ABSTRACTS

Twelfth Congress of European Chemoreception Research Organisation (ECRO XII)

Held in Zurich, Switzerland, August 25–31, 1996

1. Receptors and signalling pathways in olfactory neurons

Heinz Breer

University Stuttgart-Hohenheim, Institute of Zoophysiology, Garbenstraße 30, 70599 Stuttgart

Olfactory sensory neurons detect and discriminate thousands of odorous compounds with remarkable sensitivity and specificity; in addition, they encode the strength, duration and quality of odorous stimuli into afferent neuronal signals. Recent advances in physiology and biochemistry of olfactory receptor cells implicate second messengers as the critical link between the initial odor recognition and the electrical response. Upon interaction of odorous molecules with specific receptors, second messenger cascades are triggered via G-proteins, leading to rapid and transient second messenger 'pulses' which are supposed to elicit generator currents via direct gating of cation channels. The rapid termination of odor-induced second messenger signaling is accomplished by uncoupling the reaction cascade via a negative feedback reaction mediated by protein kinases, leading to the phosphorylation of odorant-receptors. Adaptation of chemosensory neurons due to strong odor stimuli appears to be mediated by calcium- and cGMP-dependent pathways.

Candidate receptors for odorants are members of the G-protein-coupled receptor superfamily and seem to be encoded by a large multigene family. Receptor genes are located on various chromosomes and organized in clusters. The enormous diversity of receptor genes in mammals apparently emerged during evolution from smaller gene families. Comparative studies indicate that receptors for hydrophobic and hydrophilic odorants in lower vertebrates are encoded by two distinct gene families. By means of sequence-specific antibodies putative odorant receptor proteins have been identified in olfactory cilia. Surrogate cells heterologously expressing distinct receptor types displayed a graded second messenger response to several out of a battery of odors, suggesting that the receptor types may have a relatively broad but selective ligand specificity. *In situ* hybridization studies revealed that an olfactory neuron expresses only one or a small set of receptor types and that reactive cells are organized in distinct spatial zones and clusters of the olfactory epithelium. Neurons expressing the same receptor and thus displaying the same chemospecificity appear to project their axons to the same target site (glomerulus) in the olfactory bulb; this precise patterning is considered as a basis for odor coding.

2. Odorant receptor proteins: expression in olfactory axons and olfactory bulb glomeruli supports a role in axonal guidance and/or target recognition

Christina Harrington, Michael Buckland, Nina Levy[1], Randall Reed[1] and Anne Cunningham

Neurobiology Division, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, NSW 2010, Australia and [1]Department of Molecular Biology & Genetics, Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, 725 N. Wolfe St, Baltimore, MD 21205, USA

Topographically restricted localization of odorant receptor protein mRNAs has previously been shown in both the olfactory neuroepithelium (NE) and olfactory bulb. We have studied two different odorant receptors (D3 and L5), each of which is part of a small subfamily of highly related members which we have sequenced from a rat genomic library. Polyclonal antibodies directed towards C-terminal synthetic peptides corresponding to these two receptors have defined restricted zonal expression in the NE consistent with *in situ* hybridization data of others. In addition, these antibodies label the ciliary surface—further supportive evidence that these receptors function in odorant-induced signal transduction.

We also found prominent immunohistochemical labelling using anti-receptor antibodies in axon bundles situated beneath labelled cell bodies of olfactory receptor neurons (ORNs) in the NE. When we examined the olfactory nerve layer in close proximity to the

bulb using an antibody specific for a single receptor, discrete immunoreactivity was found. We also found immunoreactivity of specific glomeruli in a pattern consistent with immunoreactive axonal processes converging on the glomerulus. As in the NE, the expression pattern was conserved bilaterally between the two olfactory bulbs.

We are attempting to define the distribution of subfamily members within ORNs in the NE and study the axonal projection of these neurons to the glomeruli. Our finding of 'odorant receptor' proteins expressed in olfactory axons and the glomeruli strongly supports the hypothesis that this receptor family has an additional functional role in axonal guidance and/or synaptic target recognition.

Supported by the Garnett Passe & Rodney Williams Memorial Foundation.

3. Putative odor receptors localize in cilia of olfactory receptor cells in rat and mouse

Bert Ph.M. Mencot, Anne M. Cunningham[1,2], Pankaj Qasba[2], Nina Levy[2] and Randy R. Reed[2]

Department of Neurobiology & Physiology, Hogan Hall, Northwestern University, Evanston, IL 60208, USA, [1]Neurobiology Division, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, NSW 2010, Australia and [2]Molecular Biology & Genetics, Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, 725 N. Wolfe St, Baltimore, MD 21205, USA

Two monospecific polyclonal antibodies generated to the C-terminal regions of peptides of two putative odor receptors in two rodent species, rat and mouse, were used to demonstrate ultrastructurally that olfactory receptor cell cilia contain these receptors. We used paraformaldehyde fixed, cryoprotected, freeze-substituted and Lowicryl-embedded olfactory epithelia in combination with immunogold labeling. The rat antibody labeled receptor cells in the anterior part of the olfactory epithelium, close to the respiratory epithelium, whereas the mouse antibody labeled such cells in a more posterior region, closer to the brain. In agreement with earlier *in situ* hybridization studies, immunopositive cells were exceedingly rare in both cases; in a linear segment, comprising ~500 cells or ~2 mm, we found maximally two positive olfactory receptor cells. Despite chemical, species and topographic differences, both antibodies behaved identically in their ultrastructural labeling pattern. They both bound to thick proximal and thin and long distal parts of the cilia; the dendritic knobs showed little if any labeling. Dendritic structures below the knobs and respiratory cilia did not immunolabel. There were no obvious differences in morphology between labeled and unlabeled olfactory receptor cells and their cilia. Sometimes, the extent of labeling could be followed along the distal parts of the cilia, i.e. we saw labeled cilium segments at a distance of ~15 μm from the knob (earlier morphometric studies suggested that the cilia can be as long as 50 μm). In both species immunogold labeling was detectable in two, sometimes three, serial sections taken through these rare cells whilst being absent in other areas, proving that the same cells were, indeed, labeled. Thus, although it has still to be established whether the labeled proteins are odor receptors, the sparse occurrence of labeled receptor cells with antibodies to putative odor receptors combined with the facts that (i) ultrastructurally, labeling is found in regions of these receptor cells that first encounter the odor molecules—specifically modified olfactory cilia—and (ii) the colocalization of these putative receptors with all subsequent olfactory signal-transducing proteins in these special ciliary regions (*Semin. Cell Biol.*, **5**, 24, 1994) provide some of the strongest evidence to date to support a special, selective and restrictive function of these proteins in olfactory processing.

Supported by NIH-NIDCD (grant RO1 DC02491 to B.Ph.M.M.), the Garnett Passe & Rodney Williams Memorial Foundation (A.M.C.) and the Howard Hughes Medical Institute (R.R.R.).

4. Olfactory adaptation: a role for cyclic GMP and carbon monoxide

Frank Zufall and Trese Leinders-Zufall

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

The primary excitation process of olfactory transduction is now well understood. In contrast, little is known about the molecular mechanisms that lead to olfactory adaptation. Recent evidence has provided some clues implicating the CO/cGMP second messenger system in sensory adaptation of salamander olfactory receptor neurons (ORNs) (Leinders-Zufall *et al.*, 1996).

To evaluate this hypothesis more rigorously we have searched for forms of adaptation with properties similar to the adaptation-like effect produced by exogenous cGMP or CO. By applying repeated odor stimuli and using perforated patch recordings under voltage-clamp we have elicited long-lasting adaptation (LLA) of the odor response in isolated salamander ORNs. LLA is positively coupled to the stimulus strength (with respect to the $K_{1/2}$ value of the odor response) and is characterized by a strong reduction in peak current amplitude to a given stimulus and a prolongation of the odor response kinetics. The reduced odor responsiveness can continue for several minutes after the end of an adequate stimulus (in the absence of odor molecules) but recovers completely so that the effect can be triggered several times during recording from a given ORN. One manifestation of LLA is that the $K_{1/2}$ value of stimulus–response curves of odor currents is shifted to higher odor concentrations and that maximum responses at saturating odor concentrations are strongly reduced. In another series of experiments we demonstrate that LLA can be abolished by reducing the external Ca^{2+} concentration to ≤1 μM, indicating that Ca^{2+} entry is required for LLA. Because the properties of LLA closely match the effects of exogenous CO/cGMP, we tested the consequences of a variety of pharmacological blockers of the cGMP second messenger system. We fmd that LLA can be uncoupled selectively from excitation and be prevented entirely in the presence of agents that act as blockers of the CO-producing enzyme heme oxygenase-2, such as zinc protoporphyrins. In contrast, specific blockers of nitric oxide synthase had no effect on LLA. A series of control experiments ruled out that the effect of the CO pathway blockers was due to unspecific actions at the level of soluble guanylyl cyclase or at the CNG channels.

These data provide direct evidence that endogenous CO mediates a long-lasting form of odor response adaptation. We conclude that CO can be released in an individual ORN in

response to odor stimuli of a given strength. This effect results in cGMP formation, followed by tonic activation of the CNG channels and Ca^{2+} entry leading to sensory adaptation. The described phenomenon could play an important role in the perceptual adaptation to odors.

Supported by the National Institute of Deafness and Other Communications Disorders.

References

Leinders-Zufall *et al.* (1996) *Proc. R. Soc. Lond. B.*, in press.

5. Influx of Ca^{2+} mediates the activation of the odorant induced inhibitory current in vertebrate olfactory receptor neurons

Bernardo Morales, Rodolfo Madrid and Juan Bacigalupo

Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

We have previously described the inhibitory responses triggered by putrid odorants in isolated olfactory neurons from the toad *C. caudiverbera* (Morales *et al.*, 1994). Inhibition is caused by an increase in a ciliary Ca^{2+}-activated K^+ conductance, inducing a hyperpolarizing receptor potential (Morales *et al.*, 1995). We have investigated the participation of extracellular Ca^{2+} on the activation of this conductance.

Electrical recordings were obtained from isolated olfactory neurons under whole-cell V-clamp. Ca^{2+} changes were monitored by confocal microscopy, with fluo-3-AM as a Ca^{2+} indicator. Odorants were applied to the cilia with a multibarreled pipette connected to a picospritzer.

The inhibitory outward current was found to depend on external Ca^{2+}. Its amplitude decreased to 66% upon reducing extracellular Ca^{2+} from 1 to 0.1 mM, and to –32% when Ca^{2+} was further reduced to 0.01 mM (V_{HOLD} = 20 mV). Nifedipine (20 μM), a Ca^{2+} channel blocker, reversibly abolished the inhibitory current, suggesting the involvement of a Ca^{2+} influx through Ca^{2+} channels. No obvious sign of an inward current induced by the putrid odorants could be observed under normal ionic conditions. However, we were able to record an odorant-triggered inward current after replacing all external permeant cations by 10 mM Ba^{2+} and 84.5 mM *N*-methyl-<S>D<D>-glucamine, and Cs^+ with K^+ in the internal solution. The currents recorded under these conditions were –4.7 pA (V_{HOLD} = –50 mV, n = 6), with a latency of ~250 ms. A small Ca^{2+} current (<1 pA) is sufficient to elevate Ca^{2+} to μM levels within the small volume of the apical structures (cilia and dendritic knob) to produce the activation of the inhibitory K^+ current.

By confocal microscopy, we showed that the putrid odors trigger a transient elevation of internal Ca^{2+}, principally confined to the apical region of olfactory neurons. This Ca^{2+} elevation was abolished by nifedipine.

Our results indicate that an odorant-induced Ca^{2+} influx is important for elevating internal Ca^{2+} and activating the inhibitory K^+ conductance.

This study was supported by FONDECYT 1960878 and TWAS 94A63 RG/BIO/LA.

References

Morales *et al.* (1994) *Proc. R. Soc. Lond. B*, **257**, 235.
Morales *et al.* (1995) *FEBS Lett.*, **359**, 41.

6. Classes of vertebrate odorant-binding proteins identified by partial amino acid sequences

Monica Ganni, Marina Garibotti, Ambretta Navarrini, Sara Paolini, Paolo Pelosi, Daniela Pes and Andrea Scaloni[1]

Istituto di Industrie Agrarie, Via S. Michele, 4, 56124 Pisa and [1]Mass Spectrometry Laboratory, CNR, Via Pansini, 5, 80131 Napoli, Italy

The extensive structural information obtained in recent years on odorant-binding proteins (OBPs) has not yet clarified the role of these proteins in olfactory transduction. However, the increasing evidence for the expression of several types of OBPs in the same animal species seems to indicate that they could be involved in the recognition of different odorant molecules.

In Lepidoptera three classes of OBPs have been identified, generally one PBP (pheromone-binding protein) and two GOBPs (general odorant-binding proteins), or two PBPs and one GOBP. In one species the two PBPs showed opposite binding specificities for two pheromones (Du and Prestwich, 1995). Results of this type strongly support the hypothesis that OBPs may be actively involved in the recognition and discrimination of chemical structures of odorants rather than performing a role of passive carriers. In Lepidoptera, the segragation of OBPs into three classes is self-evident, given the high degree of similarity between sequences of the same class, which is related to the short phylogenetic disances between species of this order.

In vertebrates the situation is more complex. First of all, different numbers of OBPs have been identified so far in each animal species: one in the cow, two in the rat and pig, three in the rabbit, four in the mouse and nine in the porcupine (belonging to two major groups). Second, the greater phylogenetic distance even between mammal species makes the task of establishing sequence homologies more difficult than in the case of Lepidoptera.

However, the available data on the amino acid sequences (most limited to the N-terminal region) provide a basis for a preliminary classification of OBPs into at least three classes. Members of the same class, expressed in different species, show significantly greater similarities than OBPs of the same species but belonging to different classes. It has also been observed that other lipocalins involved in the mechanisms of chemical communication show greater similarity to OBPs of one or the other class. The clearest example is that of urinary and salivary proteins of the mouse, which share, in some cases, >90% amino acids (limited to N-terminal regions) with OBPs of class III. We also have evidence for a further division into subclasses of OBPs of the same class, as indicated by mass spectrometry and amino acid sequencing.

References

Du and Prestwich (1995) *Biochemistry*, **34**, 8726.

7. Hyperbolic and linear dose dependence of receptor occupancy: types of chemoreceptors

Karl-Ernst Kaissling (in collaboration with John Thorson, Oxford)

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

The hyperbolic relationship between stimulus concentration and receptor occupancy was proposed by Beidler (1954); it was based on the assumption that the receptor sites at the cell membrane of the taste receptor cell are exposed to the external tastant concentration. The interaction of the tastant molecules (S) with the hypothetical receptor molecules (R) was considered as an association–dissociation (or adsorption–desorption) process which should follow the hyperbolic adsorption isotherm. For such 'concentration detectors' we find, at equilibrium, a receptor occupancy $RS/R_{total} = 1/((K_d/S) + 1)$, with $R_{total} = R + RS$ and K_d is the dissociation constant of the complex RS.

The stimulus situation is in principle different in many olfactory receptor organs because they accumulate stimulus molecules during exposure, especially if they adsorb the stimulus molecules from the air space. If desorption is negligible, constant odor concentration in air at constant air speed should result in a constant adsorptive flux ϕ of stimulus molecules to the organ ('flux detector'). During exposure the stimulus concentration at the receptor sites ought to increase steadily. In spite of this, the receptor potential reaches a steady level and declines after the end of stimulation. This suggests that the stimulus molecules are removed or deactivated as rapidly as they arrive, so that the effective stimulus concentration is constant. Thus, for adsorptive stimulus uptake the abscissa of the dose–response curve measured at constant airspeed and various stimulus concentrations is, in fact, a flux (molecule concentration per s). Again we assume that the stimulus molecules taken up by the organ interact with the receptor molecules as in taste receptors but, in addition, we need a stimulus deactivating or removing process, with a velocity constant k_3. At equilibrium, the receptor occupancy is $RS/R_{total} = \phi/(k_3 \times R_{total})$ if the deactivation is a catalytic process with the receptor molecules serving as enzymes (as suggested by Ziegelberger, 1995). This means that the receptor occupation depends linearly on the stimulus flux ϕ and the stimulus intensity for half saturation of the receptors does not reflect the dissociation constant K_d as in concentration detectors. With a given number of receptors, half-saturation depends solely on the velocity constant for stimulus deactivation k_3. Thus k_3 contributes to the specificity of the receptor cell.

For a deactivation catalyzed by a separate enzyme (E), the receptor occupancy depends linearly on the stimulus flux if the dissociation constant K_d of the stimulus–receptor complex RS equals K_m of the deactivation process. The relationship is hyperbolic if $K_d < K_m$ and steeper than linear if $K_d > K_m$. In both systems the receptor molecules are fully occupied if the flux ϕ equals the maximum rate of deactivation $v_{max} = k_3 \times E_{total}$. If ϕ exceeds v_{max}, the system will remain in saturation until the accumulated odor is sufficiently deactivated.

References

Beidler, L.M. (1954) A theory of taste stimulation. *J. Gen. Physiol.*, **38**, 133–139.

Ziegelberger, G. (1995) *Eur. J. Biochem.*, **232**, 706–711.

8. Application of statistical thermodynamics to the olfaction mechanism

A. Ben Lamine and Y. Bouazra

Faculté des Sciences de Monastir, 5000 Monastir, Tunisia

By the application of the grand canonical ensemble in statistical thermodynamics to the stimulus adsorption on the olfactory receptor sites, we established the following expression of the olfactory response:

$$R = \alpha N_M/[1 + (N_{g1/2}/N_g)^n]$$

where N_g is the stimulus concentration in air, n is the Stevens law exponent, N_M is the receptor site number per unit surface, α is a proportionality coefficient of transduction and $N_{g1/2}$ is the half-saturation concentration:

$$N_{g1/2} = \beta SVP exp(-\Delta Ea/RT)$$

in which SVP is the saturated vapor pressure of pure stimulus, ΔEa is the free enthalpy of stimulus adsorption from the dissolved state in the mucus and $\beta = 1/kT$.

