulin expression and analyzed its time course after lesion. We show that neuregulin β_1, and to a minor extent, α_2 and β_2, are transcribed in the OB and the time course of neuregulins after $ZnSO_4$ lesion follow strictly that of erbB4, suggesting that erbB4 expression may rely on neuregulin stimulation.

Supported by Cavalieri Ottolenghi Foundation and CNR.

160. Odour measurement using sensor arrays

Krishna C. Persaud

Department of Instrumentation and Analytical Science, UMIST, Manchester M60 1QD, UK

The development of an electronic multi-sensor system that could start to mimic some aspects of the biological olfaction system is currently a research objective of many laboratories. Such a system has been given the term 'electronic nose'. It can be divided into a number of components:

- an array of chemical sensors—possessing broad specificity;
- feature extraction—extraction of salient data for further analysis;
- pattern recognition—identification of sample or discrimination from other samples.

This paper reviews current developments in sensor technologies, electronics and software that will ultimately produce powerful 'smell' recognition systems.

Current research on 'electronic noses' is progressing on two fronts: the identification of gas sensitive materials and their development, and the mathematical extraction of information from an array of such sensors. The wide range of possible sample matrices and chemical cross-sensitivities mean that the development of sensors highly specific to individual chemicals is not straightforward; however, the combination of an array of broadly specific sensors and appropriate software could be used for the identification of volatile chemicals. The sensor technologies that are emerging include metal oxide semiconductors, conducting polymers, quartz crystal microbalances, surface acoustic wave devices, optical devices and various combinations of these technologies. Each of these technologies will be considered with examples of practical applications as well as limitations. Software techniques and material science are important aspects of the development of the system. Advancement in software signal processing techniques, coupled with pattern recognition, enable optimum usage of sensor responses. The use of neural network algorithms will be discussed.

161. Ca^{2+} blockage and permeation in the olfactory cyclic nucleotide-gated channel are controlled by a glutamate residue

Cristiana Picco, Paola Gavazzo, Elisabeth Eismann[1], U. Benjamin Kaupp[1] and Anna Menini

Istituto di Cibernetica e Biofisica, CNR, Via de Marini 6, 16149, Genova, Italy and [1]Institut für Biologische Informationsverarbeitung, Forschungszentrum, Julich, Germany

Olfactory transduction in vertebrates involves the activation of an enzymatic cascade in the cilia of olfactory receptor neurons,

producing an increase in cAMP which directly opens cyclic nucleotide-gated ion channels (CNG channels). In physiological conditions the opening of CNG channels causes an inward current carried by Na^+ and Ca^{2+} ions, that generates a membrane depolarization. Ca^{2+} ions permeate and at the same time block the current carried by Na^+ in a voltage-dependent manner. Ion channels directly gated by cyclic nucleotides are also present in photoreceptors and have been cloned from many different species. It has been shown that in the bovine retinal rod channel a glutamate residue, Glu363, is within the putative pore region and controls the blockage of the channel by external divalent cations. In this work we studied the role of the equivalent residue, Glu 340, in the alpha subunit of the bovine olfactory CNG channel.

Point mutations were introduced in the cDNA encoding the alpha subunit of the bovine olfactory CNG channel: Glu340 was substituted with different non-charged amino acids such as Gln, Asn, Thr, Gly, or with the other negatively charged amino acid Asp. The mRNA of wild type or mutant channels was injected into *Xenopus laevis* oocytes, which, after a few days of incubation, expressed functional olfactory CNG channels on their plasma membrane. Electrophysiological properties were studied with the patch-clamp technique in excised membrane patches. Both macroscopic and single-channel currents activated by cAMP or cGMP were measured in the presence of various Ca^{2+} concentrations. When Glu340 was substituted with non-charged amino acids the current carried by Ca^{2+} ions increased, while the extracellular Ca^{2+} blockage on the Na^+ current was greatly reduced. The conservative mutation E340D did not reduce the Ca^{2+} blockage. Moreover, mutations of the Glu 340 residue did not significantly affect the blockage by internal Ca^{2+}. These results show that Glu340 controls extracellular Ca^{2+} blockage and permeation in the alpha subunit of the bovine olfactory CNG channel.

Supported by EC grant BIO4-CT96-0593.

162. Body odour monitoring: a non-invasive method in diagnostic practice

A.M. Pisanelli, K.C. Persaud, D.H. Robertson[1] and R.J. Beynon[1]

DIAS, UMIST and [1]Department Biomolecular Sciences, UMIST, PO Box 88, Manchester M60 1QD, UK

Body odour can be a factor in human communication and can provide an important source of information involving body processes. The odours are secreted by sweat glands, faeces, expiration, saliva, breath, skin and sex organs (Inaba and Inaba, 1992, Human Body Odor. Etiology, Treatment and Related Factors, Springer Verlag).

The major physiological contributors to body odours arise from eccrine and apocrine sweat glands. Although the glandular secretions are odourless, the odours can be enhanced by dietary factors, intake of drugs and pathological processes.

Chronic or necrotic wounds, overt or covert infection, liver disease, uremia, diabetes, skin disease, abnormalities of amino acid metabolism, and enzymatic disorders that result in accumulation of metabolites, can promote characteristic body odours (Ruocco and Florio, 1995, Int. J. Dermatol., 34: 92–93).

Analytical methods such as headspace concentration, gas chromatography and a combination of GC/MS have been used to identify the chemical components of the body odour (Pause *et al.*, 1997, Physiol. Behav., 61: 957–961).

Over the last few years an innovative system, able to emulate the olfactory system has been developed (4 Persaud K.C. and Travers P. Multielement array for sensing volatiles chemical. In: Intelligent Instruments and Computers. New York, Elsevier, 147–154 (1991)). This system called an 'electronic nose' can provide on line monitoring of odours in different fields such as fermentation processes (Craven, 1996, Trends Anal. Chem., 15: 486–493), environment monitoring (Persaud *et al.*, 1996, Measure. Control, 29: 17–20) and medical field (Chandiok *et al.*, 1997, J. Clin. Pathol., 50: 790–791).

In this work we present the preliminary results of an investigation on measurement of the body odours in normal and abnormal health condition using an electronic nose, consisting of an array of 32 conducting polymers. Measurements have been carried out on patients with metabolic disorders and compared with a normal population.

This research is supported by the Wellcome Foundation (UK) and is in collaboration with Dr R. Quinvlan Oswestry Hospital, Oswestry (UK) and Dr J. Phoenix, Department of Medicine, University of Liverpool (UK).

163. The effect of number of estimated qualities on the judgement of sweetened yogurt

Ilse A. Polet, Marjon J.M. Theunissen and Jan H.A. Kroeze

Psychological Laboratory, Utrecht University, Utrecht, The Netherlands. e-mail: i.polet@fss.uu.nl

Several researchers have reported the so-called 'dumping effect': when subjects have to rate only one quality of a chemosensory mixture they tend to judge this quality higher than when they have to rate several qualities of the same mixture simultaneously (e.g. Clark and Lawless, 1994, Chem. Senses, 19: 583–594). In the present study this effect is examined for sweetened yogurt in an eating situation. Subjects rated the qualities of the yogurt under three different conditions: (I) five qualities simultaneously (sweetness, sourness, thickness, saltiness and bitterness); (II) three qualities simultaneously (sweetness, sourness and thickness); (III) one quality at a time. The third condition ('one quality') consisted of three subconditions: (IIIa) sweetness; (IIIb) sourness; and (IIIc) thickness. All subjects rated the stimuli under all conditions and thus participated in five sessions.

Two stimuli were used: yogurt with a low sucrose concentration (37.5 g sucrose/1000 g yogurt) and yogurt with a high sucrose concentration (75.0 g sucrose/1000 g yogurt). The yogurt was presented in disposable soup bowls, which contained 250 ml. Subjects were instructed to eat at their own pace and to swallow the yogurt. They rated the qualities of each sample three times: once after the first amount swallowed (t_0), once after 30 s (t_{30}) and once after 90 s (t_{90}). All qualities were rated on a 150 mm line scale with on the left side 'not at all' and on the right side 'very'.

The sweetness increased with increasing sucrose concentration, while the sourness and the thickness decreased. For sweetness adaptation was found over time. There was no effect of number of qualities that had to be estimated on the sweetness, sourness and thickness. This was true for both concentrations of sucrose and for times t_0, t_{30} and t_{90}.

164. Responses to repeated oral irritation by capsaicin, cinnemaldehyde and ethanol in PROP super-tasters and non-tasters

John Prescott and Nicola Swain-Campbell

Sensory Science Research Centre, University of Otago, Dunedin, New Zealand

Studies using capsaicin, a potent oral irritant, have shown that the intensity of oral irritation tends to grow over successive samples, a phenomenon known as sensitization. If a hiatus of 5–15 min is then introduced, the intensity of irritation produced by a subsequent capsaicin stimulus is much reduced and desensitization is said to have occurred. Studies of other irritants such as menthol, nicotine and zingerone have not consistently shown this response pattern, casting doubt on the extent to which sensitization and desensitization are general properties of trigeminal chemoreception. In addition, there is considerable individual variability in response patterns for a given irritant. The present research sought to assess response patterns to multiple irritant samples in the same subjects and to examine potential sources of individual variability. The latter includes responses to the bitter compound 6-*n*-propylthiouracil (PROP), sensitivity to which is genetically determined, producing three population groups: non-tasters, tasters and super-tasters. PROP sensitivity is an index of taste bud density and of trigeminal innervation, with non-tasters perceiving the burn of capsaicin as lower than tasters. Variability due to psychological variables, including beliefs about hot foods, and private body consciousness, a measure of awareness of physiological responses, was also examined. Two groups, non-tasters and super-tasters of PROP, received 11 consecutive solutions of three irritants—capsaicin, cinnemaldehyde and ethanol—each in a separate session. The inter-stimulus interval was 1 min, except for the period between stimuli 10 and 11, which was 11 min. Mean patterns of rated intensity differed between irritants. Responses to capsaicin changed little over the initial 10 stimuli, while those to cinnemaldehyde and ethanol showed decreasing intensity. Following the hiatus (from stimulus 10 to 11), a pronounced decrease in intensity (desensitization) was apparent for capsaicin, whereas cinnemaldehyde showed little change and ethanol showed an increase in intensity, these latter patterns suggestive of recovery from desensitization over the initial 10 stimuli. These results are discussed in terms of the differing temporal characteristics of the individual irritants. PROP super-tasters gave higher intensity ratings than non-tasters to all stimuli but the two groups did not differ in terms of response patterns. The impact of variation in the psychological variables will be discussed.

165. Olfactory bulb dopaminergic neurons: tyrosine hydroxylase expression is mediated by odor-evoked, NMDA receptor-dependent neural activity *in vitro*

A.C. Puche, and M.T. Shipley

Department of Anatomy and Neurobiology, Program in Neuroscience, University of Maryland, Baltimore, MD 21201, USA

The expression of tyrosine hydroxylase (TH) by juxtaglomerular (JG) neurons of the olfactory bulb (OB) requires innervation of the bulb by olfactory receptor neurons (ORNs). Lesion of the olfactory nerve selectively downregulates TH in JG neurons. However, glutamic acid decarboxylase expression in the same neurons is unaffected. In reversible lesions, TH expression is up-regulated as newly formed ORN axons reinnervate the OB. TH expression may be regulated by trophic factor release and/or synaptic activity from ORN axon terminals. To test these hypotheses we investigated TH expression in co-cultures of dissociated postnatal rat OB cells and embryonic olfactory neuroepithelium (OE) slice explants. TH-positive neurons in control dissociated OB cell cultures alone comprise only a small fraction of the total population of cells present in the culture. However, when OE slice explants are co-cultured with dispersed OB cells there is a mean 2.3-fold increase in the number of TH-positive neurons. Pulses of odorants applied to these OE–OB co-cultures increases TH induction by an additional 70% above unexposed OE–OB co-cultures. This model allowed the investigation of the neurotransmitters involved in TH regulation. ORN axons utilize glutamate as an excitatory neurotransmitter. Broad spectrum excitatory amino acid antagonists (kyurenic acid), or selective antagonists of the NMDA receptor (AP5) both prevented the induction of TH expression in OE–OB co-cultures. Antagonists of the AMPA/KA receptors reduce, but do not prevent, TH induction. In addition, stimulation with pulses of the glutamate agonist NMDA induced TH expression in the absence of OE. Dopaminergic neurons in culture also express both the NMDA receptors R1 and R2b. These findings indicate that *in vitro* odor stimulated glutamate release by the ORNs regulates TH expression via NMDA receptors.

Supported by NIH grant NIDCD-00347.

166. What could be the reason of unusually high thermal stability and suppressed binding ability of porcine VEGP?

H. Rabesona, T.V. Burova, H. Kostinen[1], M. Seppala[1], C.K. Jankowski[2] and T. Haertlé

Laboratoire d'Etude des Interactions des Molécules Alimentaires, Institut National de la Recherche Agronomique, B.P. 71627, F-44316, Nantes Cedex 03, France, [1]Department of Obstetrics and Gynaecology, Helsinki University Central Hospital, Haartmaninkatu 2, FIN-00290 Helsinki, Finland and [§]Faculté des Etudes Supérieures et de la Recherche, Université de Moncton, Moncton NB, Canada E1A 3E9

Thermal stability of porcine von Ebner gland protein (pVEGP) and its interaction with taste modifying compounds were studied by high-sensitivity differential (HS-DSC) and isothermal titration (ITC) calorimetry. Similarly to pig odorant binding protein (pOBP), pVEGP, under physiologic conditions, is a homodimer with a subunit molecular weight of ~17.4 kDa. However, at neutral pH its thermally induced denaturation transition occurs at surprisingly high temperature of 110°C with the enthalpy 396 kJ/mol. Such transition temperatures are characteristic of proteins from hyperthermophiles. Since pVEGP is a lipocalin, its stability parameters were compared with the corresponding values of other members of this super-family such as porcine odorant-binding protein (pOBP), bovine β-lactoglobulin (BLG), human retinol binding protein (RBP) and human glycodelin A (hGdA). The results revealed unusually high denaturation temperature of pVEGP as compared with other lipocalins (ΔT_d ~ 30–40°C). The denaturation enthalpy of pVEGP is nevertheless quite similar to that observed in case of pOBP, and slightly lower than that of RBP. Additionally, this protein is poor in disulfide bonds (only one

S–S bridge is present) which otherwise could stabilize its native structure by decreasing entropy of the unfolded form. Hence, it is likely that the observed denaturation transition of pVEGP at extremely high temperature but with relatively low enthalpy may be due to very tight binding of one or of several endogenous ligand(s) with the binding enthalpy close to zero. The isothermal calorimetric titration of pVEGP with aspartame and denatonium saccharide failed to detect any heat effect of binding at neutral pH. However, according to DSC data collected at acid pH interacting properties of pVEGP are altered. This is probably due to the partial release of endogenous ligand and liberation of potential binding sites. Additionally, the transition profile of pVEGP at acidic pH is highly asymmetric suggesting conformational heterogeneity of the protein with different amounts of endogenous ligands bound.

167. Olfactory dysfunction in Parkinson's disease is not dopamine dependent

Tomas Radil, Jan Roth[1], Evzen Ruzicka[1], Jiri Tichy[1] and Charles J. Wisocki[2]

Institute of Physiology, Czech Academy of Sciences, Prague, [1]Neurology Clinic, First Medical Faculty, Charles University, Prague, Czech Republic and [2]Monell Chemical Senses Center, Philadelphia, PA, USA

We performed two studies to verify the presence and to analyze the nature of olfactory dysfunction in Parkinson's disease (PD) patients.

We examined olfactory function in 16 PD patients (mean age 59.1 years, mean duration of PD 7.8 years, mean Hoehn and Yahr score 2.5) and in 18 gender- and age-matched healthy controls. Amyl acetate (AA) in 12 sequential dilutions was used as the odorant for examining ascending, binary, forced-choice, and ascending and descending limit-thresholds.

Testing revealed anosmia in one patient and hyposmia (a threshold equal to or higher than the AA concentration corresponding to the mean threshold value, plus two standard deviations, of the control group) in six patients and one healthy subject. Average olfactory thresholds were slightly higher in the PD group compared with the control group. The decrease in olfactory sensitivity was unrelated to the age of the patients, duration of PD, degree of motor impairment, or dose and duration of L-DOPA treatment.