The expression of R is in agreement with the Hill model (Patte *et al.*, 1989). The olfactory threshold expression N_{gt} is deduced directly from R:

$$\log N_{gt} = \log SVP + [\log(N_{at}/N_M)]/n - \Delta Ea/RT + \log\beta$$

which can explain the empirical relation established both in electrophysics and psychophysics by Laffort and his co-workers (Dravnieks and Laffort, 1972; Patte *et al.*, 1989) and containing only the first two terms. (N_{at}/N_M) is the proportion of occupied receptor sites at the threshold.

The consequent analytical expression of the power law exponent n is:

$$n = [\log(N_M/N_{at})]/[\text{p.ol.} + \log K_p - (\Delta Ea/RT) + \log\beta]$$

where p.ol. (olfactory power) is defined as $(-\log N_{gt})$ and K_p is the partition coefficient which equals SVP in the case of absence of stimulus–mucus interaction.

This expression can perfectly explain the established correlations by Laffort and Patte (1987) in both psychophysics and electrophysics (honeybee data) in considering the non-ideality of the human mucus and either the ideality or the absence of the honeybee mucus expressing the absence of any stimulus–mucus interaction.

In conclusion, the expression of the olfactory response R we established by this statistical model turns out to be quite powerful. It confirms some known empirical relations and the important role played by the adsorption phenomenon in the olfaction mechanism.

References

Dravnieks, A. and Laffort, P. (1972) In Schneider D. (ed.), *Olfaction and Taste IV*. Stuttgart, Wissenschaftliche Verlegsgesellshaft, pp. 142–148.

Laffort, P. and Patte, F. (1987) *Ann. N.Y. Acad. Sci.*, **510**, 436–439.
Patte, F. *et al.* (1989) *J. Insect. Physiol*, **35**, 667–675.

9. Olfactory coding and olfactory memory in an insect

Randolf Menzel, Jasdan Joerges, Armink Küttner and Giovanni Galizia

Freie Universität Berlin, Institut für Neurobiologie, Königin-Luise-Straße 28/30, D-14195 Berlin, Germany

Honeybees learn odors of flowers quickly and discriminate them very well. We have used behavioral, electrophysiological and optical imaging techniques to study the neural basis of olfactory coding and learning in worker bees. Olfactory receptors are arranged in a large number of pore-plates along the antenna. Their axons project into the antennal lobes, where they form synaptic contacts with local interneurons and projection neurons in 156 spherical glomeruli. The glomeruli appear as functional units of olfactory coding. We used optical imaging techniques to reveal spatio-temporal excitation patterns following olfactory stimulation of the antenna in a semi-natural preparation. Different odors lead to different patterns of glomerular excitation. The excitation pattern for each odor is basically the same at low and high odor concentrations, though less pronounced at low concentrations. Mixtures of odors induce patterns which resemble the components' patterns but deviate from a simple algebraic addition of their excitation patterns, indicating non-linear interactions between glomeruli. Pheromone-like odorants appear to induce consistent excitation patterns in different individuals, whereas general floral odors lead to different patterns in different animals. We conclude from these observations that the odor code is shaped by experience-dependent processes and thus reflects the outcome of a self-organizing neural mechanism during early adult lifetime and development.

Intracellular recordings from interneurons reveal that the odor code at the single neuron level is characterized by a temporal pattern of action potentials. Thus, the spatial code as an across-fiber code is combined with a temporal code in each olfactory neuron as a consequence of excitatory and inhibitory interactions. The temporal code changes with experience, both for non-associative learning (sensitization with sucrose) and for associative learning (appetitive conditioning using sucrose as the unconditioned stimulus). These changes appear to be the neural correlates of transient non-associative and long-lasting associative learning phenomena which are well known in bees.

Combined behavioral and pharmacological experiments revealed that both the antennal lobes and the mushroom bodies are the loci of olfactory learning. The mushroom bodies are a central brain structure of insects. We therefore conclude that olfactory memory is distributed between at least two major CNS structures, and that these structures store different aspects of the memory trace.

10. Olfactory receptor neurons for conspecific and prey-habitat volatiles in the ladybird beetle, *Harmonia akyridis*

Joseph C. Dickens

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

Ladybird beetles are predators of aphids and other insects, and thus may facilitate biocontrol of pestiferous species. *Harmonia axyridis*, a native of eastern Asia, became established in the USA for biological control purposes, but achieved pest status based on its infestations of buildings in the fall while searching for overwintering habitats. Knowledge of chemical communication in *H. axyridis* may be useful either for biological control or in regulating pestiferous populations. I report results of initial morphological and electrophysiological investigations in *H. axyridis* to determine antennal olfactory sensilla, and characteristics of receptor neurons associated with them. The antennae of *H. axyridis* consist of 11 segments, with the two distal segments somewhat enlarged to form a club. Scanning electron micrographs reveal clusters of apparent olfactory sensilla in sensory regions on the anterior surface of the distal two segments of males and females. A majority of the sensilla are located on the terminal antennal segment. At least five different types of sensilla occur in these sensory clusters. Electrophysiological recordings from individual sensilla not only verified their function as olfactory sensilla, but also revealed specificity of receptor neurons associated with them. Odorants tested included 18 volatiles which were selected based on their presence in: (i) adult males and females; (ii) prey, e.g. aphids (β-farnesene); or (iii) host plants of prey. Individual olfactory neurons were specialized for monoterpenes and sesquiterpenes found in volatile emissions of conspecifics and host plants of prey. The results are discussed with regard to the role of conspecific emissions and prey-habitat volatiles in chemical communication in the ladybird beetle.

11. Contact chemoreception related to oviposition behavior in the monarch butterfly, *Danaus plexippus*

Robert Baur, Meena Haribal[1], J. Alan A. Renwick[1] and Erich Stadler

Department of Zoology, Swiss Federal Research Station, CH-8820 Wädlenswil, Switzerland and [1]Boyce Thompson Institute at Cornell University, Tower Road, Ithaca, NY14853, USA

Behavioral events during host selection by ovipositing monarch butterflies (*Danaus plexippus*) include tapping the leaf surface with fore tarsi and touching this surface with mid tarsi ('drumming') and antennae. Occurrence of these events depends on host (*Asciepias*) species but is also highly variable among individual females. Flavonoids (quercetin-glycosides) identified from host-plant extracts are known to stimulate oviposition. Scanning electron microscopy revealed the presence of contact-chemoreceptor sensilla on all appendages that contact the leaf surface. This electrophysiological study aimed to identify the

contact chemoreceptors sensitive to the known oviposition stimuli and thus probably involved in host recognition.

Receptor cells of conspicuous sensilla grouped in clusters on female fore tarsi were found to be sensitive to the behaviorally active butanol fraction of host-plant (*Asciepias curassavica*) extract. However, these receptors generally had low sensitivity to the oviposition stimulating flavonoids identified from this fraction. The receptors on the fore tarsi were remarkably sensitive to the butanol fraction of a non-host (*Brassica oleracea*). Chemoreceptors on tarsomers 2–4 of the mid legs also responded to the behaviorally active fraction of host-plant extract and showed some sensitivity to one of the flavonoids stimulating oviposition. Similar results were obtained from some receptor cells in sensilla on the tip of the antennae. Most sensilla responded to the butanol fraction of *Asciepias* extract but only 25% were also sensitive to one of the behaviorally active flavonoids isolated from this fraction.

Electrophysiological results, in combination with behavioral observations, suggest that host selection in monarch butterflies is based on a complex pattern of decisions integrating peripheral sensory information from several types of tarsal and antennal contact chemoreceptors.

12. Development of the bee olfactory system: neuron–neuron and neuron–glia interactions

Jean Gascuel and Claudine Masson

Laboratoire de Neurobiologie Comparée des Invertébrés, INRA-CNRS, URA 1190, BP 23, Bures sur Yvette 91440, France

The developing and adult 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 (Gascuel and Masson 1991a,b).

By using hybridoma technology we generated monoclonal antibodies specific to neuron categories. Emphasis was 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 (Gascuel *et al.*, 1996).

A co-culture system of the different cell categories involved in the interaction has also 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 dynamics of the cell–cell interactions between neurons and/or between neuron and glia is in progress. Moreover, such a culture system will allow us to carry out, using monoclonals, blocking experiments in order to investigate the function of the corresponding antigens.

References

Gascuel, J. and Masson, C. (1991a) A quantitative electron microscopy study of the antennal lobe of the honeybee. *Tissue Cell*, **23**, 341–355.

Gascuel, J. and Masson, C. (1991b) Developmental study of afferented and deafferented bee antennal lobes. *J. Neurobiol.*, **22**, 795–810.

Gascuel, J., Brouillard, F., Papin, C. and Masson, C. (1996) Expression of the surface antigen A2B7 in adult and developing honey bee olfactory pathway. *Int. J. Dev. Neurosci.*, in press.

13. Cytoskeletal proteins in the antennae of the male silkmoth genus *Antheraea*

George Louis Kumar, Rosaric Maida and Thomas Axel Keil

Max-Planck-Institut für Verhaltensphysiologie, D-823 19 Seewiesen, Germany

The antenna of the male silkmoth *Antheraea polyphemus* and *A. pernyi* is a featherlike sense organ optimized for the capture of the female sex pheromone molecules. The antennal stem carries ~120 side branches with up to 500 olfactory hairs each. An olfactory hair is usually innervated by 2–3 olfactory receptor neurons (ORNs), each giving off an olfactory dendrite, in which olfactory transduction is thought to occur, a cell body and axons, the latter running through the side branches and the main antennal stem towards the brain. Like any other eukaryotic cell, ORNs have a cytoskeleton which gives them a distinct shape. Although the overall cytoskeletal morphology, especially of the olfactory dendrites, is known, the cytoskeletal proteins that structurally and functionally support the ORNs, particularly in the olfactory dendrites, have yet to be identified. We report here the identification of the major cytoskeletal proteins, namely tubulin, actin and intermediate filament-like proteins present within the olfactory dendrites, and motor proteins such as kinesin, kinesin-related proteins and unconventional myosin, in the antennal side branches of *Antheraea* by immunoblotting. We also show that the tubulins within the olfactory dendrites as well as in the antennal side branches are acetylated. Our interest in the identification of the cytoskeletal proteins especially in the olfactory dendrites arose because they exibit the following interesting features: (i) microtubules within dendrites show a hexagonal arrangement with a centre-to-centre spacing of 40–50 nm; they are connected by fine filaments and are also cross-linked to the membrane by unknown proteins; (ii) upon stimulation with high concentrations of pheromones, the microtubular cytoskeleton of the stimulated dendrite gets damaged, most probably due either to calcium or to phosphorylation of cytoskeletal proteins; (iii) the dendrites have been observed to show active movements such as elongation and contraction outside apically opened trichoid sensilla (Keil, 1993); (iv) living dendrites show membrane swellings or 'beads' that have been observed to move along the dendrites (Williams, 1988;Keil, 1993); and (v) pheromone-stimulated dendrites take up cobalt (G.L. Kumar *et al.*, unpublished observations) and transport it through the axons to the brain.

Our future studies will focus on identifying more cytoskeletal proteins within the dendrites and localizing them by immunoelectron microscopy. We also plan to investigate to what extent cytoskeletal proteins play a role in olfactory transduction in the moth antennea.

Supported by DFG-grant 'Ke 398/2-1'.

References

Keil, T.A. (1993) *Tissue Cell*, **25**, 573–587.

Williams, J.L.D. (1988) *Int. J. Insect. Morphol. Embryol.*, **17**, 145–151.

14. Monitoring tsetse host odours in the field using a mobile electrophysiological device

Karen E. Voskamp, C.J. Den Otter and N. Noorman

Sensory Physiology Group, Department of Animal Physiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands

Tsetse flies (*Glossina* spp.) are attracted from a distance to their hosts by odours emanated by the hosts. Attractants identified so far include carbon dioxide, acetone and 1-octen-3-ol from cattle breath, and various phenolic compounds from cattle urine.

To date, it has not been entirely understood how tsetse flies navigate towards the host odour. The temporal and spatial distribution of odour in the air may be important factors inducing and sustaining orientation behaviour in the flies. In the field, in Zimbawe, using a portable electrophysiological device, we were able to record the electrical responses from olfactory cells of living flies at various distances upwind and downwind of an odour source. Experiments were done at an airstrip and in riverine woodland to determine possible differences between responses in an open field and in a wooded environment. Electroantennograms (EAGs) and single-cell recordings were made.

It is believed that odour plumes may be intermittent and that odours may arrive downwind as a series of 'puffs'. We found that this indeed may occur since upwind odours evoked manifold EAGs. Comparison of various parameters of the EAGs recorded (e.g. frequency, duration, amplitude) provided information about the shape of odour plumes and suggested how tsetse flies may locate their hosts.

The EAG response revealed that in an open field flies can smell an odour source at least 10 m downwind of it, whereas in woodland they may already be able to detect the source at 60 m downwind.

15. The use of a portable EAG sensor to measure pheromone emission from dispensers for mating disruption in greenhouses

Jan N.C. van der Pers, J.W. Klijnstra[1] and J.J. de Vlieger[1]

Syntech/VDP Laboratories, PO Box 1547, 1200 BM Hilversum and [1]TNO Plastics and Rubber Research Institute, Crop Protection Research, PO Box 6031, 2600 JA Delft, The Netherlands

An important parameter for the success of mating disruption is the emission rate of the synthetic pheromone formulation and thus the concentration of pheromone released in the air. Conventional methods of measuring aerial concentrations are based on either trapping the pheromone in cartridges containing a suitable adsorbent followed by gas chromatographic analysis, or measuring the loss of pheromone in the dispensers over time. However, application of these methods is limited by their complexity and high cost. We have therefore developed a pheromone-measuring apparatus based on the electroantennogram (EAG) technique that is suitable for routine use outside the laboratory.

The device is small, lightweight and can be operated by non-specialists. A male antenna of the species under study is excised and placed in a holder provided with contact pads. Contact between the ends of the antenna and the input circuit of the amplifier is established using a non-drying electrically conductive gel. The holder with the antenna is placed in the device in a completely closed compartment. A miniature air pump maintains a constant, charcoal-filtered airflow over the antenna. Pressing a button activates a valve by which a short puff (0.3 s) of unfiltered air is passed over the antenna. After a pre-set time (5 s) a puff of air from a built-in reference cartridge containing a synthetic pheromone component is presented to the antenna. The responses to the reference are used to indicate the decline in antennal sensitivity during the measurements. The EAG signals evoked by the air puff and the reference compound are recorded on a portable tape recorder, which is also used to record verbal comments during the measurements.

In 1995 EAG measurements were conducted inside conditioned greenhouses in which sweet peppers (paprika) were grown. Pheromone dispensers for mating disruption on the noctuid moth *Chrysodeixis chalcites* were applied in half of the greenhouses at a density of 600 dispensers/ha; the other half served as non-treated controls. The non-treated greenhouses provided the reference plant odour background necessary to measure the EAG response to plant odours only. EAG measurements in pheromone-treated greenhouses were alternated with measurements in the non-treated greenhouses. The EAG response to the pheromone in the treated greenhouses was calculated by substraction of the EAG to plant odours only in the non-treated greenhouses.

In 1996 tests were started in greenhouses provided with dispensers having four increasing pheromone release rates. EAG measurements were made starting from the moment of dispenser application and continuing at regular intervals.

All EAG measurements were compared with the data obtained by release rate analysis of the dispensers used, as well as with data provided by biological assessment of the effect of the mating disruption.

From the EAG data collected so far, we conclude that the portable EAG sensor is a practical and reliable device for measuring pheromone release rates in greenhouses.

16. Three-dimensional recording: a new tool for identification of behaviourally active pheromone components

Ashraf El Sayed, Josef Gödde[1] and Heinrich Am

Swiss Federal Research Station for Horticulture and Viticulture, 8820 Wädenswil, Switzerland and [2]235753 Greifenstein-Nenderoth, Germany

With the rapid development of gas chromatography and mass spectrometry, a number of pheromone-related compounds can be detected in the sex pheromone glands. Therefore, developing highly sensitive bioassays to identify the behavioural role of these compounds is demanding. Most investigations of male flight behaviour are restricted to recording with one camera, resulting in a distorted two-dimensional view of the flight track. Since flying insects move both in the horizontal and the vertical plane, only three-dimensional recording of a male's upwind flight in the wind tunnel can provide valuable information. Simple flight parameters, e.g. straightness, track angle and linear velocity, can be used as criteria for characterization of blends and identification of behaviourally active compounds. Male *L. botrana* were found to fly faster and straighter to calling females or gland extracts than to

synthetic pheromone. Addition of certain secondary compounds to the main pheromone compound (*E*)-7,(*Z*)-9-dodecadienyl acetate was found to enhance most of the flight parameters. Using this technique, the pheromone blend of *L. botrana* was characterized.

17. The genetics of olfaction

Randall R. Reed

Department of Molecular Biology and Genetics, Howard Hughes Medical Institute, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA

Our laboratory has used molecular genetic methods to understand the mechanisms which underlie the ability of the mammalian olfactory system to identify odorants with high sensitivity and specificity. Previous work from our laboratory has demonstrated that the components of the signal transduction pathway as well as several other genes expressed in the mature neurons are regulated by the transcription factor Olf-1 (O/E-1). This factor is a member of a highly conserved family of related factors transiently expressed in many of the differentiating sensory neuronal precursors in the mouse embryo. The members of the O/E family are expressed at a time when these neurons begin to extend axonal and dendritic processes, and we hypothesize that transcriptional control of the proteins responsible for these events might be under the control of the O/E transcription factors.