Using the same procedure, we estimated the thresholds in seven hyposmic patients (mean age 60.1 years, mean duration of PD 9.0 years, mean Hoehn and Yahr score 2.8) before and after administration of apomorphine (APO; 0.05 mg/kg body wt). Domperidone was used 48 h before APO to avoid adverse effects, and all dopaminergic treatment was withdrawn 12 h before APO.

In contradiction to the marked motor amelioration, we failed to find any significant improvement in olfaction after APO.

In conclusion, olfactory dysfunctions were present in about one-third of our patients only. Duration of the disease, degree of clinical symptoms (expressed by the Columbia University Rating Scale score), and duration and dose of L-DOPA treatment did not correlate with olfactory thresholds. Furthermore, olfaction was insensitive to APO. Thus, olfaction in PD did not seem to be dependent on dopamine deficiency, and hyposmia was not a very valuable marker of PD.

168. Insulin in the olfactory system: receptor localization and evidence for local synthesis

Nancy E. Rawson, E. Tatar and P.M. Ulrich

Monell Chemical Senses Center, Philadelphia, PA 19104, USA

Levels of insulin (Ins) and insulin receptors (InsR) in the olfactory bulb are higher than elsewhere in the brains of adult animals, but little is known about the role of Ins in the olfactory system. Recent interest in the neural effects of insulin has implied that in addition to its metabolic role, it has neurotrophic and neuromodulatory effects. We have used anatomical, molecular and functional approaches to investigate the role of insulin in the olfactory system. InsR protein and mRNA is localized to a subpopulation of receptor neurons and basal cells of adult rats, and is more prevalent in the epithelium of streptozotocin-diabetic rats, indicating sensitivity to circulating levels. Surprisingly, c-peptide immunoreactivity is also present in the epithelium and olfactory bulb even after destruction of the pancreatic islets with streptozotocin. Subsequent PCR and *in situ* hybridization studies further support the notion of local synthesis of Ins within both the olfactory bulb and epithelium. Functional studies of the acute effects of Ins on olfactory receptor neurons using calcium imaging suggest that Ins can play a modulatory role in ORN responsiveness. Taken together, these data strongly suggest that Ins is essential to olfactory system structure and function and that an Ins-like molecule is synthesized in the olfactory system of adult rats.

Supported by NIH DC08276 and a Morley R. Kare fellowship.

169. Odorant responses and adaptation in isolated frog olfactory receptor cells

Johannes Reisert and H.R. Matthews

Physiological Laboratory, Downing Street, Cambridge CB2 3EG, UK

Various aspects of adaptation and underlying mechanisms were investigated in isolated frog olfactory receptor cells. The suction pipette technique was used to electrically record from these cells and rapid solution changes were applied to stimulate or expose cells to solutions of desired ionic composition.

Stimulating olfactory receptor cells for 1 s with Cineole revealed a steep dose-dependency for action potential firing. Not only did the spike frequency saturate within a 15-fold increase in odour concentration but the number of generated spikes progressively decreased with increasing concentration. In contrast the receptor current dose–response relationship did not show signs of saturation over a 300-fold Cineole range. A 4 s prepulse applied before the test pulse shifted the test pulse dose–response relationships to higher Cineole concentrations; a higher prepulse causing a larger adaptational shift. Long Cineole exposures (60 s) revealed three major response patterns. The first pattern (70% of cells tested) was characterized by regular bursts of action potentials generated every ~5 s. The second pattern (20%) showed continuous spiking throughout the odour presentation while the remaining 10% of the cells only fired spikes at the onset of stimulation. The use of ionic exchange protocols and specific channel blockers suggests the presence of a Na^+/Ca^{2+} exchanger in frog olfactory cilia. It was found that the exchanger serves as the main means of Ca^{2+} extrusion after the Cineole-induced Ca^{2+} increase and hence plays a crucial role in response termination. Without a fall in intracellular Ca^{2+}, the excitatory current through the Ca^{2+}-

activated Cl^- conductance, which is present in these cells, cannot terminate. Furthermore, the Na^+/Ca^{2+} exchanger seems to have an important function in aiding recovery from adaptation, the latter was investigated by exposing cells to Cineole twice in succession. When Na^+/Ca^{2+} exchange was prevented after the first Cineole stimulation by exposing cells to a zero Na^+ solution instead of normal Ringer the response to the second stimulus was always reduced compared with the control condition.

170. Stereoselective properties of the trigeminal nerve to discriminate between R- and S-nicotine

B. Renner, F. Meindorfner, M. Kaegler[1], N. Thuerauf[2], A. Barocka[2] and G. Kobal

Institute of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, D-91054 Erlangen, [1]Institute for Biological Research, D-51149 Cologne, Germany and [2]Department of Psychiatry, University of Erlangen-Nuremberg, D-91054 Erlangen, Germany

The aim of this study was to investigate whether nasal trigeminal receptors are able to discriminate between nicotine stereoisomers.

Twenty male Wistar rats (weight 300–500 g) were anaesthetized with urethane and artificially ventilated. Stimuli of either S- or R-nicotine vapour (stimulus duration 1350 ms, concentrations of 18–90 µg/l) were delivered to the nasal mucosa using an apparatus that allows specific stimulation without concomitant thermal or mechanical alteration of local conditions. This was achieved by embedding the nicotine stimuli in a stream of humidified nitrogen (relative humidity 80%) of constant flow and temperature (500 ml/min and 36°C, respectively). In order to obtain extracellular single cell recordings from the ipsilateral Gasserian ganglion, the right hemisphere of the brain was removed. Stimuli of R- and S-nicotine were applied randomly at different concentrations. Gaseous CO_2 was used as a control stimulus of trigeminal receptors (concentrations of 20, 40, 60, 80, 100% v/v). In an additional experiment hexamethonium was topically administered to the nasal mucosa before stimulation with R-nicotine, S-nicotine or CO_2.

Trigeminal responses were clearly different for the nicotine enantiomers. Specifically, the threshold for R-nicotine was significantly higher than that for S-nicotine. Furthermore, the responses to both R- and S-nicotine (but not to CO_2) could be suppressed by hexamethonium indicating the involvement of acetylcholine receptors in the peripheral processing of nociception. The units that responded to nicotine were identified by electrical stimulation as A-delta fibers.

By means of single cell recordings from the Gasserian ganglion, we were able to demonstrate that nasal trigeminal receptors can distinguish between nicotine enantiomers.

Supported by Philip Morris, USA.

171. Effects of trifluoromethyl ketone pheromone derivatives on olfaction and behaviour in male moths

Michel Renou, Angel Guerrero[1], Philippe Lucas, Gloria Rossel[1] and Andrée Berthier

INRA Unité de Phytopharmacie et Médiateurs Chimiques, Route de Saint Cyr, F-78026 Versailles, France and Department of Biological Organic Chemistry, CID (CSIC), Jordi Girona 18–26, 08034 Barcelona, Spain

A great diversity of pheromone analogues bearing specific chemical groups have been prepared with the aim of getting invaluable tools to understand mechanisms in olfaction and for their potentiality as pest management agents. Among them, fluorinated derivatives have been the subject of great interest due to their activity as enzyme inhibitors (Prestwich, 1986, Pestic. Sci., 37: 430–440; Parilla *et al.*, 1994, Bioorg. Med. Chem., 2: 243–252). For instance, 3-octylthio-1,1,1-trifluoropropan-2-one (OTFP) inhibits *in vitro* the conversion of Z11–16:Ac into the corresponding alcohol in extracts prepared from adult antennae. *In vivo*, OTFP strongly affects electrophysiological and behavioural responses of male moths to their sex pheromone (Renou *et al.*, 1997, Chem. Senses, 22: 407–416). In an effort to increase activity and specificity, trifluoromethyl ketones structurally related to the natural pheromones have been synthesized. Thus, the effects on olfaction and behaviour of (*Z*)-11-hexadecenyl-1,1,1-trifluoromethyl ketone (Z11–16:TFMK) have been tested in two noctuid moths, Mamestra brassicae and Sesamia nonagrioides, using (*Z*)-11-hexadecenyl acetate (Z11–16:Ac) as the main component of their sex pheromone. Dose–response curves established by EAG or SSR showed that Z11–16:TFMK has no intrinsic activity at physiologically relevant concentrations. Topical applications of Z11–16:TFMK on the antenna inhibited the responses to three pheromone components with different functional groups, Z11–16:Ac, Z11–16:OH and Z11–16:Ald. When EAG and SSR responses to Z11–16:Ac were measured after a background of Z11–16:TFMK has been created in air passing over the antennae, the following observations were made. The EAG amplitude was reduced and the repolarization time was slightly increased. The firing response of the Z11–16:Ac receptor cell was decreased, but the response kinetics was not affected. Under the same conditions, automatic recordings of behaviour showed that Z11–16:TFMK strongly inhibited the response of male moths to the sex pheromone, increasing the response latency and decreasing its duration. Thus, Z11–16:TFMK is a powerful inhibitor of pheromone communication although its physiological effects cannot be fully explained by its anti esterase activity.

172. A radar–Doppler actograph to monitor insect behaviour

Michel Renou, Jan Van der Pers[1], Andrée Berthier and Laetitia Desbarats

INRA Unité de Phytopharmacie et Médiateurs chimiques, Route de Saint Cyr, F-78026 Versailles, France and SYNTECH VDP Laboratories, POB 1547, 1200 BM Hilversum, The Netherlands

Insect behaviour in response to chemical stimulus has been extensively studied, using a variety of experimental setups. Quantification of behaviour generally relies on scoring specific activities. Further refinement of ethological tests resulted from recording flying or walking insects with video cameras or a servosphere, followed by analysis of the locomotory paths by trajectometry. These methods require either a sophisticated and expensive equipment or demand a large number of insects to achieve quantification of behaviour. Thus, other devices are required when it is necessary to screen large number of compounds. We describe an actograph based on a cheap movement detector, using the Doppler effect, connected to the A/D converter board of a computer. The performances of a prototype were evaluated by recording the responses of male

noctuid moths to short puffs of their sex pheromone. A single male moth was introduced in a glass observation chamber with a permanent air flow. The detector probe was focused on the chamber. The analogic output was high-pass filtered and fed into an acquisition board IDAC-02 (Synthec, Hilversum) in a PC-based microcomputer. Behaviour was recorded for several minutes. Video recordings were performed simultaneously to correlate the amplitude of the signal with specific behaviours showing that changes in activity were clearly recorded. The system provides a convenient actograph and offers several advantages. It is possible to obtain a quantitative measurement of the response of a single individual to a chemical stimulus. Disturbances to the animal are minimal. The system is poorly affected by external sources of noise. It can operate at very low light intensity or in complete darkness. The material commonly used to build olfactometers or insect observation chambers are transparent to the signal.

173. Transduction diversity in olfaction?

D. Restrepo, J.H. Teeter[1], F.W. Lischka[1] and R. Delay

Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO and [1]Monell Chemical Senses Center, Philadelphia, PA, USA

Stimulation of olfactory receptor neurons (ORNs) with odors can elicit an increase in the concentration of cAMP leading to opening of cyclic nucleotide-gated (CNG) channels and subsequent depolarization. Although opening of CNG channels is thought to be the main mechanism mediating signal transduction, modulation of other ion conductances by odorants leading to cell hyperpolarization has been postulated. In order to determine whether inhibitory odorant responses occur in mammals, we studied odorant responses in rat and mouse ORNs. In perforated patch recordings, we found that rat ORNs display two predominant types of responses to odors, neither of which would hyperpolarize the cell. Thirty percent of the cells responded to a mixture of odorats (mix A) with activation of a CNG conductance. In contrast, in 55% of the ORNs, stimulation with mix A inhibited a voltage-activated K^+ conductance (I_{Ko}). The effect of odorants on I_{Ko} was specific (only certain odorant mixtures inhibited I_{Ko} in each ORN). These results indicate that, in addition to opening of CNG channels, indirect suppression of a K^+ conductance (I_{Ko}) by odorants may play a role in signal transduction in rat ORNs. Consistent with these results, mix A elicited only depolarizing responses in loose patch recordings with rat ORNs. In contrast to the results in the rat, loose patch recordings indicate that mouse ORNs often respond to mix A with a hyperpolarizing current. In addition, stimulation of mouse ORNs with the cAMP phosphodiesterase inhibitor IBMX elicits either depolarizing or hyperpolarizing currents. Furthermore, stimulation of ORNs isolated from mice deficient for subunit 1 of the olfactory CNG channel (Brunett *et al.*, 1996, Neuron, 17: 681–693) respond to odorants with hyperpolarizing but not depolarizing currents, indicating that CNG channels do not participate in generation of the hyperpolarizing currents. These results indicate that multiple mechanisms participate in olfactory signal transduction in mammals.

174. Comparative ultrastructural aspects of vertebrate taste bud development

Klaus Reutter and Martin Witt[1]

Department of Anatomy, University of Tübingen, D-72074 Tübingen and [1]Department of Anatomy, Technical University of Dresden, D-01307 Dresden, Germany

To date, we do not know the micromorphology of the very early stages of taste bud (TB) development in all vertebrate groups, especially amphibia, reptiles and birds. We therefore must concentrate on the results from phylogenetically distinctly separated taxa, the teleostean fishes (*Scophthalmus maximus*, Reutter *et al.*, 1995, Chem. Senses, 20: 764) and mammals (humans, Witt and Reutter, 1996, Anat. Rec., 507–523). If we compare ultrastructurally the earliest TB primordia of fish with those of man we can establish some common characteristics:

- In fish as well as in man the first TB primordia consist of clusters of 3–4 cells which belong to one type of epithelial cell. They are situated directly on the basal lamina of a two-layered epithelium.
- The very early TB primordia contain profiles of unmyelinated nerve fibers which are rich in vesicular structures of different electron densities and which are in close contact to the primordial cells.
- Whereas the very early TB primordia comprise one type of cell of low electron density, the elder primordia consist of different electron-dense lighter and darker cells.
- The apical end structures of lighter and darker cells, the receptor villi, reach the surface of the epithelium when the TB's receptor area (fish) or the TB porus (man) has been developed.
- Afferent synapses at the bases of especially the lighter cells are found before the receptor area (fish) and the TB porus (man) are established.
- In fish and in man a dermal papilla underneath the TB primordium is not necessarily required.

The only striking difference between the TB primordia of fish and humans seems to be concerned with the basal cells. Fish have Merkel cell-like basal cells which seem to develop from glial (Schwann) cells. Mammals have no similar basal cells; their basal cells are supposed to be stem cells. In conclusion, it seems likely that in vertebrates of different systematic position (fish, mammals) TB development will follow the same morphogenetic principles, as far as these can be observed by morphological methods.

175. Odorant reception and termination in Lepidoptera

Matthew E. Rogers and Richard G. Vogt

Department of Biological Sciences, University of South Carolina, Columbia, SC 29208, USA

Lepidoptera antennae are exquisitely designed to capture volatile molecules; these volatiles include biologically active odorants such as pheromones, and molecules emitted from plants or other organisms including food signals and hazardous xenobiotic compounds. Our laboratory has identified two proteins whose

expression patterns suggest that they may function in odorant processing: SNMP-1, a neuronal membrane protein (Rogers *et al.*, 1997, J. Biol. Chem., 272: 14792–14799), and GST-msolf1, a cytosolic glutathione-*S*-transferase (GST) present in olfactory support cells.

SNMP-1 is expressed in antennal olfactory neurons and is localized in the cilia, dendrites and soma of these neurons. Developmental studies suggest that *snmp-1* mRNA levels increase significantly towards the end of adult development, coincident with the functional maturation of the antenna. EM-immunological studies in males indicate that SNMP-1 protein is present on the membrane surface of olfactory dendrites and is localized in only one of the 2–3 olfactory neurons present in pheromone-sensitive sensilla. SNMP-1 is clearly not homologous with the G-protein coupled receptors identified as olfactory receptors in vertebrates and *C. elegans*. However, SNMP-1 is homologous with the CD36 family of receptor-like membrane proteins; several members of this family bind proteinacious ligands (Calvo and Vega, 1993, J. Biol. Chem., 268: 18929–18935). Analogous to the known functions of its CD-36 counterparts, SNMP-1 may interact with odor-OBP complexes, stabilizing them at the membrane surface and thus enhancing the delivery of odor molecules to nearby receptor proteins. SNMP-1 may also function as a scavenger receptor and internalize the odor-OBP complexes into olfactory neurons.