We have identified a *C. elegans* homologue of the mammalian Olf-1 protein that displays remarkable similarity (>80% amino acid identity) over 350 amino acids. Fusion of the CeOlf-1 promoter to GFP and expression in transgenic worms results in transient expression in a specific pair of chemosensory neurons (ASI) and motor neurons of the ventral nerve cord. Genetic analysis has revealed that CeOlf-1 is the product of the *unc-3* gene. Originally isolated based on its uncoordinated phenotype, axons in the mutant make aberrant projections and ectopic synaptic connections. Defects in chemosensory processes have also been described in *unc-3* mutants. We are currently examining potential targets for this transcription factor.

Complex mechanisms must underlie the zonal restriction and cell-specific expression of olfactory receptor genes in mammals. We have used a variety of molecular genetic approaches to understand the regulation of this large family of receptors in olfactory epithelium. In particular, we have identified clusters of orthologous receptors in mouse and human, characterized their long-range genomic organization and patterns of expression, and are attempting to identify evolutionary conserved sequences important in the regulation of gene expression. Finally, we have used genetic linkage analysis to identify genes in the mouse that define the sensitivity of particular strains to the odorant isovaleric acid. Recently, we have demonstrated that a single locus present in the homozygous state is sufficient to allow a normally anosmic strain to detect the odorant. The molecular identity of the protein responsible for this gain of function is currently under investigation.

18. Induced sensitivity to androstenone and DMCMC, a non-steroid analog of androstenone, in inbred mice

Vera V. Voznessenskaya[1,2], Elena S. Chukhray[3], Oles M. Poltorack[3] and Charles J. Wysocki[2]

[1]A.N. Severtzov Institute, Academy of Science, Moscow, Russia, [2]Monell Chemical Senses Center, Philadelphia, PA, USA and [3]Moscow State University, Moscow, Russia

Inbred strains of mice that provide a model for specific anosmia to androstenone (AND) are available in the forms of NZB/B1NJ [NZB; insensitive to androstenone (AND)] and CBA/J (CBA, sensitive to AND). We previously estimated behavioral sensitivity of NZB and CBA mice to AND and 4-(4,4-dimethylcyclohexyl)-2-methylcyclohexanoone (DMCMC; a non-steroid that has AND-like characteristics to people who can smell it). NZB mice could detect 0.1% (w/v) AND in mineral oil but not 0.05% AND. CBA mice could detect AND at a concentration of 2000-fold. Sensitivity to DMCMC was 4–8 times higher than that to AND for both strains of mice. After 2 weeks of exposure (16 h/day) to AND all mice increased sensitivity to AND and to DMCMC regardless of their initial level of sensitivity. After a further week, sensitivity of NZB mice was 64–128-fold lower for AND than original estimates and those for CBA mice were 200–400-fold lower. AND exposure did not affect sensitivity of either NZB or CBA mice to amyl acetate (AA). Exposures of NZB and CBA mice to AA did not affect AND thresholds, while sensitivity to AA was slightly increased (2-fold). Alkaline phosphatase activity (APA) of membrane proteins in olfactory and vomeronasal epithelia in AND-exposed and non-exposed NZB and CBA mice was compared. Exposures to AND resulted in an increase in APA in the olfactory epithelium of CBA mice. The effect was more profound for longer AND exposures. The vomeronasal epithelium of CBA mice did not respond to AND stimulation before exposure, but did so after exposure. Preliminary data collected after exposure to AND indicate that removal of the VNO caused a 4- to 16-fold decrease in sensitivity to AND and DMCMC but not to AA in the CBA mice. Non-exposed NZB olfactory epithelium showed inhibition of APA in response to AND stimulation. AND exposures resulted in activation of APA in response to AND stimulation. Vomeronasal epithelium of NZB mice did not respond to AND stimulation either before or after AND exposure. After exposure to AND and removal of the VNO, preliminary data indicate that behavioral sensitivity to AND, DMCMC and AA in NZB mice was unchanged. We continue to explore the ramifications of exposures to odorants using behavioral and biochemical measures.

Supported by NIH FIRCA TW00495, NIH grant DC00214 and USFWS.

19. A zonal topography in the developing rat's olfactory epithelial surface

Bert Ph.M. Menco and Jennifer E. Jackson

Department of Neurobiology & Physiology, Hogan Hall, Northwestern University, Evanston, IL 60208, USA

In situ hybridization studies suggest that the rodent's olfactory

epithelium has four zones in which putative odor receptors are differentially located (*Cell*, **73**, 569,1993; *Cell*, **74**, 309, 1993). Is there a morphological basis for this zonal distribution? It is not possible to determine this in olfactory epithelium of adult mammals with scanning electron microscopy as cilia of olfactory receptor cells and microvilli of supporting cells together cover the surface with a mat of hairlets, obscuring topographic details underneath. Therefore, we elected to study embryos for that purpose. *In situ* hybridization studies on the putative odor receptors showed that the zones start to be expressed (*Eur. J. Neurosci.*, **17**, 492, 1995; *Neuron*, **15**, 779, 1995) around the same time that the olfactory epithelial surface becomes recognizably olfactory, i.e. when differentiation sets in (*J. Cell Sci.*, **78**, 283, 1985). This implied that embryos are well suited for topographic studies. In low magnification survey maps of endoturbinate IIb in fetal age groups E16 (E1 = sperm positive) and E18 we could clearly distinguish four bands by the appearance of the surface morphology. The banded patterns became obscure at E20 and E21, whereas such a pattern was not yet clearly discernible at E15. Higher magnifications showed that bands 1 and 2 were characterized by relative high densities of receptor cell knobs, whereas in bands 3 and 4 these densities were much lower. Band 1 distinguished itself from band 2 in that the outgrowth of supporting cell microvilli was precocious in the former band. Whereas supporting cell apices were rather flat in bands 1 and 2, they were dome-shaped in bands 3 and 4, and the dome expansions were much larger in band 4 than in band 3. Bands 3 and 4 were also characterized by a rim of glandular openings that extended itself linearly over all the turbinates and the septum. Finally, bands 3 and 4, and the anterior region of band 2, contained ample scattered microvillar cells that looked like vestibular hair cells. These cells had aligned microvilli that resembled stereocilia and a typical single kinocilium ending in a knob. Interestingly, apical regions of these cells were precocious in their development, as these apices had the same appearance in E16 as in E21, whereas the appearance of the apices of both olfactory receptor cells and supporting cells changed drastically in that developmental window. In conclusion, there is a distinct banded pattern as a function of development in the rat's olfactory epithelial surface. Outlines of these bands roughly match that of the zones seen by whole-mount *in situ* hybridization (*Cell*, **74**, 309, 1993) The data suggest that olfactory supporting cells, and possibly also some other olfactory epithelial cells, such as the hair-cell like microvillous cells, play a major role in the determination of these zones.

Supported by NIH-NIDCD (ROl DC02491).

20. Olfactory receptor genes: chromosomal localization and modulation of expression under normal or experimental conditions in bird embryos

M. Leibovici, K. Ladjali-Mohammedi, F. Lapointe and C. Ayer-LeLitvre[1]

Institut d'Embryologie Cellulafre et Moléculaire, CNRS UPR 9064, Nogent sur Marne, France
[1]*Present address: CNRS URA 1485, 87025, Limoges, France*

Perception of odorant molecules starts with their binding to transmembrane receptors synthesized by the olfactory neurons. In vertebrate species there are large repertoires of putative odorant receptors (OR) belonging to the seven transmembrane domain receptor families. The discrimination of odorants depends on two processes settled during embryogenesis: the choice of OR synthesized by each neuron and the organization of central synapses from neurons expressing the same OR. In an attempt to understand these processes, we have cloned several OR genes in the chick (COR) and studied their organization as well as their expression and modulation in experimental embryos.

We show that the COR are organized into clusters including genes of the same or different subfamilies (three different subfamilies have been studied). This is confirmed by the distribution of these OR genes on several chromosomes.

COR gene expression starts early in development (ES) and concerns exclusively olfactory placode-derived cells. The level of expression clearly increases after the beginning of synaptogenesis (E8) but at no stage could we detect co-expression of COR from different subfamilies in a single neuron.

COR expression in cells migrating along the olfactory nerve was transient before E8. It might be involved in axon guidance and primary organization of sensory neuron synapses with central deutoneurons in the bulb primordium.

After axotomy at E16, COR expression stops within 40 h, before any morphological sign of degeneration. We show a concomitant activation of a gene normally involved in neurogenesis. The identity of a signal relating neurodegeneration and olfactory regeneration is questioned.

For the first COR expression by the sensory neuron, our experiments point to a relative independence from the CNS which may be lost after synaptogenesis. The clustering of COR genes and the dispersion of the clusters in the genome imply complex regulatory mechanisms leading to the selection of one COR to be synthesized by one neuron.

21. Familiarization to odor drastically modifies output cells' responsiveness in the rat main olfactory bulb

N. Buonviso, M. Chaput, M. Chalansonnet and R. Gervais

Physiologie Neurosensorie, CNRS—University of Lyon I, 69622 Villeurbanne, France

Several studies have already shown that olfactory conditioning modifies responsiveness to learned odor at the level of relay neurons of the main olfactory bulb (OB). However, little is known about the effects of a simple familiarization to odor. The present study examined this question.

Rats were familiarized to one odor only: either isoamyl acetate (FAMISO group, $n = 6$) or cineole (FAM-CIN group, $n = 6$). A control naive group ($n = 31$) was not exposed to odor. Familiarization consisted of putting the animal over an odorized litter for 20 min daily for 6 consecutive days. Electrophysiological recordings of extracellular mitral/tufted (M/T) cell unitary activities were performed in anesthetized animals just after the last session of familiarization. During recording sessions, four odorants were used as odor tests: isoamyl acetate,

cineole, acetophenone and *p*-cymene. Cells responsiveness was determined using three criteria: (i) in +, 0 or – responses by comparing firing rate before versus during stimulation; (ii) according to the size of their excitatory receptive field, defined as 'small' for cells excited by only one odorant and 'large' for cells excited by all four odorants; and (iii) in nine different types according to their temporal pattern along the respiratory cycle (Buonviso *et al.*, 1992).

Results collected from 121 cells show that, according to the first two criteria only, familiarization induces dramatic changes in M/T cell responsiveness to all four odors used. Namely, the proportion of cells showing no response to the four testing odors was significantly enhanced: 32% in FAM-ISO, 15% in FAM-CIN and 4% only in the control group. When considering responsive cells, the proportion of excitatory responses decreased while the proportion of suppressive reponses increased for each odor. The receptive field classification revealed that while the control group showed 50% of cells with a 'small' excitatory receptive field, this value reached 85 and 100% in the FAM-CIN and FAM-ISO groups respectively.

In summary, familiarization to one odor dramatically modifies M/T responsiveness to all odors used by reducing the proportion of excitatory response and by decreasing the size of their receptive field. This leads to finer M/T cell tuning to odorants. Moreover, the results point to the importance of considering the animal olfactory experience in at experiment aimed at deciphering the olfactory code.

Reference

Buonviso *et al.* (1992) *J. Neurophysiol.*, **68**, 417–424.

22. Distribution of synaptic vesicle proteins in axonal and dendritic processes within the mammalian olfactory bulb glomerulus

Charles A. Greer, Hahnah J. Kasowski and Hanna Kim

Sections of Neurosurgery and Neurobiology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8039, USA

Primary afferent synapses from olfactory receptor neurons (ORNs) distribute within the olfactory bulb glomeruli where they establish synapses with the dendrites of projection neurons and interneurons. The glomerular neuropil is further defined by the presence of modulatory reciprocal dendrodendritic synaptic circuits. To examine further the distribution of these circuits within the glomerulus we have employed immunocytochemistry to localize the distribution of antibodies to the synaptic vesicle proteins synapsin I (SYN-I) and synaptophysin (SYN), and in co-localization studies have analyzed their distribution in axonal and dendritic processes within the glomemlus. In parallel, we have used electron microscopy (EM) to assess the distribution of synaptic appositions within the glomerulus. Sprague–Dawley rats, 30–50 days postnatal, were perfused with 4% paraformaldehyde and immunocytochemically processed for olfactory marker protein (OMP), SYN, SYN-I, glial fibrillary acidic protein (GFAP) and/or MAP-2. Equivalent rats were perfused with 4% paraformaldehyde and 1% glutaraldehyde and processed for routine transmission EM. OMP immunoreactive (IR) processes occupied restricted regions within the glomeruli. Double labeling for OMP and MAP-2 revealed a distinct interdigitation of axonal and dendritic processes within glomeruli. Areas not IR for either OMP or MAP-2 were either IR for GFAP, indicating a glial process, or appeared to be a blood vessel. OMP IR, though present throughout the glomerulus, was strongest in the shell or outermost portion, an impression previously gained from our analyses of DiI-stained ORN axons. MAP-2 staining was less extensive and not apparent in the glomerular shell. SYN and SYN-I also showed differential IR within the glomerulus. SYN co-localized most strongly with OMP IR processes while SYN-I co-localized most strongly with MAP-2 IR processes. Reconstructions of the area occupied by ORN axons versus dendrites from EM montages revealed continuous islands of axons within the glomerulus, accounting for 28.24 ± 1.34% of the total area, with bundles of dendritic processes interspersed. The distribution of synapses within the glomerulus further suggested the segregation of axo- and dendrodendritic interactions. The results support the hypothesis of a subcompartmental organization of the olfactory bulb glomerular neuropil.

Supported in part by NIDCD DCO0210 and NINDS NS10174 to C.A.G.

23. Hierarchy of chemical senses on selective L-lysine ingestion in rats with an L-lysine deficiency

K. Torii[1,2], T. Kondoh[2], Y. Ninomiya[3], A. Niijima[4] and T. Ono[5]

[1]Torii Nutrient-stasis Project, ERATO, JRDC, [2]Ajinomoto Co., Inc., Kawasaki 210, [3]Asahi University, Gifu 501-02, [4]Niigata University, Niigata 951 and [5]Toyama Medicine & Pharmacology University, Toyama 930-01, Japan

L-Lysine (Lys) in plasma and brain are unchanged all day long under a normal diet feeding *ad libitum*. Once rats were fed a Lys-deficient diet, the Lys level in plasma and brain declined and anorexia occurred. Following this, the animals selectively ingested Lys solution from among other amino acids beyond the amount required. It was previously confirmed that the Lys deficiency and dietary Lys intake were recognized in the lateral hypothalamic area (LHA) using functional MRI and operant-type behavior. In addition, some neural plasticity in the LHA deferentially responding to Lys in the brain and its ingestion was observed electrophysiologically. Similar plasticity was found in the nucleus tractus solitarius. However, the threshold value and sensitivity to Lys solution recording from the chorda tympani and glossopharyngeal nerves were not altered regardless of Lys deficiency. In the present study, the hierarchy of chemical senses controlling Lys solution ingestion in rats with Lys deficiency was studied. Young Wistar male rats were offered a Lys-deficient diet and exposed to eight different amino acid solutions in a choice paradigm, concurrently with a constant i.p. Lys injection, but they started to ingest Lys solution selectively. The sensitivity to Lys recording from the hepatic vagal afferent nerve of rats with Lys deficiency became enhanced specifically. So, recognition of Lys deficiency begins in the oral cavity and subsequently in the alimentary tract during digestion, and the vagus nerve plays an important role in notifying the degree of Lys deficiency into the

LHA controlling quantitative ingestion of Lys due to Lys homeostasis.

24. Identification and variation of volatile compounds in the sternal gland secretions of related and non-related male koalas (*Phascolarctos cinereus*)

M. Salamon[1,3] and N.W. Davies[2]

[1]Department of Zoology, University of Erlangen-Nürnberg, Staudtstrasse 5, 91058 Erlangen, Germany and [3]Central Science Laboratories, University of Tasmania, GPO Box 252C, Hobart 7001, Australia
[3]Present address: Institut für Medizinische Psychologie, Universität München, Goethestrasse 31, 80336 München, Germany

The koala (*Phascolarctos cinereus*) is probably one of the best recognized and most admired native Australian marsupials. It is distributed in the eastern part of the continent from southern Victoria to northern Queensland, but has never existed in Tasmania. Adult animals weigh between 8 and 14 kg, with the males up to 50% larger than the females. The koala is herbivorous and its diet contains only *Eucalyptus* leaves, which are processed with the help of a bacterial biota in the large caecum. Only the male koala shows a large sternal gland (up to 85 × 50 mm) which undergoes discernible seasonal changes in activity and is used for scent-marking on trees. First research on the semiochemical content of the gland was carried out in Queensland by Carman and Greenfield in 1981, when some compounds were identified but the results were never published.

Our study investigated the chemical composition of the sternal gland secretion of the koala by using gas chromatography–mass spectroscopy (GC-MS) and looked at the variation of scent composition in related and non-related males. Four male koala which were housed in a wildlife park in Tasmania were used and their sternal gland secretion was collected on a small piece of filter paper for analysis.

The chemical constituents of the sternal gland of the koala showed that a range of fatty acid derivatives, monoterpenes and sesquiterpenes as well as long-chain hydrocarbons were present in the secretion and some of those compounds were possibly connected to the diet. However, because of the strong dependence on *Eucalyptus* foliage feeding, experiments could not be undertaken.

High resolution GC-MS analysis revealed two oximes present in the secretion that were previously found only in the urine of guinea-pigs. Furthermore, three nitriles could be identified in considerable amounts, one of which had previously been identified in the chest gland secretion of the thick-tailed galago (*Galago crassicaudatus*). In a second step the variation in scent composition was compared between three individuals using 31 identified compounds, equalling between 20 and 25% of the total peak area. Their relative abundance in the secretion was determined by integrating their peak area and their relation was analysed using principal component analysis. The consequences of those individual variations are discussed in detail.