GST-msolf1 is an antennal specific theta class GST that appears to be uniquely expressed within the pheromone specific subdomain of the olfactory. GSTs are known to function primary in the detoxification of noxious compounds (for review see Hayes and Pulford, 1995, Crit. Rev. Biochem. Mol. Biol., 30: 445–600). However, studies with vertebrate olfactory GSTs have prompted a model suggesting that olfactory GSTs may function in odorant signal inactivation (Ben-Arie *et al.*, 1993, Biochem. J., 292: 379–384). Given the role of the olfactory system in mediating critical behaviors such as reproduction and feeding, it seems likely that the olfactory epithelium has evolved mechanisms to minimize cytoxicity as well as those to inactivate odors to minimize habituation of odorant signals. GST-msolf1 may play a role in one or both of these mechanisms.

Work supported by NIH NIDCD DC-00588 and USDA CSRS 94-37302-0615.

176. Cholinergic modulation of the rat piriform cortex activity: an optical recording study

J.F. Rosin, F. Datiche and M. Cattarelli

CNRS UPR 9054, CESG, 15 rue Hugues Picardet, F-21000 Dijon, France. e-mail: fdatiche@cesg.cnrs.fr

The piriform cortex (PCx) is the main direct projection area of the olfactory bulb (OB). It also receives centrifugal cholinergic afferents which mainly arise from the magnocellular preoptic nucleus (MCPO) of the basal forebrain. These cholinergic fibers are heterogeneously distributed according to an increasing rostro-caudal gradient along the whole PCx. In the *in vivo* rat, we investigated the influence of cholinergic inputs upon the PCx reactivity to the OB electrical stimulation. We used the optical recording method with voltage-sensitive dye (RH 795, Molecular Probes), which is a powerful tool for mapping spatio-temporal patterns of neural events. The variations of fluorescence emitted by the PCx were detected at 2 kHz through a 12 × 12 photodiode array, with a spatial resolution of 280 × 280 μm^2 per photodiode. The optical PCx responses to the OB stimulation consisted of two waves (I and II), images of monosynaptic EPSPs elicited by bulbar afferents and of the di- and polysynaptic EPSPs induced by intrinsic association fibers respectively. We provided evidence that electrical stimulation of the MCPO induced an inhibitory effect (either reduction or suppression) upon both afferent and association fiber activities. Wave I was more frequently reduced than suppressed whereas wave II was more frequently suppressed than reduced in the three antero-posterior areas considered in our analysis. Thus, a stronger modulatory effect occurred upon the intrinsic association fibers activity. Nevertheless, the afferent fiber activity was also obviously affected by the MCPO stimulation. The reduction of wave I amplitude was stronger in the posterior than in the anterior PCx whereas it was the opposite for wave II. According to the anatomical distribution of cholinergic fibers, the present *in vivo* study implies a heterogeneous cholinergic modulation of the afferent and intrinsic activities along the PCx antero-posterior extent. Acetylcholine is assumed to play a major role in memory processing. This supports the possibility of the involvement of this cortex in olfactory learning. A next step should be to investigate the localization and pharmacology of the cholinergic targets within the PCx network. Physiologically relevant bulbar stimulations would also be of interest to analyze the role of cholinergic modulation upon memory processing in the PCx.

177. Sensitivity and dynamic range of olfactory sensory neurons: role of the neuron structure and environment

Jean-Pierre Rospars and Arthur Vermeulen

Unité de Biométrie, Institut National de la Recherche Agronomique, F-78026 Versailles Cedex, France. e-mail: rospars@versailles.inra.fr

Response properties of the receptor potential at steady state were analyzed in a biophysical model of an olfactory receptor neuron (ORN) embedded in its epithelial environment. The neuron structure was described as a set of several identical dendrites (or cilia) bearing the transduction mechanisms, joined to a non-sensory part composed of the dendritic knob, the soma and the axon. The surrounding auxiliary cells, linked to the neuron with tight junctions, alter the neuron response properties by compartmentalizing the neuron environment and introducing extra-neuronal voltage sources. Analytical solutions were found to describe how the receptor potential at the non-sensory part responds to a uniform change in the odorant-dependent conductance resulting from odorant stimulation of the sensory dendrites. We investigated the influence of various geometrical and electrical parameters on the receptor-potential response in two models: isolated neuron with power supply in the dendrites bathed in a homogeneous environment (Rospars *et al.*, 1996, J. Comput. Neurosci., 3: 51–72; Vermeulen *et al.*, 1997, Bull. Math. Biol., 58: 493–512), and neuron surrounded with auxiliary cells and with extra-dendritic power supply (Rospars *et al.*, 1996, J. Comput.

Neurosci., 3: 51–72; Vermeulen *et al.*, 1997, Bull. Math. Biol., 58: 493–512; Vermeulen and Rospars, 1998, J. Comput. Neurosci., 5: 243–266).

It was found that the sensitivity (as measured by the increase in membrane conductance at half-maximum response) of the neuron model in the absence of auxiliary cells is higher but its dynamic range is narrower than in their presence. The dynamic range is wide and the sensitivity low when the input resistance of the non-sensory part is small (e.g. with a large soma) and the sensory dendrite is unbranched. Both sensitivity and dynamic range are higher for a longer dendrite. These results show that there is no optimal encoder neuron endowed simultaneously with receptor potential of large magnitude, high sensitivity and wide dynamic range. They help understand the morphology of olfactory sensilla; for example, neurons with a single long dendrite and auxiliary cells (such as the moth sex-pheromone ORNs) appear tuned for wide dynamic range, whereas neurons with several short dendrites (such as the moth 'general' odour ORNs and vertebrate ORNs) are tuned for high sensitivity.

178. Structure–odour correlations—scope and limitations

Karen J. Rossiter

Quest International, Ashford, Kent TN24 0LT, UK

Over the last century, we have had to rely on relatively tedious empirical approaches to develop compounds with desired odour properties. These have included the analysis of natural products and the synthesis of known analogues. Serendipity and the systematic synthesis of materials based on readily available feed-stocks and chemical technologies have also played an important role in the discovery of novel odorous materials. Thus, to date, the search for new fragrance ingredients has primarily been dependent on random screening with the current success rate to commercialization being in the order of one in every 300 synthesized.

Obviously, there are enormous money and time saving opportunities to be gained by the discovery of new molecules by design rather than by chance. Although the past 20 years have seen some significant breakthroughs in our understanding of the biochemistry of olfaction, it will be some time before computers can translate a gene sequence into a 3-D model of an olfactory receptor and screen thousands of odour molecules to find a few that optimally activate it. The development of structure–odour relationships (SORs) provides an alternative approach for helping us to understand and predict the odour properties of chemicals. However, SORs are complicated by the fact that related molecular structures do not, by any means, always display similar odour properties and, conversely, that substances having similar odours often belong to very different structural classes. The second unique feature of structure odour relationship work is the difficulty associated with odour measurement. It is also noticeable that whatever odour 'rules' are deduced, there are always exceptions. Trying to understand why these compounds do not fit the models may provide useful clues about the mechanism of olfaction. Despite all of these problems, the potential predictive ability of this approach is exemplified by the few examples where the use of SORs have led to the discovery of new fragrance ingredients. In this paper I would like to consider the scope and limitations of SORs from both a scientific and commercial point of view.

179. Functional anatomy of different levels of odor processing: two PET studies

J.P. Royet, J. Hudry, D. Godinot, O. Koenig[1], M.C. Gregoire[2], L. Cinotti[2], F. Lavenne[2], D. Le Bars[2], M. Vigouroux, V. Farget, A. Holley and J.C. Froment[3]

Neurosciences et Systèmes Sensoriels, University Claude-Bernard, F-69622 Villeurbanne, [1]Psychologie Cognitive, University Lumière, Lyon2, F-69676 Bron, [2]CERMEP et [3]IRM, Hôpital Neurologique, F-69003 Lyon, France

The functional anatomy of perceptual and semantic processings for odors was studied using PET scan. In a first study, the regional cerebral blood flow (rCBF) was measured in 15 normal subjects in three conditions: detection, familiarity and comestibility judgements. It was suggested that the three tasks were hierarchically organized from a superficial level to a deep, semantic, level. Odors were presented with an airflow olfactometer and synchronized with breathing. Subtraction of activation images showed that familiarity was associated with the activity of the right orbitofrontal area, the subcallosus gyrus, the left inferior and superior frontal gyri, and the anterior cingulate (Brodmann's areas 11, 25, 47, 9 and 32, respectively). Comestibility selectively activated the primary visual areas. In contrast, decreased activity was observed in these same visual areas for familiarity, and in the orbitofrontal area for comestibility. It was concluded that orbitofrontal and visual regions interact in odor processing in a complementary way depending on the task requirements.

In a second study, using a scanner with a higher spatial resolution, we examined in 12 subjects the cerebral areas activated in five olfactory tasks (detection, intensity, hedonicity, familiarity, and comestibility), and a non-olfactory control task. The preliminary results reveal that the olfactory tasks of low or high processing levels mainly activated the left prefrontal cortex. This cortical region has been shown to be involved in retrieval of information from semantic memory, and in encoding information about novel happenings into episodic memory.

A bilateral activation of the orbitofrontal area (11 and 47) was observed for familiarity, but just an activation in the left side was obtained for hedonicity, and an activation in the right side for comestibility. Primary visual areas (17/18/19) were mainly activated in hedonicity and comestibility. They could participate to the semantic processing of odors in the sense that the subjects could visualize the objects evoked by the odor to perform a cognitive olfactory task. Finally, the gyrus temporalis superior, including the entorhinal area (28U), and the gyrus fusiformis (36U) were activated in detection. In familiarity, a smaller rCBF than in the detection task was observed in the amygdala and the subcallosus gyrus (34U, 25), the hippocampus, and the para-hippocampical gyrus (19/30/35/36 GH).

180. Levels-of-processing effects on a task of olfactory naming

J.-P. Royet, O. Koenig[1], H. Paugam-Moisy[2], D. Zighed[3], D. Puzenat[2], M. Sebban[3], M. Vigouroux and J.-L. Chassé[4]

Neurosciences et Systèmes Sensoriels, University Claude-Bernard Lyon1, F-69622 Villeurbanne, [1]Psychologie Cognitive, University Lumière, Lyon2, F-69676 Bron, [2]Informatique du Parallélisme, Ecole Normal Supérieure, F-69364 Lyon, [3]Recherche en Ingénierie des Connaissances, University Lumière, Lyon2, F-69676 Bron and [4]Biométrie, Génétique et Biologie des Populations, University Claude-Bernard Lyon1, F-69622 Villeurbanne, France

Cognitive studies have shown that sensory stimuli can be analyzed at different levels, ranging from shallow analysis to deep or semantic analysis. The effects of the depth of processing on subsequent performance of odor naming were rated in the present study. The experimental procedure included two successive sessions: a first one in which subjects characterized a set of 30 odors with low or high level judgements, and a second one in which they had to name the same set of odors as soon as possible. Five experimental conditions, which differed according to the level of judgement performed for the first task, were used: four conditions of odor rating with descriptors (preprocessing conditions) and one control condition of no rating of odors prior to naming test (the subjects performed calculations). The preprocessing conditions differed from each other regarding the type of descriptors. These descriptors were referred to low-level olfactory judgements (superficial preprocessing in which the subjects rated intensity, hedonicity and familiarity of odors), high-level olfactory–gustatory–somesthetic judgements (deep1 preprocessing in which the subjects rated odors with descriptors as fruity, floral, fresh . . .) and high-level non-olfactory judgements (deep2 preprocessing in which the subjects rated odors with auditory and visual descriptors). One condition successively regrouped low- and high-level olfactory judgements (superficial-deep1 preprocessing). An empirical index of naming, dependent on the accuracy of response (veridical label, near misses, far misses and no response) and the response time of the subjects, was computed. Data showed that odor naming was modified for 18/30 odorants as a function of conditions. It was observed that for 94.5% of significant variations, the scores were higher for conditions in which the processing was hypothesized to be deeper than for other conditions. Thus, performances in naming were progressively improved as follows: control, superficial or deep2 preprocessing, deep1 preprocessing and superficial–deep1 preprocessing. Together, these data show that the deepest levels of olfactory encoding were later associated with progressively higher levels of performances of naming.

181. The glomerular activity code for odours is conserved within the honeybee *Apis mellifera*

S. Sachse, C.G. Galizia, A. Rappert and R. Menzel

Institut für Neurobiologie, Fachbereich Biologie, Freie Universität Berlin, Königin Luise Straße 28–30, D-14195 Berlin, Germany. e-mail: galizia@zedat.fu-berlin.de

Stimulation with odours leads to a specific activation of glomeruli in the antennal lobe (AL) of insects. These activity patterns can be measured *in vivo* with calcium imaging (Joerges *et al.*, 1997, Nature 387: 285; Galizia *et al.*, 1997, J. Neurosci. Methods, 76: 61). It is believed that odours are coded in the 'across-glomeruli' activity patterns in a spatial code; however, decifering this code is difficult, and the contribution of temporal aspects remains to be analyzed. Since the AL of insects is the functional analogue to the olfactory bulb in vertebrates, and both structures share many features in terms of architecture and connectivity, understanding the olfactory code of insects is also of great interest to olfactory research in vertebrates.

In honeybees there are 160 olfactory glomeruli which vary in shape and size, and are consistent between individuals. This has made it possible to create a morphological atlas (Flanagan and Mercer, 1989, Int. J. Insect Morphol. Embryol., 18: 145). We have developed a computerized version of this atlas (Galizia *et al.*, submitted for publication) which allows us to create views and sections in any plane, and thus to identify homologous glomeruli in diverse preparations.

After measuring odour-evoked glomerular activity patterns in the honeybee antennal lobe using calcium-green-AM measurements, we stained the brain with the membrane permeant dye RH795 in order to visualize the glomeruar layout. We then created a mask of the identified glomeruli, and mapped the activity patterns onto this mask. We tested a total of 20 odours in up to 10 individuals for each odour, for a total of 22 identifiable glomeruli.

We found this spatial component of the olfactory code to be conserved between individuals. For example, pentanol leads to the glomerulus T1–28 being maximally activated in 10/10 measured individuals. Heptanol has strongest activity in T1–28 and T1–17, and decanol in T1–33 with a weaker response in T1–17.

182. Does living in 'possum paradise' influence the composition of the sternal scent gland secretion of the brushtail possum (*Trichosurus vulpecula*)?

Mario Salamon

Institute of Medical Psychology, University of Munich, Germany

The brushtail possum (*Trichosurus vulpecula*) is a nocturnal, herbivorous marsupial endemic to Australia. Individuals live solitarily and dispersion is maintained by mutual avoidance, established by contact encounters and sustained by olfactory and auditory communication. One of the main features of the social system in *T. vulpecula* is the use of olfactory communication to establish home ranges and to find mates. Individuals of both sexes possess a large sternal scent gland which is used for scent marking. The diet in Australia consists primarily of the leaves of trees, and the proportion of *Eucalyptus* leaves varies from 0 to 95% and reflects the availability of other plant species. The abundance of *Trichosurus* is determined by palatable plants other than *Eucalyptus* as possums cannot feed solely on this species because of toxic plant secondary metabolites.

Between 1858 and 1900 *T. vulpecula* was deliberately introduced to New Zealand in order to establish a fur trade. In the first couple of years the national distribution of possums was even financially supported by the government. Since then *T. vulpecula* has developed into the biggest mammalian pest in New Zealand, reaching an estimated population size of 70 million individuals. The conditions in New Zealand have been highly favourable to the possum as there are no larger mammal herbivores present. Furthermore, local tree species do not contain large amounts of

phytochemicals, resulting in massive browsing damage to the endemic trees of New Zealand.

Nowadays *T. vulpecula* has successfully populated nearly 90% of the country and population densities 10 times higher than in Australia have been recorded. It is assumed that this elevated population density has an effect on the olfactory system and may influence the composition of the scent secretion.

In previous studies we could show a short-term effect of the diet on the scent content in the Australian *T. vulpecula*. Herein we report on the composition of the sternal gland secretion in Australian and New Zealand brushtail possums. Individuals were caught in Tasmania (Australia) and on the North Island of New Zealand and their scent secretion was analysed using gas chromatography-mass spectrometry (GC-MS).

We will report on the differences and similarities between the two populations which inhabit two different environments and illustrate the dramatic adaptations to the new ecological conditions. The consequences for the social behaviour of the possums as well as for the local environment will be discussed in detail.

183. The mammary pheromone of the rabbit: comparative pup responsiveness to odor cues from milk and from lactating females' abdomen

B. Schaal, G. Coureaud, D. Langlois[1] and G. Perrier[2]

Laboratoire de Comportement Animal, CNRS Ura 1291, Inra, F-37380 Nouzilly, [1]Laboratoire de Recherche sur les Ar'mes, Inra, F-21000 Dijon and [2]Etablissement National d'Enseignement Suprieur Agricole, F-21000 Dijon, France

Lactating female rabbits emit odour cues from their abdominal skin which elicit a typical behaviour sequence in newborn pups, beginning with a sharp general activation, followed by the display of search-probing motions, and ending with the oral capture of a nipple (Hudson and Distel, 1983, Behav., 79: 255). Oral seizing is also triggered by odour cues carried in fresh rabbit milk (Keil *et al.*, 1990, Physiol. Behav., 47: 225). However, the releasing efficiency of both odour sources has been characterized in very different experimental contexts, so that their functional equivalence to newborns remains unclear. The present study aimed at assessing the responses of pups exposed to the headspaces of a lactating doe's abdomen and of rabbit milk.

A testing arena was devised in which pups were exposed to the odour sources without direct contact. The orientation behaviour of independent groups of pups was timed during 2 min tests to examine: (i) their response to the headspace of either a non-familiar lactating female's abdomen (LFA) or of milk (Mi) freshly sampled from a non-familiar doe, presented in contrast with a control stimulus (scentless rabbit fur: RF); and (ii) their relative response to both odour stimuli presented simultaneously. In assay 1 (LFA versus RF), pups oriented longer to LFA, indicating that they detected it. Assay 2 (RF versus blank) evidenced that RF odour was neither attractive nor aversive, thus confirming that LFA odour alone accounted for pup attraction in assay 1. In assay 3 (LFA versus male abdomen: MA), pups displayed preference for LFA over MA, indicating that the attractive abdominal cue was emitted by lactating does. Assay 4 opposed Mi on RF versus RF to gauge whether milk alone would also elicit an attraction response; Mi on RF elicited significantly longer orientation than the control RF. Finally, assay 5 contrasted LFA versus Mi on RF, and to control for potential effects of thermal differences, assay 6 opposed LFA versus Mi on MA. In both assays, pups oriented for an approximately similar duration to either stimulus. These results indicate that the headspaces emitted by a lactating doe's abdomen or by fresh rabbit milk elicit a similar pattern of positive orientation. Both exocrine sources may thus generate functionally similar compounds hypothesized to be carrying the same pheromonal effects.

184. Visual event related potentials modulated by contextually relevant and irrelevant olfactory primes

Mehri Sarfarazi, Anne Richardson[1], John M. Behan[1] and E. Michael Sedgwick

Clinical Neurological Science, Room LF73B, General Hospital, Tremona Road, Southampton SO16 6YD and [1]Quest International, Ashford, Kent, UK

Visual evoked potentials were recorded from 16 scalp locations on 10 young subjects during presentation of a series high quality photographs on a computer screen. The photographs consisted of equal numbers of pictures of fruit (citrus and non-citrus fruits), flowers (roses and other flowers) and other objects (e.g. buildings, vehicles, animals etc.). Every picture was unique in order to avoid repetition effects. The pictures were presented under four different odour conditions: no odour, rose odour, jasmine odour or citrus odour. In order to keep the subjects alert they were asked to make a decision categorizing the visual stimulus (e.g. flower or fruit). No decision was required concerning the relationship between the visual stimulus and the odour. As was expected, the N400 peak was more negative when the picture stimulus did not match the odour. It is hypothesized that the N400 peak can be used as a measure of relatedness of a sensory stimulus to a previous or on-going prime, irrespective of the mode of the stimuli (e.g. visual or olfactory).

185. Functional innervation of dissociated olfactory bulb neurons by co-cultured explants from the olfactory epithelium

O. Schluter, M. Werner, K. Gottmann and H. Hatt

Ruhr-Universitat Bochum, Department of Cell Physiology, Universitatsstraße, 150, D-44780 Bochum, Germany

Olfactory sensory neurons project to the olfactory bulb and form synaptic contacts on mitral/tufted cells. The electrical and chemical properties of these synapses have been poorly investigated, since mostly the tissue is hardly accessible for electrophysiology in preparations *in vivo*. Therefore, we established a co-culture system that allows us to study synaptic interactions between the olfactory epithelium and olfactory bulb neurons using the patch-clamp technique.

Olfactory epithelium and olfactory bulb were obtained from embryonic rats (embryonic day 20). Dissociated olfactory bulb neurons were plated into culture dishes together with 3–5 explants of olfactory epithelium. During cultivation, fibers growing out of the explants innervated the dissociated bulb-cells within 4–7 days.

In bulb-cultures interneurons and mitral/tufted cells can be distinguished by morphological and electrophysiological characteristics. After 7–10 days *in vitro*, olfactory bulb neurons formed small aggregates containing in the center a mitral cell,

which was surrounded by several interneurons. These aggregates may represent functional units. Similar structures are known from anatomical studies in the olfactory bulb. The aggregates were strongly innervated by bundles of nerve-fibers originating from the epithelium-explants.

The electrophysiological characterization of the bulb cells was performed using the whole-cell patch-clamp technique. Under asymmetrical Cl^- concentrations, excitatory postsynaptic currents (EPSCs) were recorded at a holding potential of –60 mV and inhibitory postsynaptic currents (IPSCs) at –20 mV. Interneurons exhibited spontaneous, glutamatergic EPSCs, while mitral cells showed additionally spontaneous GABAergic IPSCs, suggesting dendrodentritic synaptic connections with surrounding interneurons.

To demonstrate a functional synaptic connection extracellular electrical stimulation of an explant was instigated and EPSCs were recorded in the mitral cells located closely to the explant. Upon stimulation bursts of EPSCs were evoked in the mitral cells indicating a functional synaptic projection from olfactory sensory neurons within the epithelium explant to the bulb-neuron.

186. Expression of KET, a new protein related to the tumour suppressor p53 in keratinocytes of the circumvallate taste papilla

Hartwig Schmale and Casimir Bamberger

Institute of Cell Biochemistry and Clinical Neurobiology, University of Hamburg, D-20246 Hamburg, Germany

Taste buds of vertebrates comprise a collection of 40–120 axonless taste receptor cells, supporting- and precursor cells. The outer margin of taste buds is formed by flattened, concave cells that border at the surrounding epithelial cells. Taste cells have a short lifetime of ~10 days and are continously replaced. Several lines of evidence indicate that taste cells and epithelial cells arise from common progenitor cells present in the local epithelium.

From a rat circumvallate (CV) taste papilla cDNA library we have isolated a cDNA clone encoding the new KET protein which exhibits significant homology to conserved parts of the tumour suppressor p53. The transcription factor p53 is implicated in cell-cycle control mechanisms that monitor the cell's health and thus prevent malignant cell proliferation. The KET gene shows remarkable homology to a molluscan p53 of the squid and therefore may represent a primordial p53 ancestor gene which appeared early in phylogenesis. The persistence of the KET gene and its conservation point to an important function that cannot be fulfilled by p53. KET as a member of the new family of p53-related proteins is expressed during embryonic development and in certain adult tissues. The expression pattern in the adult suggests that KET is involved in tissue-specific differentiation. *In situ* hybridization histochemistry revealed high levels of KET mRNA in keratinocytes of the tongue epithelium, predominantly in the basal part of the trenches of taste papillae that contain taste buds. In the CV papilla, the onion-shaped taste buds seem to be embedded in keratinocytes expressing the KET gene. Cells of the taste bud itself, including taste receptor cells which contain gustducin mRNA, do not possess KET transcripts. It is tempting to speculate that KET expression marks a special population of keratinocytes that subsequently enter the taste bud as precursor cells.

187. Selective and reversible blockage of a fatty acid odour response in the olfactory bulb of the frog

Mussadiq Shah and Krishna C. Persaud

Department of Instrumentation and Analytical Science, UMIST, PO Box 88, Manchester M60 1QD, UK

The lectin Concanavalin A (ConA), when applied to the olfactory mucosa (OM) of the frog and rat, is reported to partially inhibit electroolfactogram (EOG) responses to fatty acid odours. Control odours like isoamyl acetate were not affected (Wood *et al.*, 1982, Biochem. Soc. Trans., 781–782; Shirley *et al.*, 1987, Biochem. J., 245: 175–184).

We have now studied in the frog *Rana temporaria* whether this treatment affects the corresponding olfactory bulb (OB) response. The OB surface was impregnated with the voltage-sensitive dye (RH 414). Spatial and temporal patterns of odour response were measured by changes in dye fluorescence that occurs when OB neurons fire. The apparatus, previously described (Persaud and Minor, 1995, Chem. Senses, 20: 97–97), consisted of an epifluorescent microscope coupled to a 64 × 64 pixel CCD camera. This allowed imaging over a 0.9 mm^2 area of the OB glomerular layer to high resolution.

When frog OM was bathed with 5 mg/m ConA in Ringer's solution, the *n*-butyric acid (nBA) odour response in the OB largely disappeared while the isoamyl acetate response did not change. When this experiment was repeated in the presence of 20 mM methylmannopyroside (a ConA inhibitor), ConA failed to inhibit the nBA response. Moreover, the ConA effect was reversible. A prolonged Ringer wash of the OM after ConA treatment slowly restored the nBA response to normal. Thus the OB results seem to be compatible with the EOG results and reinforce the notion that ConA selectively prevents nBA sensitive olfactory receptor neurons from firing. Chemical modification of the OM and their effect on OB response patterns may provide a useful approach to investigate olfactory quality coding.

This study was supported by a studentship from RAM Research Ltd. We are grateful to Ernest Polak for his advice, support and encouragement.

188. Consensus and controversy in olfaction: developing an integrated conceptual framework for topographical organization and temporal processing underlying discrimination of odors and pheromones in the olfactory bulb

Gordon M. Shepherd

Section of Neurobiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510, USA

Evidence for a topographical organization underlying the processing of odor stimuli by the olfactory bulb of many vertebrate species has been provided by a variety of studies, including tritiated amino acid and HRP tract tracing, 2-deoxyglucose and c-fos mapping, voltage sensitive dyes and Ca^{2+} imaging, electrophysiological single unit and field potential recordings, and localization of olfactory receptor mRNA. Many of these studies have converged on a common set of principles relating spatial organization to odor specificity and odor concentration that applies to most vertebrates as well as invertebrates. Within this

spatial organization, it is proposed that microcircuits mediate contrast enhancement between odor modules that can contribute to odor discrimination. The results of a new method, high resolution fMRI of the rat olfactory bulb, which allows imaging of odor induced activity at the laminar level over periods of tens of seconds, support these principles. Because of the different cellular constituents of the bulbar laminae, these results also provide important clues to the cellular basis of brain images. Olfactory bulb ablations have relatively limited effects on odor discrimination; we will discuss the extent to which this is due to the redundancy built into the topographical specificity. Recent studies in several laboratories have focused on encoding of odor information by oscillatory impulse patterns. We and others have hypothesized that the generation of oscillatory patterns of odor-induced activity depends on many of the same membrane properties and synaptic microcircuits that are involved in the spatial contrast mechanisms that underlie odor discrimination. It is important to recognize that analysis of temporal coding requires knowledge of the underlying topographical organization. The predominant role of the main olfactory bulb in mediating pheromone responses in most species will be reviewed. The lecture will illustrate how, in the olfactory pathway as in other sensory pathways, a clear understanding of topographical organization is the necessary starting point for assessing mechanisms of sensory processing.

189. Sensory olfactive characterization of cosmetic raw materials

Jean-Marc Sieffermann

ENSIA—Departement Science de l'Aliment, F-91744 Massy, France

This paper presents a collaborative work which was done to improve the sensory methodology used by a cosmetic application laboratory in order to achieve an olfactive characterization of cosmetic raw materials. The object of the study was to obtain, through a small sensory expert panel, a reliable and effective way of describing the aging of those cosmetic raw materials.

The first part of the study consisted in pointing out the critical points of the pre-existant olfactive characterization, and thus according to sensory analysis methodological basic points. Good analytical sensory analysis requires to strictly separate preference appreciation from descriptive analysis. It concentrates on qualitative and quantitative evaluations, taking into account potential inter-individual differences.

The five-member expert panel was familiar with a consensual group evaluation based on a global five-point intensity rating followed by the selection of 3–5 terms chosen among 44 empiric descriptive terms. This sensory approach was questionable due to potential group leader effect and too little training on many of those descriptive terms. The terms were also not always relevant, accurate or independent, and some of them were potentially hedonic terms. Finally, products were always labelled and fully identified during evaluation.

The panel was thus first asked to change his evaluation to individual and blind assessments, using a three-step temporal evaluation with the same descriptive list followed by a global intensity evaluation. Results showed strong inter-individual differences, with a huge descriptive variability and a poorly interpretable quantitative evaluation. The absence of a precise consensual product description, despite a strong common olfactory background, was a real and perturbing surprise to the expert panelists. It proved to be the needed shock to convince the panel to proceed to further changes in the olfactory evaluation.

The second step of the study consisted then in the elaboration of eight global olfactory descriptive notions through principal component analysis, cluster analysis and expert discussion. Evaluations were pursued on the basis of blind quantitative individual ratings on fie-point quantitative scales, this time specific to each descriptor. Results showed to be very encouraging and allowed a good time dependent product characterization.

This work led to the creation of the Cosmetic Olfaction Group, a section of the French Cosmetic Society. Its aim is to provide, through small methodological changes and individual expert evaluations based on different vocabularies and experiences, the construction of a global cosmetic raw materials lexicon.

190. Expression of c-fos mRNA in the AOB of prepubertal female mice induced by purified and recombinant male major urinary proteins (MUP)

Lucia Silvotti, M. Alessandra Umiltà, Davide Beghini and Roberto Tirindelli

Istituto di Fisiologia Umana, Università di Parma, Via Volturno, 39, I-43100 Parma, Italy

The major urinary protein complex (MUP) purified from adult male mice urine is responsible for the acceleration of puberty onset in female mice, suggesting that MUP is a pheromone. This effect is also retained by MUP without its naturally bound volatile molecules, and by a MUP-related synthetic hexapeptide. Given that most pheromonal cues are processed by the accessory olfactory system, we have checked for a sign of neural activation in the accessory olfactory bulb of mice after pheromone administration. Therefore, our study was designed to verify the ability of purified MUP to induce transcription of the immediate early gene *c-fos* in the accessory olfactory bulb of prepubertal female mice. To separate the role of MUP, in eliciting pheromonal activity, from that of the natural ligands bound to it, in-situ hybridization experiments have been performed either with recombinant MUP, cloned in *Pichia pastoris*, or with the synthetic N-terminal hexapeptide of MUP, Glu-Glu-Ala-Ser-Ser-Th.

191. Bitter taste mechanisms: nicotinic and muscarinic receptors in rat taste receptor cells

S.A. Simon, G.Q. Chang, L. Liu and Y.Q. Jiao

Department of Neurobiology, Duke University Medical Center, Durham, NC 27710, USA

Alkaloids, such as nicotine and atropine, can evoke both a bitter sensation and a burning sensation when placed on lingual epithelium. In rats, we found that lingual application of many alkaloids, including nicotine, activate neurons from trigeminal nerve (for the burning sensation) and neurons from the chorda tympani and glossopharnygeal nerve (for the bitter taste sensation). RT-PCR reveals that neurons from the trigeminal ganglion contain all the known neuronal nicotinic receptor subunits present in mammals. The gustatory recordings suggest that nicotine activates receptors on taste receptor cells (TRCs). Although there have been many studies regarding the cellular mechanisms involved in bitter taste, none have directly addressed the question of the presence of nicotinic acetylcholine receptors

(nAChR) and muscarinic acetylcholine receptors (mAChRs) in TRCs. Using immunocytochemical methods, we found that TRCs in both fungiform and circumvallate papillae have both nicotinic (nAChR) and muscarinic (mAChR) receptors. Specifically, nAChRs in TRCs in both types of papillae have α7, α4 and β2 subunits. These are found in a large percentage of TRCs, are intracellular, and are on both the apical and basolateral surfaces. Muscarinic receptors are also found in most TRCs, although we have not classified the various subtypes present. These data reveal potentially new transduction mechanisms for bitter tastants involving nAChRs and mAChRs.