25. Limits for discrimination of individual odors: golden hamsters as a model

Nina Yu.Vasilieva and Robert E. Johnston

Institute for Ecological Evolution, Russian Academy of Science, Moscow, Russia and [1]Department of Psychology, Cornell University, Ithaca, NY, USA

It is well documented that the capacity to discriminate individual odortypes may underlie many aspects of social behavior. However, the abilities of animals to analyze complex mixtures are still unclear. In this study, the discriminative abilities of golden hamster males were studied using a habituation paradigm. A total of 55 males, 4 to 12 months of age, were used, both sexually experienced and naive. In habituation trials a mixture of water-diluted vaginal secretions (VS) from a number of individuals was presented; in test trials an additional individual sample was added to this mixture. The mean difference between the last habituation trial and the test trial was revealed. At the level of analysis of each particular test subject the response was scored as 'discrimination' if there was an increase in investigation time in the test trial compared with the last trial of the habituation phase.

All of the test subjects discriminated the new VS sample when it was added to the mixture consisting of samples from 2–4 individuals (Mix-2 to Mix-4). The subsequent increase in complexity of the mixture led to a highly significant decrease in the number of subjects discriminating the new odor. Seventy-seven percent of males discriminated a new odor added to Mix-5, 27.9% to Mix-6, 14% to Mix-7, 6% to Mix-8 and 0% to Mix-9. The ability to discriminate individual odortypes in this study was not found to depend on age or sexual experience; most likely it depended on individual perceptual abilities. The data received show that discriminative capacities of golden hamster males are limited by a level of complexity of the mixture—when the number of compounds exceeds six, the ability to discriminate a particular odor sample drastically decreases. These results are in line with earlier findings in rats and can be considered as a further evidence to support the 'individual-specific substance(s)' hypothesis.

26. Genes and chemosensory individuality

Gary K. Beauchamp, Alan Singer and Kunio Yamazaki

Monell Chemical Senses Center, Philadelphia, PA, USA

The ability to recognize individuals is a prerequisite for most complex social behavior. Individual recognition is also required for many physiological responses that organisms make in response to members of their own species. Many species use odors, which we have termed odortypes, for individual recognition. More than 20 years ago the scientist/physician/writer Lewis Thomas first suggested a specific genetic basis for odortypes. He postulated that genes of the major histocompatibility complex (MHC), involved in regulating immune responses via discrimination of self from nonself internally, might also provide an external marker of individuality. Specifically, he suggested that it might be possible to train a dog to sniff a person who needs a kidney transplant and then, by sniffing a large number of potential donors, locate the donor with the closest tissue-type match. Over the ensuing 20 years our laboratories and others have provided strong evidence supporting this remarkable prediction.

Most of the work on this topic has been conducted with rodents (mice and rats) and, to a far lesser degree, other species. Summarizing briefly, the following has been found: (i) Mice tend to mate preferentially with individuals differing from themselves (or more precisely, differing from their parents) at the MHC. This apparently functions to maintain heterozygosity at the MHC and to avoid inbreeding. (ii) Mothers preferentially retrieve infants of the same MHC type as themselves and their litters and, correspondingly, infants tend to be attracted to odors of self-MHC type. (iii) Odortypes are evident as early as the second trimester post-conception; the odortype of a pregnant female is thus a mixture of her own odortype and that of her fetuses. (iv) MHC odortypes are coded for by the same genes that code for cell-surface proteins that bind self and non-self peptides for display to immune cells. (v) The odorants indicative of specific odortypes are found in serum, indicating that the kidney has no essential role in their coding. (vi) Unique odortypes appear to be made up of differential proportions of volatile carboxylic acids, the precursors of which may have been bound to MHC molecules.

What role might the MHC play in human individual recognition? Work with tracking dogs indicates that people can be discriminated by odor and that the odor has a genetic basis. Recent studies in our laboratory and in several others show that individuality of human odor shares may characteristics with rodents, may be specified in part by MHC differences, may modulate odor-based preferences for individuals of the opposite sex and perhaps may even influence mate choice. These tantalizing yet tentative data warrant substantial further study but, if supported and extended, would indicate a much greater role for body odor in human behavior and physiology than generally believed.

27. Signal transduction mechanisms for bitter and umami tastes

Joseph G. Brand

Monell Chemical Senses Center, Philadelphia, PA, USA

Assessing probable transduction mechanisms for bitterness and umami provides a study in contrast. While thousands of compounds taste bitter, apparently only relatively few impart the umami taste. These basic observations are reflected in current speculations on the transduction mechanisms for these two taste modalities. Because of the large number and great structural variety of chemicals that give rise to bitterness, it is likely that there is a diversity of mechanisms for bitterness. Several hypotheses have been developed to explain this observation, ranging from those describing purely non-specific mechanisms to those that assume the existence of specific receptor/transduction sequences. Based in part on behavioral genetic observations in mice for some bitter stimuli, receptor and transduction processes for at least some stimuli should be specific. Studies using the mouse as a model system suggest that bitter taste transduction is initiated when a stimulus interacts with a receptor which then activates, via a G-protein, a phospholipase C (PLC) to produce the two potential second messengers, inositol 1,4,3-trisphosphate (IP_3) and diacylglycerol. Production of IP_3 in response to various bitter stimuli has been measured in the millisecond time frame, with peak production occurring at 50–100 ms. In addition, recent molecular biological and behavioral studies also implicate gustducin-stimulated phosphodiesterase (PDE) activity in bitter taste transduction, presumably acting through a decrease in cyclic nucleotide levels. While these two mechanisms would appear to be competing, they may be linked via gustducin, the α-subunit of which stimulates PDE while the βγ-subunit stimulates PLC. The calcium requirements of these two stimulated enzymes are probably different, however. These differences must be accounted for and may predict the time course of appearance and disappearance of potential second messengers. While there is evidence that PLC, gustducin and IP_3 are involved in the transduction of several bitter tasting compounds, it is likely that other unrelated mechanisms also transduce bitterness for other compounds. In contrast to bitterness, umami taste is apparently imparted by only a few compounds, most notably the prototypical stimulus, monosodium glutamate. Two current hypotheses attempt to explain umami taste transduction. One states that umami is transduced by a metabotropic-type glutamate receptor, with evidence coming from studies that locate the message for a GluR4 receptor specifically in taste buds. Another hypothesis states that umarni is transduced by a glutamate-gated ion channel receptor, similar to an NMDA-type receptor. Evidence for this mechanism derives from reconstitution studies and from calcium imaging studies of isolated taste buds. Continued research into these modalities will lead to a better understanding of transduction mechanisms for bitter and umami tastes.

28. IP_3—a common second messenger for many bitter compounds

A.I. Spielman[1,3], H. Nagai[2], S. Rosenzweig[1], G. Sunavala[1], M. Nakagawa[2], T. Amachi[2] and J.G. Brand[3,4]

[1]New York University College of Dentistry, New York, USA, [2]Suntory Research Center, Osaka, Japan, [3]Monell Chemical Senses Center, Philadelphia and [4]Veterans Affairs Medical Center, Philadephia, PA, USA

Bitter taste is elicited by a variety of compounds with diverse chemical structures. Several recent studies have shown the involvement of the inositol trisphosphate pathway in signal transduction of bitter compounds such as sucrose octaacetate and denatonium. The involvement of a gustducin-activated cyclic nucleotide phosphodiesterase in bitter taste transduction has also been proposed. The current investigation was undertaken to understand the signaling mechanisms of other bitter compounds, alone or as mixtures.

We have performed rapid kinetic studies using the quench flow system and taste tissue from C57BL/6J, SWR mice and Sprague–Dawley rats. Generation of inositol 1,4,5-trisphosphate (IP_3) was monitored over the first 500 ms. The bitter stimulants used were: quinine sulfate (1 mM), naringin (1 mM), caffeine (25 mM), denatonium (2–10 mM), or a combination of caffeine (25 mM) and denatonium (2 mM).

All bitter compounds alone or in combination elicited production of IP_3, while non-gustatory tissue did not respond to any of the bitter compounds. Quinine elicited an IP_3 peak between 100 and 150 ms when tested in C57BL mice, while naringin stimulated peak IP_3 production at 50 ms in the same strain. Caffeine generated an IP_3 peak in both strains of mice and rat taste

tissue at ~50–100 ms. Denatonium also elicited a peak at 50–100 ms. The mixture of denatonium and caffeine elicited an IP_3 curve which was additive when compared with separate IP_3 production profiles of each compound.

These data are comparable with previous studies done with denatonium (10 mM) and strychnine (10 mM) separately and as mixtures. They imply the existence of independent mechanisms for signal transduction of caffeine, denatonium and strychnine, and argue for specific mechanisms that, at least in part, utilize the inositol trisphosphate pathway. IP_3 appears to be a common second messenger for a number of bitter compounds.

This study was supported by MH grant DE10754 to A.I.S. and DC00356, and a grant from the Department of Veterans Affairs to J.G.B.

29. Taste studies: from chemoreceptor modeling to FMRI mapping of cerebral projections

Annick Faurion

Laboratoire de Neurobiologie Sensorielle de l'E.P.H.E., 1, avenue des olympiades, 91305 Massy, France

Converging psychophysical and electrophysiological studies of taste sensitivity in human and hamster (Faurion and Courchay, 1990; Faurion, 1993) led us to disclose the following:

(i) Interindividual differencies of sensitivity: each individual presents a different taste sensitivity profile. The distribution of individual quantitative sensitivities is wide and sometimes shows multimodal distributions, as known for PTC. They are related to differences of qualitative perceptions such as bitter or sweet taste elicited in different subjects by methyl-α-D-mannopyrannoside, for example.

(ii) A continuum of taste perceptions: without relationship to semantic taste classification, it does not support the phenomenological classification of tastants into four groups.

(iii) An extreme power of discrimination of the peripheral system of detectors: any molecule is discriminated from any other one. Receptors remain unknown due to impossible biochemical purification although recent genetical studies and cloning might provide a new outcome in the future (Abe *et al.*, 1993; Gannon *et al.*, 1996).

Based on these findings, molecular modeling of taste receptors (Froloff *et al.*, 1996) has suggested a number of model receptor sites. These structures were successfully tested in a cross-adaptation study. The idea that a unique combination of multiple receptor sites generates a unique sensation for each unique molecule (Faurion *et al.*, 1980; Faurion and MacLeod, 1982) seems more and more documented and accepted. It has also been supported recently by transduction studies (Bernhardt *et al.*, 1996).

Cerebral imaging techniques now offer new issues to taste scientists: we have depicted taste cerebral areas with fMRI showing, for the first time, a dominant hemisphere taste projection relationship (Cerf *et al.*, 1996; and this congress); in addition, time resolution of taste projections is being explored by collaborative magnetoencephalographic neuroimaging.

Taste quantitative sensitivity is modified with learning, conditioning, novelty and semantic labeling of the stimulus, and also with cancer chemotherapy. By putting all this together we will soon be able to map out the effects of learning new neophobic or new pleasant tastes in different cerebral areas. This will provide a renewed interest for integrated physiological studies of taste.

References

Abe, K., Kusakabe, Y., Tanemura, K., Emori, Y. and Arai, S. (1993) Multiple genes for G-protein-coupled receptors and their expression in lingual epithelia. *FEBS Lett.*, **316**, 253–256.

Bernhardt, S., Naim, M., Zehavi, U. and Lindeman, B. (1996) Changes in IP3 and cytosolic Ca^{++} in response to sugars and non sugar sweeteners in transduction of sweet taste in the rat. *J. Physiol.*, **490**, 352–336.

Cerf, B., Faurion, A., MacLeod, P., Van de Moortele, P.F., Le Bihan, D. (1996) Functional MRI study of human gustatory cortex. *Neuroimage*, **3**, 342.

Faurion, A. (1993) Physiology of sweet taste and molecular receptors. In Kanters and Mathlouti (eds), *Sweet Taste Chemoreception*. Applied Science Publishers, London, pp. 291–315.

Faurion, A. and MacLeod, P. (1982) Sweet taste receptor mechanisms. In Birch and Parker (eds), *Nutritive Sweeteners*. Applied Science Publishers, London, pp. 247–273.

Faurion, A. and Courchay, C. (1990) Taste as a highly discriminative system: a hamster intrapapillar single unit study with 18 compounds. *Brain Res.*, **512**, 317–332.

Faurion, A., Saito, S. and MacLeod, P. (1980) Sweet taste involves several distinct receptor mechanisms. *Chem. Senses*, **5**, 107–121.

Froloff *et al.* (1996) *Chem. Senses*, in press.

Gannon, K.S., Ninomyia, Y., Wong, O.T. and Margolskee, R.F. (1996) Gustducin knock-out mice exhibit reduced bitter taste sensitivity as evidenced by glossopharyngeal responses and conditioned taste aversion. *ACHEMS Abstr.*, p. 22.

30. From hyperpotent sweeteners to sucrose: the multipoint attachment (MPA) theory

Jean-Marie Tinti and Claude Nofre

Université Claude Bernard, Faculté de Médecine Alexis Carrel, Rue Guillaume Paradin, 69008 Lyon, France

Several families of hyperpotent sweeteners, with potencies of up to 225 000 times that of sucrose on a weight basis, have been discovered by the authors during the last two decades. After the failure of the most well-accepted models to explain the structure–activity relationships (SAR) of such potent compounds correctly, a new theory (by Boston in 1990), further amended (by Reims in 1991 and Reading in 1995), was proposed. This theory, which strongly contrasts with former models (those of Shallenberger in 1967 and Kier in 1972), is now designated as the 'multipoint attachment (MPA) theory'.

It is proposed that in the single-type human sweetness receptor (most probably a seven-pass transmembrane protein) exist eight fundamental recognition sites able to interact with sweet molecules through up to 15 elementary interactions. All of these interactions are not required simultaneously to induce a sweet taste. In the receptor, the support of these eight recognition sites was inferred from SAR to be a corresponding set of eight amino acid side chains. Three of these side chains, assigned to be Asp (or Glu)-1, Lys-2 and Asp (or Glu)-3, are involved in reinforced ionic interactions and/or hydrogen bonds (through the β- or γ-carboxylate groups of Asp or Glu and the ε-ammonium group of Lys). Another group is constituted of four threonine side chains, Thr-4, Thr-5, Thr-6 and Thr-7; each Thr side chain [$CH(CH_3)OH$] allows hydrogen bonding through the β-hydroxyl group and steric interaction (van der Waals contacts) through the β-methyl group.

The eighth receptor recognition site, represented by a serine side chain, Ser-8, acts as an H-bond donor group.

A significant innovation of the MPA theory is the lack of the hydrophobic interaction concept, which is too inaccurate to explain the SAR observed with hyperpotent sweeteners; shape, size and length of the hydrocarbon part of sweeteners, often associated with the steric fit, are now considered as more crucial factors than hydrophobicity. A steric fit means that a sweet molecule acts, through small points of contact (CH_3, CH_2 or CH groups), as a wedge between at least two of the β-methyl groups of the Thr-4, Thr-5, Thr-6 or Thr-7 side chains.

The steric fit, in synergy with the other conventional ionic interactions and hydrogen bonding, induces a conformational change of the receptor itself which, moving into an activated expanded state, thus originates the physiological response.

The SAR of some hyperpotent sweeteners are presented and interpreted as an illustration of the MPA theory.

31. Defining a hydrogen bond lifetime

Tim Astley, Gordon G. Birch[1], Michael G.B. Drew, P. Mark Rodger and Gareth R.H. Wilden

Chemistry Department and [2]Department of Food Science and Technology, University of Reading, Whiteknights, Reading RG6 6AD, UK

Hydrogen bonds (H-bonds) are responsible for a great many effects in chemistry. Specifically they have been used to explain the sweet taste properties of a number of carbohydrates and other sweet-tasting molecules in water. Also, it has been proposed that the lifetimes of these H-bonds are correlated with the strength and duration of the sweet taste response. Computer simulations offer good oppourtunities for investigating H-bonds; however, a fundamental problem has been to find an appropriate definition for the H-bond and its lifetime.

The two most common definitions (sometimes compounded together) are (i) energetic: two molecules are considered to be H-bonded if their interaction energy is lower than a given value V_{HB}; and (ii) geometric: two molecules are considered bonded if the values of pertinent internal coordinates of the dimer are within appropriate ranges. These definitions are only partially suitable to study dynamic properties of H-bonding. Indeed, water molecules show a librational motion on a timescale of 10^{-13} s superimposed on slower diffusional and rotational motions, which causes a time variation of dimer interaction parameters. Therefore, if a definition of the H-bond based on cut-off values is used, the lifetimes of bonds with parameters oscillating not too far from the H-bond definition limits can appear much shorter than they really are. This has a direct effect on the predicted lifetime of the H-bond, which, being an experimentally measurable value, would help validate any simulation techinique. Thus, the need to develop a suitable way of defining what constitutes an H-bond and its lifetime are of crucial importance.

Using D-glucose as an example, the use of rolling averages with various weighting schemes has been employed to more accurately ascertain the lifetime of H-bonds between the various hydroxyl sites on the monosaccharide and water. Initial results indicate that the lifetimes are between 10^{-11} and 10^{-10} s.

This work was supported by BBSRC(UK).

32. The mechanistic understanding of the sweetness response

Gordon G. Birch and Jane M. Westwell

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

A programme funded by the European Commission under the auspices of ECRO has been underway for 18 months. The programme involves eight European laboratories and is coordinated at the University of Reading. The first report has been issued.

The research involves the chemistry and psychophysics of sweet taste and is unique in focusing on the role of water to elucidate the sweet taste mechanism. Both theoretical and experimental studies of the sweetener–water interactions are under investigation, the former by molecular dynamics studies and the latter by solution chemistry measurements. Sensory differences between permitted sweeteners are already interpretable by structure–activity relationships and solution parameters. These results are currently being extended to complex blends of sweeteners and model formulations involving more or less ionic solutes and also flavour molecules. Their overall taste properties are ascribable to precise hydrostatic, hydrodynamic and water-compatibility effects of the solute molecules. It is anticipated that this research will make a contribution to the improvement of taste in food products.

33. The labeled-line /across-fiber pattern: on the relationship between taste fiber identity and sweet taste quality in chimpanzee

G. Hellekant, Y. Ninomiya[1], V. Danilova, D. Glaser[2] and D. Ming

The University of Wisconsin and Wisconsin Regional Primate Center, 1655 Linden Drive, Madison, WI 53706, USA, [1]Asahi University, School of Dentistry, Gifu, Japan and [2]Anthropologisches Institut der Universität Zürich, Zürich, Switzerland

It is still unresolved if taste in peripheral taste nerves is coded as an 'across-fiber pattern' or as a 'labeled-line'. The 'across-fiber pattern' argues that activity across the fibers codes for taste, while the 'labeled-line' claims that activity in a particular set of fibers elicits a taste quality.

As is well-known, gymnemic acids (GA) suppress and miraculin enhances sweet taste in humans. These effects parallel suppression and enhancement, respectively, of the summated chorda tympani (CT) response to sweet in human and chimpanzee CT by GA and miraculin. Further, in humans, GA after miraculin abolishes the sweet taste as well as the sweetness of sweeteners. In monkeys the CT enhancement induced by miraculin is abolished by GA. Thus behavioral observations and summated CT nerve recordings indicate that GA and miraculin exert similar effects in chimpanzees as in humans. Chimpanzee CT taste fibers are more specific than those of any other mammal. Cluster analysis distinguished an S-cluster, responding exclusively to sweet stimuli; a Q-cluster, sensitive to bitter tastants; and an N-cluster, stimulated by salts. The above indicates that we might be able to address this question by applying GA and miraculin on chimpanzee taste buds while recording from single CT fibers.

We found that GA suppressed or abolished the response to every sweetener in fibers of the S-cluster, but had no effect on the Q- and N-clusters. After miraculin, fibers of the S-cluster, which did not respond to acids before miraculin, responded to every acid, as well as to the sweeteners. GA abolished the miraculin-induced response to sour stimuli as well as the responses to sweeteners in the S-cluster. These results link the sweet taste quality to activity in fibers of the S-cluster. Thus our findings seem to satisfy the definition of the labeled-line theory: 'that activity in a particular fiber type represents a specific taste quality'.

34. Structure–activity relationships of sweet-tasting molecules taking into account solvent effects

Tim Astley, Gordon G. Birch, Michael G.B. Drew, P. Mark Rodger and Gareth R.H. Wilden

Departments of Chemistry and Food Science and Technology, University of Reading, Whiteknights, Reading RG6 6AD, UK

QSAR studies include an investigation of the structural and physical properties of molecules to find factors responsible for their activity. In our work on the mechanistic understanding of the sweet response, we have extended this technique to include the structural features and behaviour of the molecules in water. The taste properties of molecules are all obtained in the presence of water, and it therefore seems appropriate to concentrate on solvent properties.

We have included in our QSAR studies the results of extensive simulations of molecules in water using the DL-POLY program together with the CHARMm force field for the solute and SPC water. The Nose-Hoover with Verlet algorithm was used. We have carried out molecular dynamics calculations on a range of molecules, primarily monosaccharides, isovanillates and sulfamates, each for 200 ps and calculated their water structuring properties. Specifically useful for the QSAR studies have proved to be details from radial distribution functions and the hydrogen bond counts for both donor and acceptor atoms. We have found that these parameters for individual hydroxides groups in the carbohydrates taken either separately or in combination with those from adjacent groups gives rise to significant correlation in the QSARs.

We thank BBSRC(UK) and the EC for support (AIR3.CT94.2107).

35. Brain imaging techniques for the study of central representations of olfaction in man

G. Kobal

Department of Experimental and Clinical Pharmacology of Erlangen-Nuremberg, 91054 Erlangen, Germany

Chemosensory bioresponses of the central nervous system in man have more and more found their way into a number of fields of research. They help to establish the function of both the trigeminal and the olfactory systems. The investigation of chemosensory deficits in neurological diseases as well as deficits due to problems of the upper respiratory tract are only some of these typical ranges of application. In order to understand cerebral functional deficits it is very important to have sufficient information about the locations of the cortical generators of olfactory bioresponses. Recently new brain mapping techniques have been developed that offer powerful tools for the investigation of cortical and subcortical areas involved in the processing of chemosensory information.

While positron emission tomography requires the intravenous administration of radioactive markers, functional magnetic resonance imaging is completely non-invasive, as is magnetic source imaging (MSI). New approaches to evaluate multi-channel electroencephalogram recordings offer an additional way of analyzing the locations of cortical cell populations activated by chemosensory stimuli. Results obtained with the four techniques are demonstrated and compared with respect to their topographical and temporal resolution.

When using the MSI technique in healthy volunteers areas activated by the various odorants differed from each other, but the two hemispheres were activated symmetrically in most cases. After stimulation with vanillin, hydrogen sulfide and ethyl vanillin, activation was mainly found in the temporal lobe and the insular cortex. Surprisingly, the insular generator in the left hemisphere was not activated after stimulation with hydrogen sulfide. Additionally, we observed bilateral activation of the hippocampus during the first 600 ms after olfactory stimulation with eugenol.

36. Effects of aging on cognitive processing and evoked response representations of olfaction in man

Claire Murphy, Charlie D. Morgan, Mark W. Geisler and James Covington

San Diego State University and University of California, San Diego, 6363 Alvarado Court, Suite 101, San Diego, CA, 92120-4913, USA

Psychophysical studies of sensory responses in the elderly show impairment in olfactory function which is profound in demented populations. We seek to understand through neuropsychological testing and olfactory event-related potentials the effects of aging on cognitive processing and central representations of olfaction. We have examined the influence of age on odor identification and on odor recognition memory at short and long delays, as well as verbal recall of odors presented at short and long delays. The results indicate significant impairment relative to similar measures of verbal memory and implicate difficulties with semantic encoding as an underlying mechanism. We have recorded olfactory event-related potentials from young and elderly adults. Subjects also performed tests of memory and attention. Stimuli were delivered olfactometrically in a stream of air heated to 36.5°C and humidified to 80% RH, with a rise time of <20 ms. Subjects used velopharyngeal closure to maintain constant airflow through the nasal cavity (similar to Kobal's method). Responses were recorded from Fz, Cz and Pz of the international 10/20 system, amplified and filtered. Stimuli were presented at different concentrations and different intertrial intervals to examine the influence of these variables. The components N1, P2, N2 and P3 were examined with regard to amplitude and latency. Age effects were significant at P2

and N2 and greatest at P3. Concentration significantly affected amplitude for both old and young. Correspondence between neuropsychological measures of memory, allocation of attentional resources and the latency and amplitude of the evoked potential, particularly the late cognitive potential, strongly support a central origin for slowing of olfactory stimulus processing with age.

Supported by NIH grant DC02064 (C.M.) and training grant DC00032 (M.W.G.).

37. Influence of olfactory learning on the rat piriform cortex activity recorded with voltage-sensitive dyes

M. Cattarelli[1,3], P. Litaudon[1], A.-M. Mouly[1] and R. Sullivan[2]

[1]Physiologie Neurosensorielle, UCB-Lyon 1, 69622 Villeurbanne, France and [2]Department of Zoology, University of Oklahoma, OK 73019, USA [3]Present address: IESGI-CNRS UPR9054, 17 rue Sully, 21034 Dijon, France

Piriform cortex (PCx) seems to play a potential role in olfactory learning since long term potentiation has been observed in this structure. As learning is known to induce changes in neural networks, optical recordings with voltage-sensitive dyes were performed to monitor the neural activity on the whole PCx area with good spatial and temporal resolutions after learning acquisition. Four electrodes were chronically implanted in the olfactory bulb (OB) of the rats. In a conditioned group, rats were trained to an associative learning in which electrical stimulation of a given bulbar electrode predicted a positive reinforcement while stimulation of a different electrode predicted a negative reinforcement. In a familiarized group, rats received the same protocol of daily OB electrical stimulation but no reinforcement was associated at that time. After completion of conditioning (5–6 days), the PCx responses to OB stimulations (single shock, 200 μs, 1 mA to 100 μA) were recorded.

With the high intensity stimulation (1 mA), the amplitude of the early component (latency 17–30 ms) of the PCx signal was increased after associative learning in the posterior part of the PCx only. The probability of occurrence of a late component of the PCx signal (40–50 ms) was enhanced in both the conditioned and familiarized animals compared with the controls. However, the spread of the late component on the PCx area was larger in conditioned than in familiarized rats. In addition, conditioning selectively enhanced the probability of occurrence of a late (56–73 ms) component in response to low intensity stimulation by learned electrodes. This late wave occurred simultaneously on the whole PCx area with a constant amplitude. It can be concluded that after olfactory learning both early and late components of the PCx-evoked activity were potentiated. The origin of the late wave remains to be determined.

38. Electro-olfactograms are present when odorous stimuli have not been perceived

T. Hummel, J. Mojet[1] and G. Kobal

Department of Pharmacology, University of Erlangen-Nürnberg, Krankenhausstraße 9, 91054 Erlangen, Germany and [1]Unilever, Vlaardingen, The Netherlands

After chemical stimulation of the human olfactory epithelium it is possible to record a negative response (electro-olfactogram, EOG) which is interpreted as the summated generator potential of olfactory neurons. The aim of this investigation was to test whether the EOG is present when olfactory stimuli are not perceived by the subject.

Twelve healthy volunteers participated in the experiments (six female, six male; age 25–34 years). Stimulation was performed with vanillin and eugenol at concentrations around threshold (stimulus duration 500 ms, interval ~60 s). The stimuli were chosen on the basis of differences in transduction pathways (vanillin: IP_3; eugenol: cAMP). EOG was recorded by means of tubular electrodes (cutaneous reference contralateral bridge of the nose; impedance < 8 kΩ; bandpass DC to 30 Hz; sampling rate 125 Hz). Eye blinks were monitored via the Fp2 lead (referenced against A1). The electrode was positioned under endoscopic control; recording sites were marked on a map of the human nasal cavity.

EOG could be recorded in 4/12 subjects. Two subjects were unable to compensate the sneezing reflex; the responses of three other subjects had to be discarded due to an excessive number of artifacts. For both stimulants EOGs could be obtained even when the stimuli were not perceived. All recording sites were localized superior to the insertion of the middle turbinate. In 3/4 cases EOGs to both stimulants were obtained at the same position.

This indicates at an electrophysiological level that olfactory information is present even when stimuli are not subjectively perceived; this may provide the basis of subconscious behavioural modifications induced by odorants.

39. Psychological determinants of olfactory evoked potentials

Bettina Pause and Roman Ferstl

Institute of Psychology, University of Kiel, Olshausenstraße 62, D-24098 Kiel, Germany

It has been suggested that the recording of olfactory evoked potentials (OEP) in humans is an objective measurement of odor perception. To date, most laboratories interpret the electrical brain response to odors as a detector for odor characteristics, implying that the OEP is exogenous. Thus, OEPs are considered to reflect quality and intensity differences but to be independent of endogenous factors. However, event-related potentials of other sensory modalities have been separated into early exogenous and late endogenous components. The late components depend mainly on the amount of alertness and attention, and higher-order stimulus processing such as stimulus recognition, classification and significance.

Considering the contribution of (sub)cortical activity to the features of event-related potentials, we carried out a number of studies to try to identify the psychological determinants of the

OEP. The main finding was that the structure of the OEP seems to be very similar to the late endogenous components recorded in response to auditory or visual stimuli. We found two component complexes which varied either with the stimulus concentration and the subject's attention (N1, P2) or with the subjective stimulus meaning (late positive complex; LPC). In contrast to other modalities, the LPC elicited by odors was usually so large that the small P2 component was mostly overlapped. This indicates that the positive component, which has hitherto been described as part of the OEP, depends mainly on subjective stimulus processing such as stimulus categorization and stimulus meaning. Moreover, we were able to demonstrate that the OEP depends greatly on the amount of attention. If the subject's attention is distracted by another secondary task (e.g. counting target words in pop songs) the positivity of the OEP is almost absent. Finally, we found that the amplitude of the N1–P2 complex only depends on stimulus concentration when odors are used which activate the trigeminal system. Increasing the concentration of olfactory stimuli resulted in shorter latencies of the N1 component, indicating a faster response by the central nervous system.

We conclude from our experiments that the human electrical brain activity evoked by odors mainly reflects the psychological relevance of the odors and the attentional status of the subject.

These studies were supported by a grant from the Stiftung Volkswagenwerk.

40. Human cortical taste area analyzed by MEG's early component and fMRI

T. Kobayakawa[1], B. Ceff[2], H. Endo[1], A. Faurion[2], S. Ayabe-Kanamura[1,3], P. MacLeod[2], H. Ogawa[4], P.F. Van de Moortele[5], T. Takeda[1], D. Le Bihan[5] and S. Saito[1]

[1]National Institute of Bioscience and Human-Technology, 1-1, Higashi, Tsukuba, Ibaraki, 305 Japan, [2]Laboratoire de Neurobiologie Sensorielle, 1, avenue des Olympiades, 91305 Massy, France, [3]University of Tsukuba, [4]Kumamoto University, Japan and [5]Service Hospitalier Frederic Joliot, CEA, 91304 Orsay, France

Gustatory cortical areas (GCAs) are well known in the rat and monkey but information on the human GCA is scarce. Recent advances in brain imaging techniques, e.g. magnetoencephalography (MEG), functional magnetic resonance imaging (fMRI) and positron emission tomography, enable the location of activated brain regions in the conscious human. A MEG study was undertaken in Japan together with a fMRI study in France to localize taste-related areas in the human. We developed a gustatory stimulation device giving a stimulus rise-time of ~20 ms. Magnetic fields (MFs) evoked by gustatory stimulation with 1 M NaCl and 3 mM saccharin were recorded using a 64-channel whole-cortex SQUID system (CTF System Inc., Canada). Stimulus duration was ~400 ms, and the interstimulus interval was ~30 s. The temperature of the tastant was ~36°C. During the recording of the MEG the subject, wearing ear plugs, kept his eyes open and watched a fixation point. MFs were sampled at a rate of 250 Hz with a low-pass filter of 40 Hz. Forty trials were presented to a subject per session. The averaged onset latency of MFs was 93 ms for NaCl and 172 ms for saccharin; no response was obtained for water. A large correlation coefficient was noted between the difference of onset MFs latencies in two tastants and that of behavioral reaction times, and the response to saccharin was delayed or abolished after treatment of a subject's tongue with a sweet-suppressing agent. The findings indicate that the MFs obtained were caused by gustatory rather than somatic stimulation. On assumption of equivalent current dipole (ECD) of MFs on each hemisphere, we estimated the location of the ECD on the subject's MRI, and found it at the transition between the operculum and the insula. NaCl, aspartame and quinine hydrochloride gave activations in the left and right insula, left and right inferior frontal gyrus—pars opercularis and pars orbitalis—postcentral gyrus and temporal gyrus. The activation of the left and right insula confirmed the ECDs by MEG. From the fMRI and MEG findings, the insula was recognized as a gustatory related area. Temporal information from MEG also indicates that the transition between the operculum and the insula is the primary GCA evoked by taste stimulation.

41. Coding of information in odour mixtures

D.G. Laing, A. Jinks, I. Hutchinson and N. Oram

Centre For Advanced Food Research, University of Western Sydney, Bourke Street, Richmond, NSW 2753, Australia

Recent research in this laboratory and by Laing and Livermore has indicated that humans use both spatial and temporal coding processes to discriminate and identify the components in odour mixtures. Such processes, however, although allowing rapid identification of a complex mixture such as 'chocolate', limit the identification of components in mixtures to a maximum of four. Currently, the mechanisms that underlie the massive loss of information about the components in a complex mixture are largely unknown.

As part of our investigations into the processing of odour mixtures, the role of transduction pathways used by odorants and their effects on odour interactions has been explored and are described here. In studies with binary mixtures, produced in a computer-controlled olfactometer, the interactions of odorants that have been reported to induce release of either C-AMP or IP_3 as second messengers in non-human species have been investigated. Mixtures contained like-pathway odorants or unlike-pathway odorants. The odorants were presented to subjects either in mixtures or in series at intervals of between 100 and 600 ms, and the task of the subjects was to indicate which odorant was perceived first, or to rate the intensity of each odorant. The results indicate that temporal coding occurred with each of several odor pairs, and usually resulted in the 'slower' odorant being suppressed to a greater extent than the 'faster' odorant. In addition, the type of odorant (C-AMP or IP_3 inducer) did not affect the outcome.

In a second series of studies with ternary mixtures and similar experimental conditions, subjects had great difficulty in determining which odorant was perceived first unless the critical time interval between the first and last perceived odorants was ~500 ms. This dramatic fall off in the ability to discriminate and identify odorants in ternary mixtures when measured by a technique very different to that employed by Laing and Livermore again emphasizes the massive interaction and loss of information about mixture components that occurs once a mixture contains three or more components, regardless of the types of odorants in a

mixture. The latter results will be discussed in terms of the possible underlying mechanisms.