This work is supported by grants from NIH (DC-01065) and the Philip Morris Corp.

192. Receptor neuron projections in the macroglomerular complex of the antennal lobe in the male moth, *Helicoverpa armigera*

Hanne Th. Skiri, Tor J. Almaas and Hanna Mustaparta

Department of Zoology, Norwegian University of Science and Technology, N-7034 Trondheim, Norway. e-mail: hannesk@stud.ntnu.no

The macroglomerular complex (MGC) of the antennal lobe in the male moth *Helicoverpa armigera* consists of three compartments: one large unit (cumulus) at the entrance of the antennal nerve and two smaller, medially located units. The projections of different receptor neuron types in the three compartments were studied by electrophysiological recordings from cut hairs of the male specific sensilla trichodea type 1 during stimulation with biologically significant compounds. The neurons were then stained with cobalt-lycin applied via a second electrode, during stimulation with the potent compound. Successful stainings revealed single axon terminals in different MGC compartments after histological treatments and sectioning of the brain. Receptor neurons responding to antennal stimulation with the major pheromone component, (*Z*)-11-hexadecenal, were often colocalized with another receptor neuron which did not respond to the test compounds. Cobalt-lycin applied to these sensilla usually resulted in one stained axon terminating in the cumulus. In some cases a second neuron was stained which projected in an ordinary glomerulus close to the MGC. From another sensillum type responses were obtained to stimulation with the second pheromone component (*Z*)-9-hexadecenal and the interspecific signal (*Z*)-9-tetradecenal as well as to higher concentrations of (*Z*)-11-hexadecenol. Cobalt-lycin applied to these sensilla revealed two stained axons, each terminating in one of the two medial compartments. No receptor neuron responded to antennal stimulation with (*Z*)-11-hexadecenyl acetate, a biologically significant signal used by the American related species possessing specific receptor neurons for this compound.

193. Does intranasal administration of zinc sulfate produce anosmia in rats?

Burton Slotnick, Polly Anne Glover and Natalya Bodyak

Department of Psychology, American University, Washington, DC 20018, USA

Intranasal administration of zinc sulfate is the most commonly used procedure to produce a peripheral anosmia. This method has been used to test the role of smell in reproductive behavior, baítshyness, homing behavior of pigeons, gustation and appetite, learning and photoperiodic responsiveness, among other behaviors. However, there are reasons to question the validity of the method. Histological studies demonstrate that some regions of the olfactory epithelium (OE) are spared after $ZnSO_4$ treatment (e.g. Stewart *et al.*, 1983, Dev. Brain Res.; Winnans and Powers, 1977, Brain Res.) and, in the only olfactometric study available, treated rats recovered near normal function in a few days (Slotnick and Guttman, 1977, J. Comp Physiol. Psychol). In the present study rats were trained in an olfactometer to detect ethyl acetate (0.02–0.00002% of vapor saturation), administered 5% zinc sulfate (50–500 µl/nostril) and tested in a single post-treatment session 24, 48 or 96 h later. These zinc volumes filled the nasal cavity, and excess fluid drained from the external naris and nasopharynx. Every one of eight rats so tested achieved criterion performance (85% correct) on all or all but the lowest concentrations of EA. Two rats, tested on two-odor discrimination tasks, performed about as well as controls. Immediately after behavior tests the OE was treated with horseradish peroxidase (HRP) and the olfactory bulbs were processed 24 h later. In each case there was anterograde transport of HRP to most OB glomeruli. In other studies 5% $ZnSO_4$ was injected directly into the olfactory sac and HRP was given 24–96 h later. In no case did treatment eliminate axonal transport to >60% of OB glomeruli. We conclude that intranasal application of $ZnSO_4$, as generally practised, neither destroys the entire olfactory epithelium nor produces anosmia.

194. Greater superficial petrosal nerve responses in Sprague–Dawley, Fischer 344 and developmentally sodium restricted rats

Suzanne I. Sollars and David L. Hill

Department of Psychology, University of Virginia, Charlottesville, VA 22903, USA. e-mail: sis2n@virginia.edu

Much research has examined gustatory response properties of the chorda tympani nerve (CT) in rats and hamsters: in adulthood, during development, following various experimental manipulations such as sodium deprivation or CT transection, and across different strains. Far fewer studies have investigated greater superficial petrosal nerve (GSP) taste responses, and no studies have been published which examine GSP taste profiles that vary from those of standard adult rats and hamsters. The present series of experiments was designed to establish normative data on sodium sensitivity and amiloride suppression of GSP responses, and to begin characterization of GSP responses in situations that differ from that of the normal adult rat. Experiment 1: adult Sprague–Dawley rats were used to establish normative data and serve as a control group for the following studies. Experiment 2: adult Fischer 344 rats were used because they differ from other strains of rat in that they have an aversion toward normally preferred NaCl solutions and a heightened amplitude of CT responses to sodium. Experiment 3: adult rats that were deprived of sodium from embryonic day 3 were used because they have decreased CT responses to sodium. In all experiments, the GSP was exposed in the tympanic bulla and neurophysiological responses were obtained to a series of solutions (NaCl; sodium acetate, NaAc; NH_4Cl; and sucrose) applied to the palate in water followed by NaCl and NaAc series in 100 µM amiloride. Responses were analyzed relative to an NH_4Cl response standard.

In all experiments, the GSP responded strongly to NaCl and sucrose, and there was substantial suppression of sodium responses by the sodium channel blocker amiloride. The amplitude of Fischer 344 GSP sodium responses was significantly higher and more strongly suppressed by amiloride than the responses of control rats. In contrast, rats with developmental sodium restriction did not differ from control rats in their GSP responses or amiloride sensitivity. The results collectively demonstrate that GSP responses share similarities with CT responses, such as sodium and amiloride sensitivity. However, there is strong indication that these two nerves differ in their development and in their regulation by environmental factors. This is particularly interesting because the GSP and CT share many neuroanatomical characteristics as well as neurophysiological properties.

Supported by NIH grants DC03500 (S.I.S.), DC02406 and DC00407 (D.L.H.).

195. Kinetics of second messengers in bitter taste

A.I. Spielman

New York University College of Dentistry, New York, NY, USA

Bitter taste is elicited by a diverse group of compounds. Accordingly, recent studies have implicated a variety of mechanisms in bitter taste transduction. One of the simplest mechanisms would involve a direct blockage of a potassium channel. Such is the signaling for potassium or quinine. Another mechanism may involve direct activation of G proteins, for instance by quinine. A widely used mechanism may involve the inositol trisphosphate pathway, generation of the second messenger IP_3 and release of intracellular calcium (sucrose octaacetate, strychnine, denatonium). The involvement of a gustducin-activated cyclic nucleotide phosphodiesterase in bitter taste transduction has also been proposed. This pathway would lower the level of cyclic GMP and cAMP. Finally, an additional mechanism may involve production of cyclic nucleotides (cAMP and cGMP) through inhibition of phosphodiesterases (caffeine, theophylline). Most of these mechanisms require rapid and transient increase or drop of specific second messengers, such as IP_3, cAMP and cGMP, respectively.

The current study reviews specific examples of second messenger formation in response to sucrose octaacetate, strychnine, denatonium, quinine, caffeine and theophylline. We have studied such events with the aid of the quench flow technique by monitoring subsecond kinetics in taste and nongustatory control tissue from mouse tongue (SWR strain). Second messenger activation starts as early as 25–50 ms after stimulation, peaks at 50–100 ms and returns to basline level within 100–500 ms after stimulation.

Our data demonstrate that second messenger formation is rapid, transitory and primarily gustatory tissue specific, although in some instances (caffeine, theophylline) nongustatory tissue also demonstrates a small degree of response.

This study was supported by NIH grant DE10754.

196. Do insects consider what they smell?

Nicholas J. Strausfeld

Arizona Research Laboratory Division of Neurobiology, University of Arizona, Tucson, AZ 85721, USA. e-mail: flybrain@neurobio.arizona.edu

Research on the neural basis of insect olfaction has been dominated by studies at either the peripheral level of the antennal lobe. However, it is likely that in arthropods, as in vertebrates, an animal's reaction to any olfactory signal can be dependent on other sensory modalities (including other odorants), as well as on experience, and on behaviors that are planned by the brain but not yet consummated.

Since 1850, there has been much speculation about whether insects are 'intelligent', meaning whether an individual is capable of performing a completely novel adaptive behavior. Researchers who have supported this notion single out social insects. And many of the same investigators single out a region of the brain that is larger in social insects than in non-social insects. This brain region comprises the paired mushroom bodies. Many recent investigators also claim that these centers are of crucial importance in learning and memory, thus adding to their mystique.

My laboratory has been investigating the cellular organization and physiology of the mushroom bodies in the cockroach *Periplaneta americana*. Here I report new results that demonstrate (i) common principles of mushroom body design across taxa; (ii) fundamental physiological properties of these centers in combining odorant information with information about other sensory cues; and (iii) a new hypothesis about the role of the mushroom bodies in behavioral choice. The last is suggested by the organization of mushroom body and antennal lobe outputs. The former carry contextual data about odorants to the lateral protocerebrum, where they converge with antennal lobe afferents carrying 'pure' odorant data. The confluence of these two information channels suggests that olfactory behavior cannot be interpreted on the basis of antennal lobe outputs alone. To comprehend the meaning of an odor to an insect requires experiments that will determine how the brain compares a pure odor with the same odor that has been contextualized with other modalities.

197. Olfactory receptors: expression patterns and topographic projections

J. Strotmann, S. Konzelmann, A. Beck, P. Mombaerts[1], P. Feinstein[1] and H. Breer

University of Stuttgart-Hohenheim, Institute of Physiology, D-70593 Stuttgart, Germany and [1]Rockefeller University, New York, USA

The notion that spatial segregation of olfactory input may be used to encode olfactory information was supported by the observation that stimulation with particular odors elicits distinct patterns of reactivity in the olfactory epithelium, indicative for a topographic pattern of responsiveness due to a spatial segregation of sensory neurons with distinct receptor types. Employing receptor specific probes in *in situ* hybridization studies, it has been shown that in the olfactory epithelium most odorant receptors are expressed in one of several broad, complementary zones (Strotmann *et al.*, 1994, Cell Tissue Res., 278: 11–20). Within their zones receptor-expressing cells are restricted to the layer of mature neurons in the lower two-thirds of the epithelium; however, it remains unclear whether they display any vertical or horizontal patterning within the epithelium. Detailed analyses revealed that neurons expressing a distinct receptor type are preferentially located in a particular laminar zone of the epithelium. In contrast to the broad distribution within the epithelium, the axonal projections of neurons expressing a particular receptor seem to converge upon common glomeruli within the olfactory bulb. This notion was recently

extended by a genetic approach which allows us to image the axons of individual olfactory neurons expressing a distinct receptor gene. It was possible to visualize that neurons expressing a given receptor project their axons to common target glomeruli within the bulb. Recently, a subfamily of receptor types was discovered which is expressed in neurons that are not restricted to one of the broad zones, but are clustered in a small region of the epithelium at a rather high density. While the axons of dispersed neurons converge onto a common glomerulus, could it be possible that the axons of clustered neurons diverge and project towards a variety of different glomeruli? This question was approached using the gene-targeting strategy which allows us to translate the receptor along with a reporter protein. Whole mount as well as serial section analysis will allow us to visualize and follow the projection of axons from the clustered population of olfactory neurons.

198. A mammalian olfactory receptor gene subfamily expressed in clustered sensory neurons

Jörg Strotmann, Anja Beck, Reiner Hoppe, Stefan Kubick, Peter Mombaerts[1], Paul Feinstein[1] and Heinz Breer

University of Stuttgart-Hohenheim, Institute of Physiology, D-70593 Stuttgart, Germany and [1]Rockefeller University, New York, USA

Most of the olfactory receptor subtypes are expressed in sensory neurons which segregate in rather broad rostro-caudal zones. The dispersed distribution of receptor-expressing neurons in large zones is difficult to reconcile with the local 'hot spots' of physiological responsiveness upon stimulation with distinct odors. It has recently been discovered that in rat one particular olfactory receptor gene exhibits a unique expression pattern; it is expressed only in neurons which are clustered in a small area of the nasal neuroepithelium. In comparative studies OR37-related genes were found in other rodents, too; these genes are also expressed in a cluster of cells located at a very similar position within the nasal cavity. Extended studies revealed that even in opposum, related genes exist and are expressed in neurons restricted to a small area of the epithelium. Southern blot analyses indicated that OR37-related genes appear to be present only in the genome of mammalian species; no such receptors were found in non-mammalian species, such as amphibia or fish (Kubick *et al.*, 1997, J. Neurochem., 69: 465–475). Molecular cloning approaches led to the discovery of several OR37-related genes which apparently constitute a small gene family. All five mouse OR37-subtypes were found to be expressed in the same small area at the tip of central turbinates. Alignment of receptor sequences revealed that the receptor protein encoded by the OR37-related genes exhibits some unique structural features, notably an extension of the third extracellular loop by an insertion of six amino acid residues. The encoded isoforms of the OR37-receptor show remarkable differences in the charged amino acids in their third extracellular loop; these structural differences may correspond to important functional variants. Genomic analysis revealed that there are at least five OR37-related genes which appear to be part of a receptor gene cluster on the same chromosome. Exploring the structural motifs in the upstream region of genes expressed in topographical clusters rather than in zones may provide some insight in the principles of gene regulation and the mechanisms governing the spatial expression of the OR37 receptors.

199. Effect of nature of learning and nature of stimuli on performance at a recognition test

Claire Sulmont, Sylvie Issanchou and E.P. Koster[1]

Laboratoire de Recherches sur les Arômes, INRA and [1]Centre Européen des Sciences du Goût, Dijon, France

In order to study the link between odour memory and food preference, we wanted to select subjects with a 'good' memory for odours and subjects with a 'bad' memory for odours. Odour memory of ~150 subjects was tested by a recognition test. This test consists of two sessions. During a first session, subjects smelled nine target odours. One week later, they had to recognize these odours among nine distractor odours. Recognition performance was determined according to signal detection theory (Banks, 1970, Psychol. Bull., 2: 81–99), i.e. by computing hit and false alarm rates in d' scores. The higher d' is, the better the odour memory. The effect of two factors on recognition performance was assessed: (i) nature of learning: during the first session, subjects are either given no special instruction (implicit memorization of odours) or are told to memorize the target odours (explicit memorization of odours); and (ii) nature of stimuli: odours were either familiar (subjects memorize and recall information seen in a particular context, the laboratory, but have encountered these odours in their everyday life) or unfamiliar (subjects memorize and recall new information, i.e. odours that they have never encountered previously).

Each subject performed thre recognition tests: an implicit recognition test (half of the subjects implicitly learned familiar odours and the other half unfamiliar odours), an explicit recognition test with familiar odours and an explicit recognition test with unfamiliar odours.

Results show that elderly subjects' performances were poorer than youngest subjects' performances. No sex effect was clearly observed. Recognition performances seemed to decrease from the first test to the last test. This decrease may be due to a lassitude of subjects. Subjects' performances between tests are not correlated. Implicit/explicit learning and familiar/unfamiliar odours seem to involved different mnesic systems. It is also possible that the recognition test is not reliable and could be highly dependent on the odour sets. Further studies may validate or may not these hypotheses.