42. 'Anosmic olfaction'—a blindsight-like phenomenon in normal subjects

Tomas Radil[1,2] and Charles J. Wysocki[2]

[1]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic and [2]Monell Chemical Senses Center, Philadelphia, PA, USA

Experiments were conducted to determine whether threshold concentrations of odorants could be detected, under certain conditions, with reliable accuracy while subjects were apparently not aware of the perceptual features of the stimulus. The experiments were performed in the following way: first, using squeeze bottles, detection thresholds were estimated for amyl acetate (resembling the pleasant smell of bananas) by using an ascending binary forced-choice method (the criterion being defined as the lowest concentration correctly differentiated from an odorless blank seven times in sequence). The threshold concentration, five lower concentrations and two higher concentration steps in the binary dilution series were presented in a pair with a blank, each ten times, in random order. The task of the subjects was to differentiate whether the first or the second bottle contained the odorant. Feedback information was provided on judgement correctness in every trial. Additionally, subjects marked on a continuous scale whether they were certain (at one extreme) or guessing (at the opposite extreme). A training procedure preceded the actual experiment. The results revealed that at the threshold concentration and at two lower concentration steps the subjects detected the majority of stimuli correctly, while they claimed that their choices were primarily based on guessing. Pilot experiments with butyric acid (an unpleasant odor at higher concentrations) showed similar results. The results suggest that unconscious olfactory detection occurs in normal subjects. The phenomenon of 'anosmic olfaction' appears to be analogous to that of 'blindsight', found when the primary visual cortex is excluded from processing visual information. A role for 'anosmic olfaction' in normal olfactory function remains to be determined.

43. Specific androstenone-anosmia in patients with impaired sperm production

Jacob E. Steiner, Irvin Hirsch[1], Keith N. Van Arsdalen[2] and Charles J. Wysocki[3]

Department of Oral Biology, Hebrew University-Hadassah Faculty of Dental Medicine, Jerusalem, Israel, [2]Department. of Urology, Thomas Jefferson University Medical College, Philadelphia, PA, [3]Department of Urology, Hospital of the University of Pennsylvania, Philaadelphia, PA and [4]Monell Chemical Senses Center, Philadelphia, PA, USA

Olfactory cues play a central role in mammalian reproduction. Humans, however, seem to be less dependent on odor cues. In hypogonadotropic hypogonadism (Kallman's syndrome; prevalent in males) total failure of sperm production is associated with general anosmia. Perhaps less extreme reproductive malfunctions, e.g. oligospermia, might be associated with impaired, but not absent, olfaction. In a preliminary study olfactory performance of oligospermic patients was found not to be different from that of controls when tested with common household odorants. There is considerable variation in the ability to detect the odor of androstenone. In an Israeli sample, ~30% failed to detect the compound's odor. Androstenone was one of the scratch and sniff samples in the 1986 National Geographic Smell Survey; 71 patients with severely impaired spermatogenesis and 64 controls were tested with this instrument. Results showed that the number of androstenone-anosmics among patients was significantly higher than among controls. To re-examine these findings, 50 severely oligospermic patients and 50 fertile controls were tested in a forced choice method. Results revealed a significantly higher number of androstenone-anosmics among patients than among controls. This suggests that certain types of reproductive malfunctions seem to be associated with anosmia specific to androstenone. Further investigation is needed to assess whether female reproductive failures might also be associated with similar specific anosmia and to study the mechanisms underlying the association in males.

Supported in part by NIH grant DC00298.

44. Tongue growth in children

I. Hutchinson, E. Temple and D.G. Laing

Centre for Advanced Food Research, University of Western Sydney, Bourke Street, Richmond, NSW 2753, Australia

Recent research in this laboratory has compared the taste sensitivity of children with that of adults. The responses to stimulation of small areas on the anterior surface of the tongue have been studied. However, little is known about the childhood growth of the tongue and, in particular, the growth of the taste-sensitive regions of the tongue. The aim of this study was to determine the extent of tongue growth during childhood, particularly the growth of the taste-sensitive anterior region that contains fungiform papillae.

Measurements were made from 232 subjects (122 female, 110 male) between the ages of 4 and 66 years. Five tongue measurements were made on each subject, and from these measurements two areas were calculated: the anterior area, which contains fungiform papillae (taste-sensitive), and the more posterior area with no fungiform papillae (relatively taste-insensitive).

The dimensions of the 'sensitive' area in children under the age of 8 years were significantly smaller than those of older children and adults. From 8 to 16 years the 'sensitive' area did not increase in size significantly. In contrast, the posterior region continued to grow until the age of 11 years, with little growth thereafter.

The results indicate that the 'taste-sensitive' area (receptive field) near the tip of the tongue undergoes most of its growth by 8 years of age, whereas the more posterior 'taste-insensitive' area continues to grow for a number of years; this pattern of growth appears to parallel that of the brain and the mandible respectively. These findings have implications for psychophysical studies of the development of taste sensitivity on the tongue.

Supported by ARC and MRC.

45. The odor influence on left and right nostril patency

Vittorio G. Bizzozero

Laboratory of Experimental Rhinology, Hospital of the University of Geneva, Geneva, Switzerland

The nasal cycle consists of an alternating left–right phenomenon of congestion–decongestion of the nasal mucosa. This is thought to be regulated by the autonomic nervous system: sympathetic dominance in one nostril, producing vasoconstriction and decongestion, occurs simultaneously with parasympathetic dominance in the other nostril, producing vasodilatation and congestion.

Autonomic functions can be influenced by many areas of the brain and many of these seem to occur by way of the hypothalamus. Werntz and others showed that the hemispheric activity was directly related to the rhythmicity of the nasal cycle: a relatively greater EEG value in one hemisphere correlated with predominant airflow in the contralateral nostril, defining a new interrelationship between cerebral dominance and peripheral autonomic nervous function. The cycle is found in 70–80% of adult humans, but a significant reciprocity of the phases in the same nostril has been found in 56% and a significant periodicity in both nostrils has been shown in only 22% of subjects.

An important question in olfaction concerns the relationship between the properties of a molecule and its influence in nasal patency. One experiment was performed to determine whether an odor could modify differently the left and the right nostril patency, suggesting a difference in left and right capability to discriminate or react to an odor. The nasal congestion was first measured bilaterally by a rhinomanometer for 10 measures every 3 min, followed by 10 3-min measures; in this second part, the subjects were asked to inhale phenylethyl alcohol before each measure. The results show a difference only for the left nostril (a significant difference in 3/4 of subjects), the odorant producing an increase in the nasal congestion, the right nostril showing no difference between the two series of measures (with and without odorant). These findings could suggest a difference in the nasal patency in perceiving and discriminating odor quality. The left hemisphere could be more concerned by the semiological and identification aspects of an olfactory stimulus.

46. Taste- and odor reactivity in heroin addicts

E. Perl, E.N. Shufman[1], J.E. Steiner[2] and S. Luger[2]

'Talbieh' Mental Hospital, Jerusalem, [1]The Jerusalem Institute for the Treatment of Substance Abuse, Jerusalem and [2]Department of Oral Biology, Hebrew University-Hadassah Faculty of Dental Medicine, Jerusalem, Israel

Opiates, in general, and heroin, in particular, are known to induce compulsive drug-seeking and drug-taking, and also to produce addictive behavior. Addiction is accompanied by psychobiological processes, which lead to modulation and even distortion of perception of sensory stimuli. Gustatory and olfactory stimuli are hedonically polarized, and are therefore most appropriate for the assessment of the organism's adequate reactivity to 'useful' and 'harmful' chemosensory events. Previous studies revealed that psychophysical and self-estimates and stimulus-triggered reflectory responses (of facial expressions) mirror with comparable reliability the hedonics of the perceived taste and odor sensations. In the present study both cognitive and reflectory facial-expressive behavioral responses of a group of heroin-addicts were recorded and compared with those of a group of detoxified former addicts and with a group of matching control examinees. Results show that all three groups differentiated significantly between 'indifferent', 'pleasant' and 'aversive' tastes and odors, as reflected by both parameters. Active addicts estimated sweet taste and savory smells as being somewhat more pleasant, and bitter and sour tastes and a putrid odor as less unpleasant than did the other two groups. The reflectory facial displays of addicts, as assessed by two independent observers, were less expressive and discriminative than those of the two other groups. Taste- and odor-induced facial displays are known to be controlled primarily by the brainstem. The findings reported here, therefore, indicate that heroin-addiction affects brain mechanisms, which mirror taste- and odor hedonics, suppressing the reflectory sensory–motor coordinations anchored in the brainstem and modulating cognitive reactions controlled by the cortex.

Supported by a grant from the Israel Anti-Dmg Authority.

47. Context in sensory testing

Herbert L. Meiselman

US Army Natick RD&E Center, Natick, MA 01760-5020, USA

Traditionally, we have tried to explain differences in food choice and in food acceptability by studying the food itself or the individual who is eating the food. The latter involves the physiology and psychology of the individual, while the former involves the sensory characteristics of the food. However, in recent years we have come to realize that a third important determinant of food choice and food acceptability is the context or situation in which food is consumed. Context involves all of those influences which are outside an eating event. For convenience I have divided those contextual influences into those involving the food, the individual and the environment. In addition, the temporal dimension must be added. This paper will review the contextual influences involving food, especially those involved in food combinations as found in meals, and in meal combinations as found in diets. Food variety will be discussed as a possible mechanism for the relationship of sensory factors and food choice. Next, the role of individual attitudes, traits and expectations will be reviewed. Expectations appear to play a major role in food choice and acceptance, and appear to interact with sensory evaluation of foods. Several studies will be presented which highlight the powerful role of expectations. Finally, the role of environmental influences will be examined, stressing those factors found in normally occurring meals as opposed to laboratory meals. Again, studies conducted in different situations will illustrate the effects which can be achieved with situational variables. This overview of the role of context in sensory testing will conclude with recommendations concerning theory and methodology.

48. Sensory-specific satiety: comparison of gustatory and kinesthetic components

Jean-Xavier Guinard and Patrice Brun

Department of Food Science and Technology, University of California, Davis, CA 95616, USA

During a meal, the pleasantness of and desire to eat foods which have been eaten declines more than those of foods which have not been eaten. Because these changes are partly specific to the sensory properties of each food, it has been called 'sensory-specific satiety' (SSS). The gustatory qualities of saltiness and sweetness have been clearly shown to trigger (sensory-specific) satiety. Whether sensory modalities other than taste are involved in SSS remains unclear. In this study the respective contributions of taste (saltiness and sweetness) and texture (mostly the hardness dimension) to SSS were compared. Thirty-two subjects (16 male and 16 female young, normal-weight adults) rated a number of parameters, including pleasantness of taste, pleasantness of texture and desire to eat, on visual analog scales for eight test foods, were then given one of the foods to eat *ad libitum* for lunch, and re-rated the same parameters for the eight foods 2 and 20 min after the end of the meal. The experimental sets of eight test foods and four lunch foods were balanced for taste quality (salty versus sweet) and texture quality (hard versus soft). Test foods were crackers (hard/salty), guacamole (soft/salty), ham and cheese sandwich on white bread (soft/salty), ham and cheese sandwich on baguette (hard/salty), yogurt (soft/sweet), carrots (hard/sweet), apple sauce (soft/sweet) and apples (hard/sweet). Lunch foods were the hard and soft versions of a salty food (ham and cheese sandwich on baguette versus white bread) or of a sweet food (apples versus apple sauce). SSS was observed for both saltiness and sweetness (e.g. desire to eat sweet test foods decreased significantly after eating a sweet lunch food and similarly for salty foods), and to a lesser yet significant extent for texture (e.g. desire to eat hard test foods decreased after eating a hard lunch food and similarly for soft foods). The same trends were observed for pleasantness of taste or texture (decrease after consumption of a food with the same taste or texture quality). It is concluded that kinesthetic-specific satiety is a significant component of satiety, yet it is not as strong as gustatory (saltiness or sweetness)-specific satiety.

49. The dimensionality of bitterness in beer

Bonnie M. King and Nathalie Moreau

Quest International, PO Box 2, 1400 CA Busstim, Holland

Bitterness in beer was studied by different types of sensory methods: 2-AFC, intensity rating and time–intensity. When the eight parameters abstracted from time–intensity curves were subjected to a principal component analysis per panelist, it was evident that some panelists needed more dimensions to describe the beer samples than others. Parameters like maximum intensity (I_{max}) and the area under the curve were significantly correlated with the first dimension for all panelists. On the other hand, panelists showed individual behavior with respect to parameters such as intensity at swallowing (I_{swal}) and the ratio of I_{swal}/I_{max}, as well as the total duration of bitterness.

For some panelists, time-related parameters were designated as one dimension while intensity-related parameters were correlated with another dimension. Other panelists showed a dimension related to swallowing. In most cases the correlation of parameters with dimensions was not so easy to explain. Among panelists requiring three dimensions, two groups could be distinguished. The eigenvalues for the first group showed almost equal weighing of each dimension, whereas for the second group the first dimension was weighed more heavily than the second, and the second was weighed more heavily than the third.

These results will be discussed in terms of bitter perception in other samples and the PTC/PROP taster status of the panelists. The more a panelist is aware of temporal aspects of bitterness in the samples to be evaluated, the more important it becomes to specify how he/she should perform a 2-AFC test or at what moment intensity should be rated.

50. Characterization of flavour release in chewing gums using the time–intensity method

Isabelle Cayeux and Marie-Clarté Vignon

Firmenich SA, 1 Route des Jeunes, CH-1211 Geneva 8, Switzerland

Flavour release in chewing gums with different formulations was studied using the time–intensity method in comparison with 'home-tests'. 'Home-tests' consisted of rating time–intensity parameters on a category scale.

Sensory tests were performed to study the influence on flavour release of: different tonalities in a market product; different dosage levels in a nectarine chewing gum; and two carbohydrate type-based matrices used for flavour encapsulation (compared with liquid flavour) in peppermint and tutti-frutti chewing gums.

Chewing gums were evaluated by 19 trained time–intensity panelists. Results indicated that the type of tonality and the flavour dosage have an influence on flavour release perception. The flavour encapsulation for peppermint has a highly significant influence on flavour release perception, affecting maximum intensity and lastingness. Panel average results were obtained by arithmetic mean and by the normalization procedure proposed by MacFie and Liu (1992). Through repetition tests and comparison with 'home-tests', the coherence of the time–intensity results has been fully validated. Time–intensity is a reliable method to characterize flavour release in chewing gums.

References

MacFie, H.J.H. and Liu, Y.H. (1992) Developments in the analysis of time–intensity curves. *Food Technol.*, November.

51. Recent advances in the analysis of preference and sensory data

Hal MacFie

Institute of Food Research, Reading Laboratory, Earley Gate, Reading RG6 6BZ, UK

Relating sensory properties to chemical and physical relations sounds so easy. A sensory panel makes careful assessment of the

foods, beverages or fragrances, and these data are then simply regressed onto the battery of physical and chemical measures that have been made in parallel. Some of the common problems encountered in relating sensory to instrumental measures and some of the solutions that have been used recently to overcome them are presented here.

Problem 1. **Too many sensory and instrumental variables for the number of samples tasted.**

The use of multivariate techniques such as principal components regression to reduce the number of explanatory variables is well understood. However, the problem of too many response variables is still not well treated. It will be argued that a detailed examination of the correlation structure of the sensory variables will lead to simplified hypothesis testing and that the question is more one of finding key instrumental variables than complex regression equations.

Problem 2. **Relations between sensory and instrumental are nonlinear.**

The use of optimal scaling techniques will be illustrated, although problems over degrees of freedom and significance testing remain. The use of neural network techniques to indicate the presence of nonlinearity and to assist in the search for meaningful transformations that give interpretable solutions will be discussed.

In searching for quantitative structure–activity relations between perceived aroma and compound structure, the possibility for tight regions of high activity embedded in areas of inactivity needs to be considered. A simple method to detect such areas, based on principal components analysis, will be illustrated.

Problem 3: **Preference–instrumental relations, not sensory–instrumental relations are what is required.**

The use of preference mapping to relate preference structures to sensory dimensions gives the potential to set up preference–instrumental relations. We are strong proponents of internal preference mapping, but we will propose a new model which, in addition to finding consumer segments based on preference, tests hypotheses about whether consumers are focusing on key sensory variables or synthesizing many sensory attributes into one or two conceptual dimensions.

52. Representation of odor quality on a spherical surface in three-dimensional stimulus space

Sarah E. Kemp[1], Rie Ishii[2], Michael O'Mahony[2] and Avery N. Gilbert[1,3]

[1]Givaudan-Roure Fragrances, Teaneck, NJ 07666 and [2]Department of Food Science and Technology, University of California, Davis, CA 95616, USA

[3]Present address: Synesthetics, Inc., Montclair, NJ 07043, USA

The organization of odor quality perception is a major unresolved problem in olfactory psychophysics. The structure of odor quality stimulus space was investigated by (i) using a hypothesis-driven version of multidimensional scaling (MD S); (ii) equating the perceived intensity of the odor stimuli; and (iii) employing a free-sorting technique to obtain a similarity judgement matrix. A traditional system of odor 'families' used by perfumers provided the predicted groupings by which the MDS results were interpreted; stimuli were selected to provide multiple examples from nine 'families', along with four miscellaneous odors. Odor stimulus concentrations were adjusted in prior sensory testing by a separate panel (n = 15) of judges using a magnitude estimation procedure. The free-sorting technique eliminated the need for exhaustive pairwise presentation of the odor stimuli, and permitted many odors to be tested in one session. In a first experiment, subjects (n = 9) sorted 43 odors, presented in unlabeled amber glass jars, into as many groups as they liked. In a second experiment new subjects (n = 10) sorted 47 odors (the odors from the first experiment plus four new odors from a new 'family'). The results of MDS analysis suggest that odor qualities are represented as projections on a spherical surface in three-dimensional stimulus space. Odor quality representations were stable across the perturbation of the stimulus set, and across individual differences in cognitive bias. The odor sphere provides an empirical, sensory-based means of validating and refining odor quality classifications.

53. Olfactory imaging: a priming experiment

E.P. Köster, O. van der Stelt, R.R. Nixdorf[1] and M.R.I. Linschoten[2]

Utrecht University, Heidelberglaan 2, 3564 CS Utrecht, [1]Heineken Breweries, Zoeterwoude, The Netherlands and [2]Rocky Mountain Taste and Smell Center, Denver, CO, USA

Do people who indicate that they have olfactory imagination perform better in a priming match–non-match reaction task than people who say they have no olfactory images? Two groups (high and low imagers) of 12 subjects (six male, six female) each were selected from a group of 31 on the basis of the difference in their scores on an enlarged version of the Bett's imagery scale. All subjects were submitted to four priming tasks in which three odours (O) and three corresponding odour words (W) were used as primes and as targets (W–O, W–W, O–W, O–O). The order of the conditions was different for each person, but counterbalanced for the two groups (Hi and Lo). The interval between the prime and the target was 2 s. Intensity-matched supra-threshold odour stimuli (5 s) were presented with a constant flow olfactometer through a nose piece. Odour words (5 s) were presented on an opal glass screen in front of the subject. After the target half of the subjects in each group pressed a right button for a match (M) or a left button for a non-match (NM). For the other half the position of the buttons was reversed. The reaction times (Rts) started on target onset. M and NM targets were both presented four times in each of three sessions for all three odours. For a given prime each of the two NM stimuli were used equally often. The number of missing values was low (0.3%) and the number of incorrect answers was not significantly higher for the Lo group (1.5%) than for the Hi group (1.2 %). A MANOVA on the Rts (prime, target, sex, Hi-Lo, MNM) showed main effects for prime (W < O) and target (W < O), and a significant interaction between target and Hi-Lo (F = 5.03, P = 0.036). This was due to the fact that the Hi

and Lo groups differed in their Rts on odour targets (Hi < Lo) but not on word targets. On average the difference is in the order of 250 ms. This indicates that imaginary preparation during the interval is effective in the Hi group. With O targets NM is faster than M, whereas with W targets the reverse is true (Target × M-NM, $F = 11.95$, $P = 0.002$).

54. An experimental look at transnatal olfactory continuity in human newborns

Luc Marlier, Benoist Schaal[1] and Robert Soussignan[2]

Laboratoire de Psychophysique Sensorielle, LN2C, CNRS URA 1939, Université Louis Pasteur, 67000 Strasbourg, [1]Unité de Comportement, CNRS URA 1291, Station de Physiologie de la Reproduction, 37380 Nouzilly and [2]Laboratoire de Psychobiologie du Developpement, EPHE, 75006 Paris, France

The olfactory cognition of human newborns can be approached through their abilities to differentially process odour qualities bearing contrasting psychobiological dimensions. Previous work has demonstrated that newborns preferentially orient their head towards odours they have been exposed to postnatally in either associative or non-associative conditions. The present series of experiments examines the relative reinforcing value of odour substrates newborns experienced just before birth [i.e. amniotic fluid (AF)] and after birth [either colostrum (CO) or artificial milk (AM)].

Three paired-choice odour tests were conducted in three groups of 2-day-old breast-fed (BRF) newborns (AF versus blank; CO versus blank; AF versus CO). Both AF and CO elicited positive head orientation when presented along with the blank (Schaal *et al.*, 1995), indicating that both odours are detectable and attractive to newborns. However, no differential head orientation duration was noted when the AF odour was presented in competition with the CO odour. This suggests that AF and CO were treated as sensorily and/or hedonically equivalent at this early age. Such a chemosensory similarity hypothesis being untenable between AF and AM, we predicted that, in contrast with BRF babies, same-age bottle-fed (BOF) newborns would evince differential responses to these odours. BOF infants were attracted toward either AF or AM odours presented against a blank; further, they oriented longer to the AF odour when exposed to the AF/AM contrast.

These results permit the following conclusions to be drawn: (i) according to the head-orientation response, 2-day-old infants are able to express a relative preference between two familiar stimuli that are attractive; and (ii) BRF and BOF infants show a distinctive pattern of orientation response in the test opposing familiar amniotic and post-amniotic odours. While BRF subjects do not differentiate them, BOF do so. These results will be discussed in terms of the infants' perception of the chemosensory similarity between the odours of amniotic and colostral fluids, but not between the amniotic fluid and artificial milk.

This research was supported in part by a grant from the DGAL, Paris.

References

Schaal, B., Marlier, L. and Soussignan, R. (1995) Responsiveness to the odour of amniotic fluid in the human neonate. *Biol. Neonate*, **67**, 397–406.

55. Behavioural clues to olfactory memory

Dan Bret and Isabelle Gallice

Laboratoire de Physiologie Neurosensorielle, 69622 Villeurbanne, France

Ten normal human subjects stimulated by 10 odours (lavander, creosote, pyridine, camphor, β-mercaptoethanol, menthol, ethyl acetoactate, cumarin, *n*-butanol and vanillin) showed stereotyped ocular behaviour which allowed inference of its logical support. They appear to be behavioural correlates of memory recall, but which type of memory is involved? Our subjects were asked to report three elements for each odour sample: (i) its identification, (ii) its connotation and (iii) associated memories. This test involves both episodic and semantic declarative memory. We applied the test procedure to five subjects suffering from Alzheimer's dementia, and regularly observed two major behavioural changes suggesting difficulty in declarative memory recall. Specific eye movements disappeared, and subjects appeared unable to perform the gestural task of smelling an odour sample on a dipstick. This involves complex motor procedures learnt during ontogenesis (coordinating respiration and hand movement, changing presentation distance according to sensitivity, changing nostrils, pausing etc.). Their loss thus involves both declarative and procedural memory, extending the observations that patients with Alzheimer's disease show a loss of olfactive sensitivity (Doty *et al.*, 1987). Two of our patients were re-tested after tacrine (anti-cholinesterase) treatment, with improvement in both declarative and procedural memory functions with a partial return of appropriate gesture and eye movements.These findings suggest that the use of stored memories involves an evokation of a mental image, less intense and more ephemeral than a real visual scene. The two image systems could be in competition, and the stereotyped eye movements would reduce visual sense input and so permit a temporary predominance of the mental image. Dealing with olfactory information is no easy task, especially for untrained subjects, and, under our test conditions, normal subjects but not the dementia patients showed exaggerated eye movements. Olfactory testing provides a well adapted method for studying not only odour perception, but also complex human behaviours including memorization and disease processes.

References

Doty, R.L., Reyes, P. and Gregor, T. (1987) *Brain Res. Bull.*, **18**, 597–600.

56. Long-term memory of odours: experts' behaviour

Philippe Beguin

Faculté de Psychologie et des Sciences de l'Education, Place Cardinal Mercier, 10, B-1348 Louvain-la-Neuve, Belgium

Memory of odours seems both excellent and weak. Excellent at evoking vivid memories of a distant past but weak in a simple recognition task where a set of stimuli is first encoded and shortly after presented with lures. This weakness could arise from the lack

of interest given to olfactory information. Indeed, if brain architecture is specific for olfaction, a functional explanation cannot be discarded. The lack of interest might thwart the building of a conceptual grid leading to negative effects on perceptual strategies and on a simple recognition task. We observed that experts in olfaction fare significantly better in the simple recognition task than novices. We suspect that experts develop a semantic code that facilitates quick and simple encoding of odours while novices strategies remain episodic.

57. Effects of hexenoic acid and vaginal secretion on dream content and sleep quality

Regina E. Maiworm, Kirsten Delfs, Hanns Hatt[1], Christian Reyher[2] and Christian Becker-Carus

Department of Psychology I, University of Münster, Fliednerstraße 21, D 48167 Münster, [1]Department of Cell Physiology, Ruhr-Universität Bochum, Universitätsstraße 150, D-447980 Bochum and [2]MPI Biophysikalische Chemie, Postfach 3129, D-37019 Göttingen, Germany

Humans respond to auditory, visual, tactile and also olfactory stimuli in sleep (Badia *et al.*, 1990). The question was whether human-associated odours could influence dream content and physiological parameters (respiration rate, heart rate) or not. In a previous study by Hatt, Willer and Goodewill significant effects of scatol and orange odour, but not of a mixture of axillary and vaginal secretion, on physiological parameters could be observed. Twelve male subjects slept for four nights in the sleep laboratory. During the nights heart rate, respiration, EEG (C3, F4), EOG and EMG were continuously measured on a Beckmann polygraph. The first night was to get accommodated to the sleep laboratory conditions. In the following three nights 3-*trans*-methyl-hexenoic acid (Givaudan Roure), vaginal secretion or control (air) was applied during the first deep sleep phase and the second and third REM phases. After 7 min the odour application was stopped and the subjects were awakened and interviewed (dream content, pleasantness of their dreams). All subjects had no sleep problems, were non-smokers, aged 20–30 years and had a heterosexual orientation. At the end of the study both stimuli were presented again and the men were interviewed (pleasantness of the odour, what it reminded them of). The vaginal secretion was collected from four donors (aged 24–29 years) at the time of ovulation and were pooled. (The ovulation was determined by a hormone assay.)

Hexenoic acid and vaginal secretion have a stronger influence on the power of the delta wave during deep sleep than during REM sleep. The reaction varies depending on the individual. Vaginal secretion and hexenoic acid seem to influence respiration and heart rate.

References

Badia, P., Wesenstein, N., Lammers, W., Culpepper, J. and Harsh, J. (1990) Responsiveness to olfactory stimuli presented in sleep. *Physiol. Behav.*, **48**, 87–90.

58. Discrimination ability for structurally related odorants in squirrel monkeys and humans: a comparative approach

Matthias Laska and Daniela Freyer

Department of Medical Psychology, University of Munich Medical School, Goethestraße 31, D-80336 Munich, Germany

Studies of structure–activity relationships suggest that within a group of structurally related substances molecular features like carbon chain length or steric conformation may determine odor quality and thus perceived similarity between members of a given class of chemicals. One useful means of assessing possible correlations between odor quality and molecular properties which is applicable to both human and non-human species is to test the discriminability of structurally related odorants.

Using a behavioral paradigm designed to stimulate olfactory-guided foraging behavior and based on the multiple discrimination of simultaneously presented odor stimuli in a manipulation task, we investigated the ability of squirrel monkeys to discriminate between aliphatic esters. This group of substances comprises odorous compounds predominant in a variety of tropical fruits and thus is presumably of biological significance for these frugivorous primates.

Five squirrel monkeys were trained to distinguish isoamyl acetate (S+) from *n*- and iso-forms of other acetic esters (ethyl acetate to decyl acetate) and from other esters carrying the isoamyl group (isoamyl propionate to isoamyl capronate) as S–. Their performance in these tasks was then compared with their ability to discriminate in a control task (isoamyl acetate versus (–)-carvone) which was interspersed between the critical trials.

We found that all five animals were clearly able to discriminate between all odor pairs tested. However, three of the odors used as S– presented significantly more difficulty to the monkeys than the control and all other tasks: two of these (isobutyl acetate and isoamyl propionate) are direct neighbours of isoamyl acetate in the corresponding homologous series, suggesting that carbon chain length may be correlated with perceived odor similarity, whereas the third odor (*n*-amyl acetate) is an isomeric form of the S+, suggesting that the two odorants were perceived as qualitatively similar despite different steric conformation of their respective functional groups.

Using a triple forced-choice procedure, human subjects were tested on the same tasks in parallel and showed an almost identical pattern of discrimination ability compared with the squirrel monkeys.

Thus, the results of this study provide evidence of a well-developed olfactory discrimination ability in squirrel monkeys for aliphatic esters and support the assumption that human and non-human primates may share common principles of odor quality perception.

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

59. Recalling sweet taste intensities in the presence and absence of other tastes

Mika Vanne, Hely Tuorila and Pentti Laurinen[1]

Departments of Food Technology and [1] Psychology, University of Helsinki, PO Box 27 (Viikki B), FIN-00014 Helsinki, Finland

This study assessed the effect of salty, sour and bitter tastes on the memory for sweet taste intensities during 125 h. Three concentrations of sucrose (0.125, 0.250 or 0.500 M) were dissolved in four media: water only and water with 0.125 M sodium chloride, 0.002 M citric acid or 0.002 M caffeine. Subjects ($n = 46$) were divided into four groups. Each group performed the memory task with the three concentrations of sucrose in one medium (water, salty, sour or bitter). *Ad libitum* mixing, in which the subjects' task was to reproduce the intensity of a previously tasted stimulus, was used as the testing method. The three sucrose concentrations served as target concentrations in the *ad libitum* mixing. In the first of the six sessions subjects received all three concentrations of sucrose in one medium for tasting. After tasting each concentration the subjects reproduced the subjective taste intensity by mixing portions of low (0 M) and high (1 M) concentrations of sucrose and tasting and retasting the resulting mixture. The reproduction of taste intensities was repeated five times after 12 min and 1, 5, 25 and 125 h based on memory from the first tasting session. The mixed sucrose concentrations were analysed refractometrically. Overall, the subjects overestimated the two lower concentrations and slightly underestimated the highest one. The overestimation of the two lowest concentrations was seen particularly in the water and sour groups. Over time the intensities of reproduced sucrose concentrations increased until 25 h. In the salty and bitter groups the intensities of reproductions continued to increase after that. The water and sour groups tended to overestimate the concentrations but the salty and bitter groups reached approximately the standard. Hence, both the time elapsed from tasting and the medium in which sweetness was tasted affected the way in which the intensity of sweetness was recalled. The reason for the differential effect of media is unclear. Different perceptual features of the stimuli are probable explanations.

60. Adaptation, differential sensitivity and mouth movements: studies with sucrose solutions

M.J.M. Theunissen and J.H.A. Kroeze

Psychological Laboratory, Psychonomics Department, Utrecht University, Heidelberglaan 2, 3584 CS Utrecht, The Netherlands

The primary function of chewing or mouth movements is to break down food to particles that are small enough to be swallowed. It is conceivable, however, that these movements can also influence taste perception. In two experiments the effect of mouth movements on taste adaptation and on the differential sensitivity to sucrose was investigated.

The just noticeable difference (jnd) for sucrose was established in three conditions: a condition with no mouth movements, one with a standardized rate of mouth movements and one in which the subject was free to choose her own rate of mouth movements. No significant differences between the jnds were found. The Weber fractions varied between 0.12 and 0.17.

In a second experiment the degree of adaptation to five concentrations of sucrose was measured while subjects used four different rates of mouth movement. It was found that when mouth movements were made there was less adaptation than when there was no mouth movement; however, the rate of movement did not appear to influence the degree of adaptation. Furthermore, concentration was found to have an effect. In the no movement condition more adaptation occurred at higher concentrations, whereas in the movement conditions the opposite effect took place; that is, there was a decrease in the degree of adaptation with increasing sucrose concentration. These phenomena might be explained by the stimulated tongue area or by taste constancy.

61. Psychophysical characteristics of binary mixtures of bulk and intense sweeteners

Marie-Odile Portmann and David Kilcast

Leatherhead Food RA, Randalls Road, Leatherhead, Surrey KT22 7RY, UK

The psychophysical characteristics of three synergistic sweetener mixture—maltitol–cyclamate, sucrose–cyclamate and maltitol–acesulfamK—were investigated using time–intensity and sensory profile methods.

The time–intensity curves were recorded and the temporal parameters (I_{max}, T_{max}, area, lag, T_r and rate) were calculated. The maximum sweetness intensity (I_{max}) was found to be higher for all mixtures than for the maltitol or sucrose solutions, confirming the synergistic effect for these mixtures. However, the sweetness response (area) was only amplified for the mixtures containing cyclamate ($P < 0.01$). In addition, the rate of release (rate) did not increase significantly, except for the maltitol–acesulfamK mixture ($P < 0.05$). For the other temporal parameters, statistically significant differences between single solutions and mixtures were not observed.

When acesulfamK and cyclamate were mixed with maltitol or sucrose, a significant reduction in their negative sensory attributes (burnt-sugar, bitter and liquorice flavours, bitter and liquorice aftertastes) was observed. In addition, acid flavour was significantly reduced ($P < 0.01$) for acesulfamK–maltitol and cyclamate–sucrose mixtures. Furthermore, for the mixture acesulfamK–maltitol, the intensities of the attributes metallic ($P < 0.01$), irritant ($P < 0.01$) and astringent ($P < 0.001$) were reduced. However, mixing sucrose or maltitol with cyclamate or acesulfamK did not significantly affect the flavour attributes caramel and menthol, or the mouthfeel attributes smoothness, body, drying and cooling.

This study is part of the EU programme (AIR 3.CT 94.2107) on 'The mechanistic understanding of the sweet response'.

62. Detection thresholds for the basic tastes: the effects of age

Jos Mojet, Elly Christ and Han Heidema

Unilever Research Laboratory, Olivier van Noortlaan 120, 3133 AT Vlaardingen, The Netherlands

Detection thresholds for 10 tastants (two per basic taste) of 21 elderly subjects (age 60–75 years) and 21 young subjects (age 18–33 years) were determined using the 2AFC-five-in-a-row method in ascending order. For selection of the individually adjusted stimulus ranges 14 concentrations of sodium chloride, potassium chloride, sucrose, aspartame, acetic acid, citric acid, caffeine, quinine chloride, monosodium glutamate (MSG) and inosine-5′-monophosphate (IMP) each were prepared by successive 0.2 log dilutions with distilled water. Each subject tasted the ten tastants six times over a period of 6 days.

MANOVA showed significant effects of age, gender and replication. The elderly people were less sensitive for all tastants. The age effect was least pronounced for sweet and sour and most pronounced for salt, bitter and umami. In general women had lower thresholds than men. However, the interaction effect of age × gender revealed that especially the older men were less sensitive. They showed the highest threshold for every tastant. In comparison with the concentrations detected by the young men and women, the concentration of the tastants needed to be multiplied by 1.15–5.75 for the older men and by 1–2.75 for the older women for aspartame–IMP respectively.