200. Olfaction and affective state: is odor evaluation and perception altered in selective responsiveness to milk odors in human newborns? Influences of prandial state and stimulus familiarity

Robert Soussignan, Benoist Schaal[1] and Luc Marlier[2]

Laboratoire de Psychobiologie du Développement, Cognition et Communication, EPHE-CNRS, EP 1595, Paris, [1]Laboratoire de Comportement Animal, INRA-CNRS URA 1291, Nouzilly and [2]Laboratoire de Psychophysique Sensorielle, CNRS EP 618, Université Louis Pasteur, Strasbourg, France

A change in the nutritional state has been shown to modulate the hedonic polarity of tastes or food-related odors. The term alliesthesia has been coined to describe this phenomenon (Cabanac, 1971, Science, 173: 1103–1107.). For example, in adult humans, eating ad-lib meals produces a negative alliesthesia to

alimentary odors with a maximal unpleasant perception within 45–60 min following ingestion (Duclaux *et al.*, 1973, Physiol. Behav., 10: 1029–1033).

We examined whether olfactory alliesthesia is functional in 3-day-old bottle-fed infants (n = 14). We investigated the effects of a motivational shift (from hunger to satiety) on the responses of the newborns to artificial and food-related odors as a function of stimulus familiarity. In experiment 1, videotaped facial movements and autonomic (respiration and heart rates, RR and HR) responses to five olfactory stimuli (familiar regular formula, unfamiliar regular formula, protein hydrolysate formula, vanillin, control) were recorded 50 min before and after bottle-feeding during episodes of irregular sleep. RR discriminated the odor stimuli from the control stimulus, indicating clear olfactory detection. Furthermore, the subjects reacted with a greater HR change only when they were exposed to their familiar formula during the post-prandial condition. The measurement of facial movements with the Baby-Facial Action Coding System indicated that disgust actions were more often evoked by the odor of regular formulas (familiar or unfamiliar) than by the other olfactory stimuli during the postprandial condition. In experiment 2, untrained adult observers were able to decode differential hedonic signals from the videotaped infants' facial responses to the odors as a function of the infants' motivational state.

These findings reveal that a process of negative post-ingestive alliesthesia may operate during the first days of life.

201. Male sex pheromone perception in the female cockroach, *Nauphoeta cinerea*: difference in the number of glomeruli on right and left deutocerebrum

L. Sreng

CNRS, LNB 'Communication chimique', 31, Chem. Joseph Aiguier, F-13402 Marseille Cedex 20, France

The macroglomerular complex observed in males of many insect species has been implicated in perception of female sex pheromone. The purpose of this study was to compare the neuroanatomy (glomeruli) of antennal lobes (deutocerebrum) in male and female *Nauphoeta cinerea*. Unlike most cockroach species, female *Nauphoeta cinerea* perceive two sex pheromone signals—one for long-distance attraction and one for induction of copulation (aphrodisiac). Long-distance sex pheromone is produced by male sternal gland and aphrodisiac sex pheromone by male tergal gland. Both male sex pheromones are composed mainly of three compounds: acetoin, 2-methylthiazoline and 4-ethyl-2-methoxyphenol. We compared deutocerebrum in five females and five males and found no macroglomerular complex. Conversely, we did observe a difference in the number of glomeruli. In all males, almost the same number of glomeruli were observed on the right and left deutocerebrum: (92–102). In 4/5 females, the number of glomeruli was higher on the right than left deutocerebrum (97–103 versus 93–100). In one female, the number of glomeruli was the same on both sides but the right deutocerebrum was larger than the left. These results suggest that the presence of more glomeruli on the right deutocerebrum in females may be correlated with perception of male sex pheromone.

202. Predator-induced defences in *Daphnia*: kairomones are prey pheromones that needs activation by predators

Ole B. Stabell, Fortune Ogbebo and Raul Primicerio

Department of Aquatic Resources and Environmental Biology, NFH, University of Tromsø, Norway

Inducible defences based on chemical signals are common among aquatic animals. In particular, the phenomenon has been extensively studied in 'water fleas' (i.e. *Daphnia*). If parthenogenetic mother individuals are exposed to odours from predators when carrying their eggs, the resulting offspring will develop elongated tail or neck spines, or 'helmets' (i.e. a morphological change in head form), compared with offspring from unexposed *Daphnia*. Morphological changes of this kind are commonly denoted 'inducible defences', and appear to limit the chances of prey being captured by predators. The releasing chemical factors responsible for the morphological changes in prey animals have hitherto been classified as kairomones, since water extracts from injured *Daphnia* will not induce such morphological changes. We have shown that the signal substances in question are actually pheromones that originate from conspecific prey, but are present in the prey in an inactive form and are activated during the passage through the intestine of a predator. Our work incorporates primer pheromone effects of these chemical signals, but releaser effects in the form of behavioural alarm signalling are probably also present. The discovery demands the recognition of a new functional class of pheromones, which we have tentatively denoted 'quiescent scents'.

203. Exploring the microclimate: detection of carbon dioxide gradients by herbivorous insects

G. Stange

Visual Sciences, Research School of Biological Sciences, The Australian National University, Canberra, ACT 0200, Australia

Atmospheric carbon dioxide is a pivotal point of the carbon cycle to which all land organisms are linked. Respiring plants and animals constitute CO_2 sources and assimilating plant organs constitute sinks.Therefore, the ability to detect atmospheric CO_2 gradients is of adaptive value, and it is not surprising that it is widespread in land arthropods. In haematophages, CO_2-receptor neurons are consistently present and the gradients associated with plumes of the breath of vertebrate hosts are essential for long-range orientation.

Among winged herbivorous insects, sensory organs that detect CO_2 are particularly strongly expressed in the Lepidoptera; quite recently, CO_2 receptors were also identified in tephritid fruit flies. One function of those receptors could be the measurement of the metabolic activity of a host, and hence its suitability as a larval food source. In this case, it should be possible to modify the behaviour of ovipositing female herbivores by exposing them to artificial CO_2 gradients.

It is shown, for two complementary examples, that such gradients do indeed modify behaviour. Females of the moth *Cactoblastis cactorum* normally oviposit on their host, the cactus *Opuntia stricta*, at the time of day when the plant assimilates and thus forms a natural CO_2 sink. They prefer to oviposit on untreated host plants rather than on hosts that are exposed to

plumes of CO_2-enriched air. Females of the tephritid fruit fly, *Bactrocera tryoni*, normally oviposit on ripening fruits that are strongly respiring and thus form a natural CO_2 source. An artificial source that releases CO_2 at a rate comparable to the rate of release by a small fruit is sufficient, by itself, to attract flies and to evoke oviposition behaviour. In choice experiments, pieces of fruit that are exposed to plumes of CO_2-enriched air are preferred over controls.

204. Plant odour receptor neurons of the same types in two related moth species (*Heliothis virescens* and *Helicoverpa armigera*)

Marit Stranden, Tonette Røstelien, Anna-Karin Borg-Karlson[1] and Hanna Mustaparta

Department of Zoology, Norwegian University of Science and Technology, N-7034 Trondheim, Norway and [1]Organic Chemistry, The Royal Institute of Technology, Stockholm, Sweden. e-mail: marits@nvg.ntnu.no

Receptor neuron responses to plant odours have been recorded in two allopatric species of heliothine moths, *Heliothis virescens* and *Helicoverpa armigera*, by gas chromatography (GC) linked to electrophysiological recordings from single receptor neurons. Plant volatiles was collected by aeration of intact and cut plant materials, including host and non-host plants of sunflower, tomato, chili pepper, orange, wild briar, spruce and juniper. In addition, standards with synthetic compounds were used. Each neuron was tested for the various plant volatile mixtures, via both a polar and a non-polar column. The two columns, installed with a split at the end, led half of the effluent to the GC-detector and the other half over the insect antenna. The chemical structure of the potent compounds were then determined by gas chromatography linked with mass spectrometry (GC-MS). Most receptor neurons responded selectively to one or two components which were often present in several of the volatile mixtures. A large number (70–80%) of the neurons recorded from *H. virescens* showed selective response to the same component, appearing late in the gas chromatogram and shown by GC-MS to be a sesquiterpene. The same type of receptor neuron was also found most frequently in *H. armigera*, suggesting that this compound is an important odour cue for the two heliothine moths. Another receptor neuron type, responding selectively to two monoterpenoids, identified by GC-MS as *trans*-β-ocimen and β-myrcen, was also found in both species of moths. In this case, the neurons were retested with synthetic materials which confirmed the identification.

205. Taste adaptation during the eating of sweetened yogurt

Marjon J.M. Theunissen, Ilse A. Polet, Jan H.A. Kroeze and Hendrik N.J. Schifferstein[1]

Psychological Laboratory, Utrecht University, Heidelberglaan 2, 3584 CS Utrecht and [1]Marketing and Consumer Behaviour Group, Department of Economics and Management, Agricultural University, Hollandseweg 1, 6706 KN Wageningen, The Netherlands. e-mail: m.theunissen@fss.uu.nl

Taste adaptation, a gradual decline of taste intensity with prolonged stimulation, is frequently observed in laboratory experiments. However, during normal eating the taste of food does not seem to decrease or disappear. We studied whether taste adaptation occurs when subjects eat yogurt, sweetened with two concentrations of sucrose (3.75 and 7.5%). During 90 s subjects could eat as much yogurt as they wanted, and judged the taste intensity at 5, 35 and 95 s. In addition, we examined whether taste adaptation measured with a filter paper method is related to taste adaptation when eating yogurt.

During the eating of yogurt, sweetness intensity declined with time, whereas sourness intensity did not. This may be due to a higher rate of recovery from sourness adaptation. In addition, release from mixture suppression may have completely counteracted sourness adaptation, whereas the effect of release from sweetness suppression may be smaller than the effect of adaptation to sweetness.

The taste adaptation in the 'yogurt task' was only slightly correlated to taste adaptation measured with filter paper. During yogurt eating, the presence of saliva, the interactions between tastants and odorants and other factors influence the time course of taste intensity. Therefore, results from standard laboratory experiments about adaptation have limited relevance to the prediction of the time course of the taste intensity when eating real foods.

206. Inhibition effect in mixtures of butyric acid and R-(+)-pulegone modelled with the interaction model MBO

T. Thomas-Danguin, E. Rallet[1] and M. Chastrette[1]

Centre Européen des Sciences du Goût, CNRS UPR 9054 Groupe Physiologie de la Chimioréception, 15, rue Hugues Picardet, F-21000 Dijon and [1]Laboratoire de Chimie Organique Physique et Synthétique, CNRS UMR 5622, Université Claude Bernard Lyon 1, 43, Bd du 11 nov. 1918, F-69622 Villeurbanne Cedex, France

The MBO model which has been recently developed to account for the perceived odour intensity in binary mixtures is derived from the Hill equation previously used for pure compounds. It is based on the influence of the amount of one component on the perception of the second compound and vice versa. This interaction model has been successfully applied to published data concerning binary mixtures and especially those of butyric acid and R-(+)-pulegone (Moskowitz, 1979).

As previously observed by Moskowitz, the obtained modelling showed that there is an inhibition effect in mixtures of butyric acid and R-(+)-pulegone. However, predictions showed that there is a range of concentration of the two compounds, for which the inhibition effect is the highest. Moreover, the interaction parameters of the MBO model indicated that it is the R-(+)-pulegone that partially inhibits the perception of the intensity of butyric acid. On the contrary, the interaction parameter of the butyric acid indicated that there is no influence of butyric acid on the perception of R-(+)-pulegone.

In order to verify these predictions of the MBO model, we asked 28 subjects to evaluate the intensity of 20 mixtures of butyric acid and R-(+)-pulegone, chosen in the right range of concentration of the two compounds according to the model. The mixtures were presented to the subjects in 250 ml bottles containing a small ball of cotton wool. Intensity was evaluated on a graphic scale.

The results we obtained agreed with the data of Moskowitz, despite our using a different method. These experimental data also

confirmed the predictions of the MBO model and showed that there is a range of concentration of butyric acid and R-(+)-pulegone where the inhibition is maximum. This range had thus been predicted by the model and experimentally verified. Moreover, the experimental data we obtained confirmed the non-reciprocal inhibitory effect of R-(+)-pulegone on butyric acid.

This study confirmed that the MBO model could be very useful to study binary mixtures. On the one hand, it gives good representations of experimental data and also allows making predictions, such as find concentrations for a maximum inhibitory effect, and on the other hand, the interaction parameters of the MBO model could provide information on the inhibition process.

207. VNO receptors

Roberto Tirindelli, Lucia Silvotti, Sara Martini and Nicholas Ryba[1]

Istituto di Fisiologia Umana, Università di Parma, Italy and [1]National Institutes of Health, NIDR, Bethesda, MD, USA

Two large multigene families of putative G-protein linked receptors expressed in distinct subpopulations of neurons in the vomeronasal organ have been identified. These receptors probably mediate pheromone detection. The most surprising aspects of these findings are that there are so many receptors of two very different classes and that the receptors are unrelated to their counterparts in the main olfactory epithelium. This suggests that many active ligands are likely to exert effects through the vomeronasal organ. Parallel experiments addressing the nature of these ligands indicate a role for some proteins as well as small molecules as functional mammalian pheromones. In combination, these results start to suggest a molecular basis for mammalian pheromone signalling.

208. Central mechanisms for umami taste preference and aversion

Kunio Torii, Takashi Kondoh, Miro Smriga, Masato Mori and Taketoshi Ono[1]

Ajinomoto Co., Inc., Kawasaki and [1]Toyama Med. Pharm. University, Toyama, Japan

A preference for umami taste material, i.e. monosodium L-glutamate (MSG; <2.5% w/w) was observed when protein nutrition was within normal limits, regardless of rat strain. Once rats fell into protein malnutrition, i.e. deficiency for the essential L-amino acid lysine (Lys), preferences for Lys were found. Using functional MRI and an operant behaviour paradigm, we found that neurons in the lateral hypothalamic area (LHA) responded differentially to Lys and MSG when a Lys-deficient diet was offered. However, the neurons responded solely to 2.5% MSG when rats were fed a normal diet. These effects indicate that umami taste responsive neurons in the LHA may control the appetite for dietary protein and/or maintain protein homeostasis. Next, the concentration of L-glutamate in blood and brain are controlled all day, when rats are fed on high protein diet containing a large amount of L-glutamate. The aversion for MSG occurs whenever animals ingest MSG concentrated beyond its homeostatic control by the small intestine and liver. Aversion for MSG (>3% w/w) was examined. The degree of aversion was variable, depending upon the strain of rat tested. In Brown Norway (BN) rats an aversion was not found and Sprague–Dawley (SD) rats displayed weak aversion. In contrast, in Long–Evans Agouti (LEA) rats with hepatic dysfunction, aversions occurred. After dissection of gastric branches of the vagal nerve, MSG became aversive to SD rats, just like to LEA rats. This suggests that the vagus could suppress unpleasant symptoms caused by high concentrations of MSG at the gastric–pyloric area. This effect was confirmed by use of the conditioned taste aversion paradigm. A small volume of 5% MSG solution administered into the pyloric–duodenum area of LEA rats reduced saccharin solution intake as the unconditioned stimulus. These phenomena were protected by MSG dissolved in L-amino acids mixture, but were strengthened by coexisting L-glutamate metabolites. Thus, the aversion for highly concentrated MSG might be coupled with the homeostatic capacity of glutamate in the alimentary organs, mainly the liver.

209. Hormonal regulation of male and female mice olfaction

V. Troitskaya, S. Melnik and E. Nosova

Department of Zoology, State University, Nizhny Novgorod, Russia

It is well known that pheromones can regulate many physiological processes in animals and humans and, on the contrary, hormones can modulate changes in olfaction. This may be different perception of odours, or a lower or higher level of olfactory sensitivity. Changes of olfactory thresholds to isovaleric acid were investigated in male and female laboratory mice. We chose some stations of an animals life when their hormonal level was obviously different. Thresholds were fixed during the estrus cycle in females, after castration in males, in different age of males and females, and after injections of testosterone to females and castrated males. Females had maximal sensitivity to isovaleric acid during proestrus and minimal during metestrus ($P < 0.05$). Injections of testosterone broke the estrus cycle and caused an increase of the thresholds. In the first case changes of olfactory sensitivity were very rapid (within 4 days); in the second, the obvious changes could be registered only after 2 or 3 weeks of testosterone injections. The value of thresholds to isovaleric acid of intact males decreased after castration, but it was a long process (>3 months). Injection of testosterone deleted the effect of castration almost totally. We noticed that individual sensitivity of males to isovaleric acid differed significantly and had a high correlation with anogenital distance (–0.89). That means that olfactory sensitivity depends on individual hormonal levels, because there is a correlation between the anogenital distance and the hormonal level. Thresholds of isovaleric perception depended also on the age of the animals. Animals 1 month in age had the lowest thresholds, those of 10 months were significantly higher. The results of these experiments show that there are evidently some mechanisms of hormonal regulation of olfaction. The first of them is the regulation of the neurogenesis in the olfactory epithelium, and the result of this regulation is changes in an amount of the olfactory receptor cells. The other mechanisms may be connected with the processes in olfactory receptors or in the central nervous system.

210. Morphology and electrophysiological properties of hamster vomeronasal receptor cells

D. Trotier and K.B. Døving[1]

Neurobiologie Sensorielle, 1 avenue des Olympiades F-91300 Massy, Physiologie Oro-Faciale, JE 359, Université Paris 7, 2 place Jussieu, F-75251 Paris, France and [1]Department of Biology, University of Oslo, Box 1051, N-0316, Norway. e-mail: trotier@bisance.citi2.fr

We isolated microvillous receptor neurons by enzymatic dissociation of male hamster vomeronasal organs. Isolated neurons kept their natural bipolar shape, with a short axon, and presented numerous microvilli at the apex of the dendrite. The length of the dendrite varied from ~30 to 140 µm.

Electrophysiological recordings were obtained by sealing (>40 GΩ) the tip of the patch pipette on the somatic membrane. Cell-attached recordings indicated that most cells were nearly silent at rest, with only occasional and transient depolarizations inducing spiking. Resting potentials, measured in the whole cell configuration at room temperature, ranged from about –60 to –75 mV. Sometimes spontaneous opening of ionic channels transiently depolarized the membrane which elicited full size action potentials.

Current clamp experiments illustrated the high sensitivity of these neurons to very small depolarizing currents, suggesting that transduction currents in the range of only 1–5 pA could be operational. The duration of the repetitive firing observed in tonic cells in response to low current intensities (1–3 pA) decreased for larger intensities. Some cells elicited repetitive action potentials during the injection of 10 s depolarizing ramps of current as low as 0.1 pA/s. Cells of the phasic type elicited only a few initial action potentials during step depolarizations.

Various membrane conductances activated by membrane depolarization have been consistently observed and characterized in voltage-clamp conditions. Hyperpolarization of the membrane revealed the presence of the inward rectifying current H and another inward rectifying current.

Intracellular injection of IP_3, a putative second messenger involved in the reception of aphrodisin, a natural pheromone or pheromone carrier for male hamster, elicited an inward transient current in some cells.

211. Host-odour sensitivity in female *Anopheles* mosquitoes with different host preferences

Ingrid V.F. Van den Broek and Cornelis J. Den Otter

Group Sensory Organs and Behaviour, Department of Animal Physiology, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands. e-mail: broekivf@biol.rug.nl

Single sensillum recordings were performed from sensilla trichodea and grooved pegs on the antennae of female *Anopheles* mosquitoes. Responses to vapours from acetic, propionic, butyric, isobutyric and isovaleric acid, 1-octen-3-ol, 3- and 4-methyl-phenol, DL-lactic acid, ammonium hydroxide, butylamine, acetone and water were recorded.

In recordings from sensilla trichodea, spikes from one or two olfactory cells were seen. The majority of these cells responded upon stimulation with the fatty acids, 1-octen-3-ol and the phenols. On stimulation with fatty acids and phenols either excitation or inhibition of spike activity was found, whereas the responses to 1-octen-3-ol were always excitatory. Some trichodea cells responded with shortlasting excitation to ammonium hydroxide, butylamine or acetone. The trichodea cells did not respond to DL-lactic acid and water.

In recordings from grooved pegs spikes from different cells could not always be separated. High excitatory responses were found on stimulation with ammonium hydroxide, acetone and butylamine, whereas weak responses to L-lactic acid (excitatory) and fatty acids (excitatory and inhibitory) were recorded. Approximately two-thirds of the grooved pegs responded to water with an increase in spike activity. The sensilla did not respond to 1-octen-3-ol and the phenols.

A comparative study was done on the sensitivities of sensilla trichodea of four Anopheles species with different host preferences, namely the anthropophilic *Anopheles gambiae* s.s., the zoophilic *A. quadriannulatus* and *A. maculipennis* atroparvus, and the more opportunistic *A. arabiensis*. It appeared that the anthropophilic species is more sensitive to fatty acids, while the two zoophilic *Anopheles* species respond stronger to 1-octen-3-ol. The sensilla trichodea cells of the opportunistic *A. arabiensis* showed responses which were intermediate between those of the anthropophilic and zoophilic species.

At present, a comparison is made of the sensitivities of grooved pegs in *A. gambiae* s.s. and *A. quadriannulatus*. Furthermore, responses of the two types of sensilla to cow manure, urine and skin wash and limburger cheese (attractive for *A. gambiae*) are studied.

212. Do accelerated aging tests for beer give the same sensory profile as natural aging?

Bonnie M. van der Pers-King

Quest International Nederland BV, PO Box 2, 1400 CA Bussum, The Netherlands

Brewers, especially those who export their products, would like to increase flavor stability. Beer staling is a common problem characterized by complex changes in flavor that correspond to the vast diversity of fresh beer flavors. Most lagers show a typical disruption in the bitter:sweet ratio such that bitterness decreases with age and sweetness increases. The sweet impression is often enhanced by the development of caramel flavor, which can also be described as toffee or burned sugar. These changes develop naturally over the months of storage that usually elapse between production and consumption. In order to establish shelf-life as quickly and as accurately as possible, forced aging treatments are applied and models are constructed to relate the two aging conditions.

Beer was forced-aged by storing it at 40°C. Concentrations of iso-α-acids were determined by high performance liquid chromatography at regular time intervals and compared with the concentrations obtained under natural aging at 19°C. The aging model obtained from these data was evaluated by difference testing and by comparing sensory profiles of beer aged naturally and beer aged using the accelerated conditions. There is reason to believe that forced aging increases the rate of reaction for some staling mechanisms such that the resulting imbalance of flavor compounds results in a different sensory impression than that obtained by natural aging.

213. Olfaction/emotion connexion: comparison between autonomic and verbal responses

Evelyne Vernet-Maury, Ouafae Alaouï-Ismaïli, Sophie Rousmans, Olivier Robin, Georges Delhomme[1] and André Dittmar[1]

Université Claude Bernard Lyon I, CNRS Sciences de la Cognition and [1]Microcapteurs Microsystèmes Biomédicaux, CNRS-LPM, INSA Lyon, Bat. 401, F-69621 Villeurbanne Cedex, France

Human emotion and action are embodied phenomena and the responses of this corporeal body can help reveal the mechanisms underlying human behavior and especially decision making. Autonomic nervous system (ANS) activity was recorded in 60 subjects in response to 12 odorants. After the experiment, subjects were instructed to situate odorants on an 11-point hedonic scale and to define what type of basic emotion was evoked by each stimulation. Taking into account previous results, ANS analysis allowed the determination of the basic emotion corresponding to each odorant. ANS evaluation showed that pleasant odorants induce mainly happiness and surprise and unpleasant ones evoke mainly anger, disgust and even fear. These results first confirm the correlation recently found between hedonic scores and basic emotions estimated from the ANS pattern. These findings support the hypothesis that pleasant odorants can improve mood and increase various types of approach behavior. Secondly, these results again evidenced the fact that autonomic response can distinguish between basic emotions. The second train of results of this study and the main one is the congruence (fitness) between subjective and objective evaluation related to pleasantly connoted odorants as opposed to a weak correlation related to unpleasant odorants. This result, i.e. opposition between pleasant and unpleasant-connoted odorants, is reinforced by that obtained with eugenol odorant which is variably scored on the hedonic axis and shows a tendency to be significantly different. In conclusion, these results show quite a good correlation between conscious (verbal) and unconscious (ANS) basic emotions when positive emotions are analyzed, while with regard to negative emotions, autonomic estimation provides a better estimation as more different emotions were evidenced. This may be explained by the fact that inducing emotion in the laboratory is ethically problematic. Emotions are private events. They are often associated with a loss of control and can activate memories of past individual experiences. Moreover, negative emotions are painful experiences that subjects might repress in their verbal expression. The main difference concerns anger: while this represents the main negative emotion revealed by analysis of autonomic responses, it does not appear to be easily expressed through the verbal channel. One limitation may be the verbal paucity of subjects in describing odorants, emotions and some cognitive inhibitory phenomena that further research would have to explain.

214. Apoptosis in the development and morphogenesis of the vomeronasal organ and olfactory epithelium of mouse

Samuele Voyron, Paola Cappello, Paolo Giacobini, Glauco Tarozzo and Aldo Fasolo

Department of Human and Animal Biology, University of Torino, via Accademia Albertina 17, I-10123 Torino, Italy

This study provides further information upon differential cell proliferation and cell death process by apoptosis occurring during mouse development, both in the vomeronasal organ (VNO) and in the main olfactory epithelium (OE). Apoptotic cells were detected by *in situ* nick translation (ISNT) and Tdt-mediated dUTP nick end labelling (TUNEL). Using the TUNEL method we demonstrate that dying cells are very abundant in non-sensory vomeronasal organ (NS-VNO), particularly in early stages of development, while in sensory epithelium (S-VNO) the apoptotic index does not considerably change, at least until birth. The cell proliferation, studied with bromodeoxyuridine (BrdU) labelling, appeared randomly distributed throughout the VNO of fetal mice. The adult-like confinement of proliferating cells at the boundaries between S-VNO and NS-VNO was achieved only after birth. Quantitation of BrdU-labelled cells indicates that proliferation is rather stable in both components during morphogenesis. Our data, therefore, demonstrate the occurrence of differential processes of apoptosis and proliferation during development of the mouse VNO.

At all the stages investigated (E12–E19, P1, P8, adult), INST-labelled cells and TUNEL-labelled cells were encountered in the OE. We show that the apoptotic process presents two peaks, one quite early during the invagination of the olfactory placode and the second one at E16. The second peak is followed by a sharp decrease from E18 to E19 and down to low values in the postnatal (P1, P8) and adult stages. Double-labelling experiments show that apoptotic cell death involves not only OMP-immunoreactive (IR), carnosine-IR mature olfactory neurons but also GAP43-IR immature olfactory neurons. According to our data, two distinguishable processes of cell death control can be envisaged: one taking place when the olfactory epithelium is not yet or poorly connected to the olfactory bulb, and one more strictly dependent on the interplay between the olfactory neurons and the olfactory bulb.

Supported by MURST, Cavalieri Ottolenghi Foundation and CNR.

215. Plasticity of rodent chemical communication

Vera V. Voznessenskaya and Charles J. Wysocki[1]

Institute of Ecology & Evolution, Russian Academy of Sciences, 33 Leninski prospect, 117071 Moscow, Russia and [1]Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104, USA

Unlike many other sensory systems, nasal chemoreception appears to be dynamic across the lifetime of an organism. Individual experiences with odors interact with genetic propensities to yield a measurable phenotype. Using rodents as model systems, we have determined that inbred strains of mice provide an excellent genetic model of variation in sensitivity to odorants.These mice could be sensitized to the pig pheromone, androstenone (AND), regardless of their initial level of sensitivity to the compound. Such induction is correlated with changes in the sensory epithelium as determined biochemically and electrophysiologically. Using different, unrelated odorants and complex mixtures, such as conspecific and heterospecific urine, we have shown that induced olfactory sensitivity is a general phenomenon, which reflects the plasticity of animal chemical communication (Voznessenskaya *et al.*, 1995). Modulation of olfactory sensitivity by environmental factors as well as induction of sensitivity to odorants and con- and heterospecific excretions considerably influences an organism's adaptability. Manipulation with odor environment in *Mus musculus* and *Rattus norvegicus* revealed an existence of critical

period in neonatal development for imprinting odor. Relative to age-matched controls, AND exposures during days (14–28) after birth produced a >600-fold increase in sensitivity to AND, while the same exposures during adulthood or during days 1–16, 28–42 after birth produced a 16-fold increase in sensitivity relative to the same control group. The present results are consistent with previous findings with rats and mice, which revealed a similar critical period during days 14–28 for affecting sensitivity to and recognition of individual-specific urine in adulthood (Sokolov and Voznessenskaya, 1997). Induced sensitivity to odorants persisted for relatively long periods. In our experiments with AND elevated sensitivity to the compound was recorded 8.5 months after exposures were completed. Exposures to odorants of older animals (1 year or older) did not considerably affect sensitivity to these odors. Hence, it appears that there is a critical period 2 weeks after birth, just after the eyes open, during which odor imprinting can be modulated. Developmental changes in olfactory sensitivity during different life periods are discussed.

216. The role of early olfactory experience in sensitivity to mammalian pheromone androstenone

Vera V. Voznessenskaya, Natalia Yu. Feoktistova and Charles J. Wysocki[1]

Institute of Ecology & Evolution, Russian Academy of Sciences, 33 Leninski prospect, 117071 Moscow, Russia and [1]Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104, USA. e-mail: vera@voznes.msk.ru

Neonatal exposures to odorants influence function of the olfactory system to produce changes in responses to these stimuli. Modulation of olfactory sensitivity by environmental factors as well as induction of sensitivity to some odorants considerably influences an organism's adaptability. Our previous findings with rats and mice revealed a critical period during days 14–28 after birth for affecting sensitivity to and recognition of individual-specific urine in adulthood. Exposures of CBA/J mice (highly sensitive to androstenone, AND) to AND during different periods in early ontogenesis also confirmed the existence of critical period for imprinting odor. To further explore the phenomenon of sensitization to odorants we exposed NZB/B1NJ (NZB) mice, which are insensitive to AND (the difference in AND sensitivity between CBA and NZB mice is at least 2000-fold), to 0.1% AND during days 1–14 ($n = 8$), 14–28 ($n = 8$) or 28–42 ($n = 8$) after birth. Similarly exposed adult NZB mice (9–11 weeks of age; $n = 8$) served as controls. NZB mice exposed to the diluent during days 14–28 ($n = 8$) were additional controls. Y-maze training and testing commenced 5 weeks after exposures. Fluid deprived mice were trained to avoid the AND-containing Y-maze arm. Quinine-tainted water, paired with AND, produced the aversion. Sweet water was the reward for correct choices during training and testing trials. AND-exposures during days 1–14 to NZB mice did not affect AND sensitivity; thresholds did not differ significantly from either control group. Relative to the age-matched controls, AND-exposures during days 14–28 produced a >17-fold increase in sensitivity to AND, while the same exposures during adulthood produced a 2- to 4-fold increase in sensitivity relative to the same control group. AND exposures during days 28–42 also produced a 2- to 4-fold increase in sensitivity. Though for highly sensitive to AND CBA mice we obtained a much greater increase in sensitivity after exposures during the critical period (>600-fold), our present results are consistent with previous findings. Regardless of the initial level of sensitivity to the odorant, the most profound effect was observed after exposures during 2 weeks after birth, just after the eyes open.

Supported by NIH TW00495, DC00298 and DC00214, and RFBR 960451060 and 980448864.

217. Functional expression of human odorant receptors

Ch. Wetzel, Ch. Wellerdieck, M. Oles, G. Gisselmann and H. Hatt

Ruhr-Universität Bochum, Department of Cell Physiology, Universitätsstraße 150, D-44780 Bochum, Germany

The ability of the mammalian olfactory system to detect and discriminate thousands of different odorants has led to intensive efforts to understand the underlying molecular mechanisms. The first members of the putative olfactory receptor gene superfamily were cloned from the rat (Buck and Axel, 1991, Cell, 65: 175–187). Evidence that this family encodes odorant receptors causes from the exclusive expression in the olfactory epithelium.

Ca-imaging experiments (furA-2), and immunohistochemical and Western blot results indicated that we were able to express odorant receptors of the zebrafish and of the nematode *C. elegans* functionally in human embryonic kidney cell line (HEK 293) (Wellerdieck *et al.*, 1997, Chem. Senses, 22: 467–476).

We constructed a eukaryotic expression vector, pSMyc, which contains a CMV promoter and a membrane import sequence followed by a *myc*-tag. The receptor encoding DNA has been cloned into this vector to reveal a fusion protein tagged at the extracellular N-terminal site. HEK 293 cells have been transfected with these plasmids. For further investigations stable cell lines have been established.

Here we provide the first documentation of a functional expression of a human (OR 1740) olfactory receptor protein. Application of a mixture of hundred different odorants elicited a transient increase in intracellular [Ca] at stable or transient transfected HEK 293 cells. By subdividing the odorant mixture into smaller groups we could identify a single component which represented the only effective substance: helional. Testing some structurally closely related molecules, we found only one other compound which also could activate the receptor (heliotropyl acetone). In contrast, mock transfected cells or cells transfected with fish receptors showed no changes in intracellular [Ca]. In the presence of PLC blocker (U73122) the response was abolished.

Immunohistochemical experiments transfected cells, which have been treated with the 9E10 antibody against the extracellular *myc*-tag, showed a staining of the cell membrane. Transfected cells were harvested and analyzed by SDS-PAGE and in Western blot experiments. Treatment with the 9E10 antibody revealed a 50 kDa band representing the odorant receptors. Recently we could also get a functional expression of OR1740 receptor protein in *Xenopus laevis* oocytes. Oocytes injected with the mRNA of the odorant receptor responded under voltage clamp conditions to the application of helional.

We thank Henkel AG for providing us with the odorant mixtures. Work supported by the DFG (We 2298/1-1).

218. Naming pictures, naming smells

Theresa L. White[1,2] and Steve Van Toller[2]

[1]Clinical Olfactory Research Center, Syracuse, NY, USA and [2]University of Warwick, Coventry, UK

Traditionally, a lower overall memory performance level is associated with olfactory stimuli when compared with visual stimuli (e.g. Davis, 1975, J. Exp. Psychol.: Hum. Learn. Mem., 134–142; Lawless, 1978, Percept. Psychophys., 493–495; Larsson & Bäckman, 1993, Psychol. Aging, 582–588). Since poor labels can lead to a deterioration of performance on an olfactory recognition task (Engen and Ross, 1973, J. Exp. Psychol., 221–227), it may be possible to account for the memory performance difference between visual and olfactory stimuli through level of verbalization. Two experiments were designed to examine the verbalizations associated with verbal and olfactory stimuli.

The first experiment examined labeling consistency, or the ability to apply the same label to the same stimulus on more than one occasion. Subjects in this experiment were asked nine times to verbally generate a single label for each of 10 (either olfactory or visual) stimuli that would 'help to remember it later'. Responses were transformed to a measure of information transmitted for each subject in each group. Average information transmitted for the pictures (2.74 bits) was significantly (*t*-test, $t = 6.08$, df = 18, $P < 0.01$) greater than for the odorants (1.61 bits). This result suggested that people were much more accurate and reliable at naming the pictures than the odours.

The second experiment was identical to the first experiment, but was performed after subjects had extensive experience (4 h during which they performed a separate task that did not involve naming) with the stimuli to be labeled. Although the information transmitted increased for both types of stimuli in comparison to experiment 1, the information transmitted by the pictures (3.26 bits) remained significantly higher (Student's $t = 10.37$, df = 18, $P < 0.01$) than that of the odours (1.99 bits).

The discrepancy in consistency of verbalization between visual and olfactory stimuli suggests that the differential recognition performance in previous literature may be accounted for (at least in part) by differential verbalization.

Supported in part by NIH grant number 9-PO1 DC00220.

219. The projections of physiologically identified and morphologically characterized olfactory sensory neurons to the macroglomerulus (MGC) of male *Antheraea polyphemus* (Lepidoptera: Saturniidae)

Les Williams[1,2], George Kumar[2] and Bill Hansson[3]

[1]Zoologisches Institut, Neurobiologie, Ludwig Maximilians University, Luisenstraße 14, D-80333 München, [2]Max-Planck-Institut für Verhaltensphysiologie, D-82319 Starnberg, Germany and [3]Department of Ecology, Lund University, Helgonavägen 5, S-22362 Lund, Sweden

About 60 000 olfactory trichoid sensilla (hairs) are distributed over the branches of the entire antenna of the male silkmoth *Antheraea polyphemus*. In general the long hairs house the dendrites (one thick, the other thin) of two receptor neurons and the short hairs, three (one thick and two thin). The axons of these neurons project to a specialized neuropile region of the antennal lobe, the macroglomerular complex (MGC). Primary processing of information about both the conspecific female sex pheromone blend and interspecific pheromone components occurs here. Anatomically the MGC can be separated into three basic regions of neuropile: glomerulus a (or cumulus), glomerulus b (or toroid) and the accessory glomeruli c. An acetate, (*E*,*Z*)-6,11-hexadecadienyl acetate (AC1), which excites the thicker dendrite, and an aldehyde (*E*,*Z*)-6,11-hexadecadienal (AL), exciting one of the two thinner dendrites, are the two volatile components of the sex pheromone blend of *A. polyphemus*. A third component, (*E*,*Z*)-4,9-tetradecadienyl acetate (AC2), first identified in the related species *A. pernyi*, can excite the second thin dendrite. Using cobalt-lysine filled microelectrodes in combination with the tip-recording method, the cells reponsive to these three components were identified and their projection patterns were traced to the antennal lobe. Each physiologically identified termination was found to correspond to one or other of the three basic morphological forms, namely, 'bifid', 'chandelier-like' and 'stellate or simple', discernable in the MGC. The following findings are significant:

- AC1 sensitive neurons have 'bifid' terminal morphologies.
- AL sensitive neurons have 'chandelier-like' terminal morphologies.
- AC2 sensitive neurons have 'stellate or simple' terminal morphologies.
- AC1 and AL terminations are found in roughly equal proportions in each of both the a and b glomeruli, but are not present in the c glomerulus.
- AC2 terminations have been found in the b and the c glomeruli, never in the a glomerulus.

These features firstly, allow recognition of the different terminations in mixed neuropile, and secondly, have important implications for the organization of the first-order olfactory interneurons within the subcompartments of the MGC.

220. Structural and immunohistochemical properties of human taste buds during development

M. Witt, M. Kasper and K. Reutter[1]

Department of Anatomy, Technical University Dresden and [1]Department of Anatomy, University of Tübingen, Germany. e-mail: mwitt@rcs.urz.tu-dresden.de

The conditions and mechanisms under which taste bud primordia develop are only poorly understood. This work will review structural and immunohistochemical properties of human taste buds and their adjacent tissues, including mesenchyme, non-gustatory epithelium and nerve fibers during embryonic and fetal development. By postovulatory week 8, nerve fibers invade the lingual epithelium, from which different types of taste bud cells differentiate, the latter forming onion-shaped cell clusters. The disrupted basal lamina allows the interaction of the early-forming gemmal with mesenchymal cells. At the surface of the epithelium, epithelial cells separate the taste bud anlage from the external environment until approximately week 13–15, when most taste pores develop. The importance of the interface between the taste bud anlage and both the non-gustatory surrounding epithelium and mesenchyme is evidenced by the distinct immunoreactivity of marginally located taste bud cells (perigemmal cells) to vimentin

and CD44. Vimentin is an intermediate filament protein that occurs abundantly in derivatives of mesoderm, but not regularly in epithelial tissues. One of the factors initiating or accompanying vimentin expression in epithelial cells may be discontinuities or 'disturbances' in epithelium during development or dedifferentiation. We observed a distinct immunoreactivity to vimentin mainly in perigemmal epithelial cells of developing taste buds. The immediate adjacency of a taste bud anlage to perigemmal cells might reflect a common role of these cells with regard to cell lineage. On the other hand, vimentin expression also may be a sign of dedifferentiation in aging gemmal cells, some of which perhaps migrate to bud periphery. Another example of the relation of taste bud anlagen with neighboring tissues is the molecular expression of intercellular matrix proteins, such as hyaluronic acid. Our results indicate a role for the hyaluronate receptor CD44 in epithelial remodeling during increased cell turnover that is distinctly different from that seen in mature taste buds. The localization of CD44 and vimentin in perigemmal cells of early taste bud anlagen suggests these cells are intimately involved in the process of early taste bud differentiation.

221. Patch clamping of mouse taste cells *in situ*

Kiyonori Yoshii

Department of Biochemical Engineering and Science, Kyushu Institute of Technology, Iizuka, Fukuoka 820-8502, Japan. e-mail: yoshii@bse.kyutech.ac.jp

We investigated the localization, distribution and pharmacology of voltage-gated channels of mouse taste bud cells under *in situ* voltage-clamp conditions. The *in situ* recordings we developed consisted of a peeled tongue epithelium mounted on a platform placed under the water-immersed objective of an upright microscope. A physiological saline solution irrigated the basolateral membranes of taste bud cells, and taste stimuli or deionized water irrigated their receptor membranes. The peeled epithelium on the platform separated these solutions, and protected basolateral membranes from deionized water and taste stimuli. Taste bud cells adapted to deionized water evoked action potentials or voltage-clamp currents in response to 10 mM HCl, 200 mM NaCl or 10 mM quinine dissolved in deionized water. Mouse taste bud cells evoked TTX-sensitive Na currents, TEA-sensitive K currents, and LVA and HVA Ca channel currents on depolarization, and TEA-sensitive inward rectifier currents on hyperpolarization. The HVA currents were sensitive to 'omega'-Aga IVA, 'omega'-CgTx GVIA and DHPs. The application of blockers on basolateral membranes blocked the respective voltage gated currents, whereas that on receptor membranes had no effect. Increased Ba^{2+} or Ca^{2+} ion concentration on basolateral membranes enhanced LVA and HVA currents but the increase on receptor membranes left these currents unchanged. These results indicate that almost all of these voltage-gated channels exist on basolateral membranes, which are different from amphibian taste cells. In each taste bud, voltage-gated channels distributed in a ring shape. When single taste buds are divided into four concentric circles with the same width, the magnitude of Na, K and inward rectifier currents recorded in the second circle from the center (first) one were significantly larger than those recorded in the outer (fourth) one. Also, HVA currents obtained from the third concentric circle were significantly larger than those from the center and the outer one. Na, K and inward rectifier channels generate action potentials, and HVA Ca channels are needed to release neurotransmitters. The present results thus show that many taste receptor cells exist in the middle concentric circles. We discuss the mechanism of how to control the distribution of these voltage-gated channels in each taste bud.

222. Immunohistochemical investigations of the mouse vomeronasal organ during postnatal development

Carlo Zancanaro, Carla Mucignat-Caretta[1], Flavia Merigo, Andrea Cavaggioni[1] and Francesco Osculati

Institute of Anatomy and Histology, University of Verona and [1]Institute of Human Physiology, University of Padua, Italy

The vomeronasal organ (VNO) starts to develop at embyonic day 12, but its maturation goes on in the postnatal life up to the third month in order to become fully competent for its complex bio-behavioural functions.

This work investigated, by means of immunohistochemistry, the developmental steps of the VNO from postnatal day 1 to 2 months. Mice aged 24 h, 8, 15 and 21 days, and 2 months were anesthetized with ether and barbiturates, and perfused through the heart with 4% paraformaldehyde. The head was dissected out, decalcified with 2.75 or 3.72% EDTA in 0.1M phosphate buffer and embedded in paraffin. Seven-millimeter sections were deparaffinized, and immunohistochemistry performed according to standard methods using primary antibodies against nitric oxide synthase (NOS), neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), atrial natriuretic peptide (ANP), neuron specific enolase (NSE) and protein gene product 9.5 (PGP).

Results show that the vomeronasal neuroepithelium is already labeled with the anti-PGP and anti-NSE antibodies at 24 h of age. The antibodies for neuropeptides did not react with the sensory epithelium at any age; however, sparse labeled fibers where found in the tonaca propria beneath the neuroepithelium. In particular, CGRP immunoreactive (ir) fibers were evident there at 21 days and 2 months. CGRP-ir fibers were also found at that age in the tonaca propria of the non-receptor epithelium, together with intra-epithelial fibers. The region of vomeronasal glands was labeled with the anti-NPY antibody at 24 h, then also ANP-, GGRP- and NOS-positive fibers appeared. At 24 h of age the anlage of the vomeronasal vascular pump showed some staining with the anti-NOS, -NPY and -ANP antibodies. The staining with the anti-NOS and -ANP antibodies then increased up to 2 months, when GCRP-ir fibers were also clearly found around pump vessels.

According to these results the vomeronasal epithelium contains mature neurons at 24 h of age. In the tonaca propria of the neuroepithelium nerve fibers probably originating from elsewhere are present, which contain regulatory peptides. The development of the vomeronasal pump, which take place during postnatal life, is associated with the expression of NOS and ANP. In the VNO the full expression of CGRP in nerve fibers is delayed at least to the second month of extrauterine life.

223. Characterization of a putative pheromone receptor showing affinity to the pheromone binding protein and the main pheromone component of *Antheraea polyphemus*

Gunde Ziegelberger and Rosario Maida

Max-Planck-Institut für Verhaltensphysiologie Seewiesen, D-82319 Starnberg, Germany

The highly specific and sensitive sex pheromone response of the male silkmoth *Antheraea polyphemus* is believed to depend on the activation of specific receptor proteins present in the dendritic membrane of pheromone sensitive sensilla trichodea. Our strategy in searching for these postulated receptors was based on the presumed affinity between the pheromone carrying pheromone-binding protein (PBP) present in the sensillum lymph and the membrane-bound receptors. We incubated native, Sepharose-coupled PBP (nPBP) with detergent lysates of olfactory hair homogenates and used the fractions obtained from the affinity column in binding assays with tritiated (*E*,*Z*)-6,11-hexadecadienyl acetate, the main component of the female sex pheromone. One membrane-specific protein of the nPBP-bound fraction was able to bind the radiolabeled pheromone, as detected by autoradiography or by the c.p.m.-profile of a sliced native gel. This pheromone receptor candidate was specific to male sensory hairs and not present in female antennae or brain.

In another set of experiments we used recombinant PBP (rPBP) for the affinity chromatography in the presence of a single pheromone component. When (*E*,*Z*)-6,11-hexadecadienyl acetate was present, a male sensory hair specific protein with an apparent molecular weight of 70 kDa was eluted from the rPBP-Sepharose column. In the presence of the minor pheromone component (*E*,*Z*)-6,11-hexadecadienal, no protein was recovered from the affinity column. Analogous experiments using female antennae did not reveal any protein with affinity to rPBP.

In both sets of affinity chromatography a male and membrane specific protein was captured which is the first putative pheromone receptor that shows binding to the adequate stimulus.

224. Epithelial application of the probable alarm pheromone results in responses of goldfish olfactory bulb relay neurons

H. Peter Zippel and Susanne Wilcke

Physiol. Inst. der Universität, Humboldtallee 23, D-37073 Göttingen, Germany

The fish alarm pheromone system is characterized by distinctive epidermal club cells that contain the alarm pheromone, probably Hypoxanthine-3(N)-oxide. Physiological responses from both types relay neurons (mitral cells, MC; ruffed cells, RC) were recorded extracellularly and simultaneously in the plexiform layer using a single tungsten microelectrode (AM 5770; 10–12 MΩ). Stimuli [hypoxanthine-3(N)-oxide, 10^{-7}–10^{-13} M; hypoxanthine, 10^{-7}–10^{-9} M; a preovulatory pheromone: 17,20α-dihydroxy-4-pregnen-3-one, 10^{-9} M; an ovulatory pheromone: prostanglandin $F_{2\alpha}$, 10^{-7}–10^{-9} M; a food stimulus: Arg 10^{-7}–10^{-10} M; and a control stimulus: hydrocortisone 10^{-9} M) were respectively applied for 15 s to the olfactory epithelium. During the 180 s interstimulus intervals a laminary water current of similar velocity (1 ml/s) was applied. During interstimulus intervals MC responded with higher, and frequently burstlike impulse rates. The impulse rates of RC were low, and each RC potential triggered a summed granule cell (GC) potential. During stimulation excitation of MC resulted in the simultaneous inhibition of RC, and inhibition of MC in excitation of RC. Even the lowest concentration resulted in significant and contrasting interactions in relay neurons. In contrast to EOG recordings, application of the probable alarm pheromone (which had no recordable effect in EOG; Sorensen, personal communication) resulted in a similar effectiveness as the preovulatory and the ovulatory pheromones, and the amino acid. Hypoxanthine was a slightly lesser effective stimulus, and application of the control stimulus hydrocortisone rarely resulted in recordable effects.

Supported by DFG, Zi 112/7-1.