The significant effect of replication indicates a clear learning effect of practice. The young men learnt best, meaning that their thresholds lowered most over the six replications, while the older men learnt least, meaning that for some tastants (acetic and citric acid, caffeine and quinine) they achieve lower thresholds over the six concentrations, but for other tastants (KCl and MSG) they even displayed higher thresholds. This study indicates that repeated threshold tests are necessary to really understand the way the mechanism works.

63. Importance of the procedure used to evaluate olfactory similarities/dissimilarities by humans

Pascal Callegari, Isabelle Lesschaeve[1] and Sylvette Tinette

CNRS-UPR 9054, I.E.S.G.I.—Groupe Physiologie de la Chimioreception, ENSBANA, 1 Esplanade Erasme, 21000 Dijon and [1]Laboratoire de Recherches sur les Aromes, INRA, 17 rue Sully, 21000 Dijon, France

In a previous study, Callegari and Rouault showed a difference in repeatability performances for a limited number of olfactory similarities/dissimilarities data sets. The data from Dravnieks *et al.* were more accurate than those from Schutz or Wright and Michels. The experimental procedure differed in two points (i) similarity judgements (e.g. on a nine point scale, 0 = not similar at all and 9 = completely similar) in the Schutz and Wright and Michels studies versus dissimilarity judgements (the reverse scale) for Dravnieks *et al.*; and (ii) pair presentation (all pairs are rated in random order) in the Schutz and Wright and Michels studies versus the reference system (one odor is designated as the reference and odorants are rated against this reference; each of the odors served as the reference once) for Dravnieks *et al.*

To state if one of these two aspects was responsible of the difference in repeatability performances, the following experiment was designed. Twenty-eight panelists were selected to compare the olfactory quality of seven volatile compounds. These odorants were chosen to cover the entire similarity/dissimilarity scale; they were diluted in odorless mineral oil and dispersed on an absorbent material into 60 ml brown test flasks.

The concentrations were tested to be iso-intensitive as far as possible. The similarity/dissimilarity of the seven compounds was rated using either the pair presentation or the reference system. Four groups of seven persons were formed to perform each of these procedures.

The first results showed no significant difference between similarity/dissimilarity judgements. However, repeatability performances were found to differ between the two presentation modes (df 341, $t = 2.704$, $P < 0.01$). These results will be fully discussed in the poster.

64. Influence of alcohol on odor detection of guaiacol

C. Dacremont, F. Sauvageot and V. Leprince

ENSBANA, 1 esplanade Erasme, Umversité de Bourgogne, 21000 Dijon, France

In the third issue of *Chemical Senses*, Engen *et al.* showed that the ingestion of 0.7 ml of ethanol per kg body wt 20 min before a 4 h session resulted in improving the detectability of guaiacol. Detectability of guaiacol was studied by sniffing solutions, each assessor performing 500 A–not A tests. In our study we used the same experimental procedure as Engen *et al.* except for five points: (i) in this experiment the seven assessors were tested under both conditions with and without alcohol (Engen used two groups of assessors—one for each condition); (ii) for ingestion, ethanol was added in apple juice and not in orange juice; (iii) guaiacol solutions were sniffed in AFNOR glasses (Engen used an equilibrium sniffer, which was much more sophisticated); (iv) 0.03 p.p.m. solution of guaiacol was prepared with water and not ethyl phthalate; and (v) our assessors ingested smaller amounts of ethanol: 0.5 versus 0.7 ml per kg body wt.

The assessors performed two sessions. During the first session, four assessors were tested under the alcohol condition and three under the non-alcohol condition. The second session took place 1 week later and conditions were reversed for the assessors. The seven assessors were tested in the same room and did not know whether they were given alcohol or not. The amount of alcohol in the blood of each assessor was measured every 20 min with a breath analyzer.

Our results are different from those of Engen *et al.* No effect of alcohol ingestion was found either for hits or for false alarms. These results are confirmed by d' values: 2.23 for the ethanol condition and 2.10 for the non-ethanol condition. The same conclusion is drawn from β values: 6.83 and 9.21 for the alcohol and non-alcohol conditions respectively. d' and β values were determined for each individual subject. In the experiment of Engen *et al.*, the d' values were similar to those we got (2.16 and 1.55). This means that the difficulty of the task was the same in the

two experiments. The difference might be due to differences of sensitivity between the two groups of assessors in Engen *et al.*'s experiment.

65. Taste sensitivity during learning: relation to the hedonic status of the stimulus

A. Faurion and A.M. Pillias

Neurobiologie Sensorielle, 1, avenue des Olympiades, 91305 Massy, France

Previous experiments have been devoted to the measure of taste sensitivity of subjects in a stabilized situation, after data acquired during the learning period had all been discarded. This study was designed to understand the parameters affecting the apparent variation of sensitivity during learning of either neophobic or hedonic positive taste stimuli. Neophobic tastants were 5'-guanosine monophosphate and taurine; hedonic positive tastants were glycyrrhizic acid and D-threonine. Subjects were 30 young healthy subjects. The experiment lasted for 10 weeks after preliminary familiarization with the psychophysical tests on other molecules (4–6 weeks, sucrose and quinine). Isointense concentrations were measured repetitively during 2 × 2 h sessions per week. Forced choice pair comparison between the stimulus and a reference of 1.7 g/l NaCl was associated to the up and down procedure of Dixon. Qualitative description of all four solutions on a free profiling basis and hedonic ratings were assessed once a week. Food intake from morning until time of experiment was recorded for each subject at all sessions. The hormonal status of women was recorded. Thresholds were measured, after all experiments were completed, for each molecule and every subject.

Data show that the isointense concentration diminishes with learning for a hedonic positive stimulus whereas it increases for a neophobic stimulus. Our interpretation is that, during learning or conditioning situations, the cognitive aspect of intensity perception is not constant but depends on hedonism. If the stimulus rating is negative, its intensity is maximized and the iso-intense concentration is lowered. When neophobia disappears the iso-intense concentration increases, then stabilizes. For pleasantly rated stimuli the iso-intensity measured is biased towards higher concentrations. With repetitions, the subject learns to assess a more objective iso-intense concentration and the stimulus may lose some of its hedonic power, as shown on individual curves.

These results are interpreted in the context of Chang and Scott's modification of coding in the NTS and Yamamoto *et al.*'s c-fos observation in the pons during conditioned taste aversion. We suggest now that a modification of taste coding in the first two relays by efferents descending from hypothalamus, amygdala or other areas related to visceral information or emotion may induce modifications of coding in cortical taste projections as well. We can see a reflection of these neuronal modifications in quantitative measurements as well as qualitative psychophysical assessments.

66. Ability to discriminate between familiar natural odor mixtures and modified ones

N. Godinot and G. Sicard

Laboratoire de Physiologie Neurosensorielle, Université Claude Bernard Lyon 1, CNRS -ERS 5643, Neuroscience et Olfaction, 43 Bd du 11 Novembre 1918, F 69622 Villeurbanne, France

Little is known about the ability to detect slight odor changes relative to differences in the composition of complex mixtures. We chose to investigate the ability of naive subjects to detect the odor variations induced by the addition of various compounds to familiar odors.

Two different essential oils were used as the familiar odors: lavender and sandalwood oil. For each of these two oils 12 different odor variations were induced by the addition of one of four different compounds at three different concentrations. The added compounds were linalool, cedryl acetate, carvone and tetrahydrothiophene. Linalool and cedryl acetate were chosen because they are already present in the lavender and in the sandalwood oil respectively: their additions would induce small modifications of the odors and could exaggerate pre-existing characters of the oils. In contrast, carvone and tetrahydrothiophene were chosen to induce a variation due to a new odorant character.

We used a triangular test to evaluate the discriminatory abilities of 41 panelists. The three samples of a triad contained the same odorant oil, but in one of them a pure odorant was added. The panelist had to identify the odd sample in the triad. Each panelist had to assess the 12 triads corresponding to the 12 modified oils. Among the 41 panelists, 21 were tested with the lavender oil and 20 were tested with the sandalwood oil.

We observed a large variability in the discrimination performances of the panelists. Nevertheless, they discriminated mixtures including carvone or tetrahydrothiophene but not those including linalool or cedryl acetate, even when the concentration of tetrahydrothiophene was inferior to its threshold concentration.

67. Does beidler's mixture equation predict the bitterness of quinine hydrochloride and caffeine mixtures?

Jean-Xavier Guinard and Charlene Wee

Department of Food Science and Technology, University of California, Davis, CA 95616, USA

Three equiratio mixture types of quinine hydrochloride (QUI) and caffeine (CAF) (0.75/0.25, 0.50/0.50 and 0.25/0.75 QUI/CAF) were compared for perceived bitterness intensity to quinine hydrochloride (the 'reference stimulus') at two concentrations of the reference stimulus (1.33×10^{-5} and 5.31×10^{-5} M QUI) using the method of constant stimuli. Eight subjects participated in six sessions during each of which they compared the bitterness of one of the three mixtures with that of one of the two reference stimuli. In each session subjects tasted 14 pairs of stimuli (seven concentrations of the mixture presented first or second in the pair versus the reference stimulus, in randomized order).

The concentrations of the points of subjective equality determined experimentally were compared with those derived from

Beidler's mixture equation. The results suggest that Beidler's mixture equation may accurately describe the taste interaction between QUI and CAF, but not necessarily so at both bitterness intensities (low and high). Implications for the chemoreception and transduction of the bitterness of quinine hydrochloride and caffeine are discussed.

68. The frequency of odor events as a measure of odor nuisance

M. Hangartner

Institute of Hygiene and Applied Ergonomics, ETH Zurich, Switzerland

The frequency of plant-specific odor events over a given period of time is assumed to be a suitable method to assess the exposure to ambient odor. The validity of such frequency investigations relies on obtaining a representative sample which in turn depends on randomized but equally distributed observations over the time period. Several investigations were made in the surroundings of industrial plants such as food processing, chemical industry, grass drying etc. Ambient odors were assessed by panelists with respect to intensity, quality and hedonic tone on one side, and the community annoyance by public survey on the other. The correlation between odor frequency and extent of annoyance was 0.8, but the results show that equal odor frequencies may evoke different nuisance levels in the population. It is evident that the type of plant and its hedonic odor tone play an important role. Based on these results, an evaluation scheme for excessive odor events is derived.

69. The effect of defatted coconut powder and coconut oil on the salt and bitter taste of potassium chloride

M. Imhof and C.J.K. Henry

School of Biological and Molecular Sciences, Oxford Brookes University, Oxord OX3 0BP, UK

Potassium chloride (KCl) is currently the most commonly used substitute for salt (NaCl). However, widespread use of KCl is limited because of its bitter aftertaste. We have conducted sensory tests which suggest that coconut powder (CP) may have the potential to increase the acceptability of KCl in food, by suppressing its bitterness (Imhof *et al.*, 1996). This study was conducted to identify the component(s) within CP responsible for this result. Two components of CP—coconut oil (CO) and defatted coconut powder (DCP)—were evaluated for their effect on KCl bitterness and saltiness, in a rice-based food vehicle. Palm kernel oil (PKO) was used as a control. Rice samples were cooked using 40 g of rice and 80 g of water, with either CP, DCP, CO or PKO, and in combination with NaCl or KCl, as specified in the table. Twenty-eight untrained panellists (mean age 26.8 years) attended two taste sessions. In each session subjects were presented twice with 15 samples, all of which contained 3g of rice. Ratings were assigned for salt and bitter taste intensity, using 100 mm line scales.

The results indicate that the fat fraction of the CP may be responsible for the reduction in bitter taste intensity of KCl. A similar effect was also produced by PKO. Both CO and PKO contain a high proportion of saturated fatty acids. Further studies are in progress to investigate whether fat within foods can influence the acceptability of KCl as a salt substitute.

References

Imhof, M., Henry, C.J.K. and Mela, D.J. (1996) Improving the acceptability of potassium chloride as a salt substitute, using coconut powder. *Proc. Nutr. Soc.*, SC34 (Coleraine).

70. Changes in taste perception of bitterness following a long-term mental workload

Takako Inui, Mamiko Ozaki[1] and Masashi Nakagawa

Suntory Ltd Institute for Fundamental Research, Shirnamoto-cho, Mishima-gun, Osaka 618 and [1]Department of Biology, Faculty of Science, Osaka 560, Japan

Taste perception depends on the chemical and physical properties of tastants, but may also depend on the physiological and psychological conditions of those who do the tasting. We studied the evaluation of taste sensation by using the time–intensity (TI) method to determine whether the taste sensation of bitterness was reduced after a mental workload. We thereby focused on the relationships between mental stress and bitter taste.

In this study the taste sensation of bitterness after a long-term mental workload was tested and the amount of residual bitter compound in human saliva was analysed quantitatively. Quinine sulfate (1.82×10^{-5} M) was used as the bitter tasting sample. We also investigated the alteration in the salivary protein composition before and after the mental workload.

Subjects performed mental tasks for 40 min on a personal computer as the long-term mental workload. The TI evaluation showed that after long-term mental work the sensation of bitterness was reduced, and that there was more residual quinine in the saliva than before the work was undertaken. The results therefore showed good conformity between the TI evaluation and the quantitative analysis of the bitter compound in the saliva.

In addition, we found a protein which apparently increased after the workload. In most cases, the protein content was transitorily increased after the workload and reduced to the control level within a few minutes. This result suggests that this protein could be related to mental stress or a reduction of the bitter taste sensation. However, more study is needed to prove this hypothesis.

71. The effect of consumer information on the evaluation of natural yogurt

Hendrik N.J. Schifferstein and Adriaan Kole

TNO Nutrition and Food Research Institute, PO Box 360, 3700 AJ Zeist and Department of Marketing and Marketing Research, Agricultural University, Hollandseweg 1, 6706 KN Wageningen, The Netherlands

Sensory evaluations of food products in realistic consumption situations are affected by consumer expectations. The expected characteristics of a specific product result from the informational cues available at the time of consumption and the interpretation of those cues.

To study the way subjects integrate different types of information, we determined sensory judgements, expectation judgements and overall product evaluations for four different types of natural yogurt. These four types of yogurt are marketed in The Netherlands with different types of claims (health claims, sensory claims, production claims). We examined the interrelationships between the different types of data. In addition, we studied the effect of realistic exposure (product description or package) on the expectations and overall product evaluations.

The perceived characteristics showed much more variation between yogurt types than the expected characteristics. The expected properties did not differ between subjects who received product descriptions and subjects who received product packages. However, the type of exposure did affect the way the non-sensory information was integrated in the overall evaluation. The latter finding indicates that an explanation of these data in terms of assimilation and contrast would be oversimplified.

72. A method for assessing the similarity of olfactory stimulants

Kerstin Krauel, Bettina M. Pause and Roman Ferstl

Institute of Psychology, University of Kiel, Olshausenstraße 62, D-24098 Kiel, Germany

Odor similarity can be defined in terms of quality, intensity or hedonic evaluation, or as how well two odors can be discriminated. If odor similarity serves as an experimental variable, e.g. in the assessment of odor memory, these factors need to be carefully addressed. This is especially true for event-related potential studies employing odors as stimulants, as the parameters of the chemosensory event-related potential (CSERP) are considerably modulated by the intensity and hedonic tone of an odor. A further crucial variable in the recording of CSERPs is the amount of trigeminal stimulation: CSERPs in response to trigeminal and olfactory odors have been found to differ in terms of topography and reactivity to experimental variations, e.g. stimulus concentration. The goal of this study was to identify odor combinations of olfactory and trigeminal stimulants that vary in the degree of qualitative similarity but are similar in intensity and valence. Allylcaproate, butylcyclohexylacetate, eugenol, linalool and phenylethyl alcohol (group 1, mainly olfactory stimulants) as well as isoamylacetate, menthol and octylacetate (group 2, mainly trigeminal stimulants) were chosen as odorants. For all odors 1:50 and 1:500 dilutions (solvent:diethylphthalate) were prepared. Within both groups each odor was combined with every other odor of the group, separately for both dilutions.

Ten male and 10 female subjects (age: 21–43 years) participated in the study. The subjects' ability to discriminate the odors was measured using a three-alternative forced-choice test. The subject's task was to decide which bottle contained the odd odor. They were further instructed (i) to assign one of seven descriptors (burnt, flowery, fruity, minty, resinous, spicy, woody) to each odor of the combination and (ii) to evaluate the degree of dissimilarity between the odors of the combination (scale from 0–4). The subjects were also asked which odor they perceived as more intense and more pleasant. After the discrimination test subjects rated the familiarity and valence of each odor.

The different approaches to odor similarity provided a complex picture of the evaluation of odors presented alone and in the context of other odors. It was possible to identify combinations that are lop-sided concerning intensity and valence ratings. It was found that descriptors that were chosen to characterize odors judged as most dissimilar did not overlap. However, the descriptors used for the different odors seemed to vary with the odorous context. Further results concerning the influence of gender and concentration on the evaluation of odor similarity is discussed.

73. Study on the interaction between intensity and hedonic value of odorants

Tao Jiang and Paul Laffort[1], Claude Buriet and Luc Mejean

U308 INSERM, rue Lionnois, Nancy and [1]CNRS-UPR 9054, Group of Physiology of Chemoreception, Dijon, France

It is well known that the hedonic value of an odorant can vary as a function of the odorant intensity. In this work we try to study how the concentration of an odorant and the perceived intensity influence the estimation of the hedonic value. Twenty students were asked to estimate a perceived intensity and to give an hedonic value of the odorants. Ten of them evaluated butanol and linalool and the others evaluated butanol and carvone. Each odorant was diluted with distilled water and presented in a large open buttle at six different concentrations. The order of the stimuli was at random. The results show:

(i) The hedonic value for butanol decreases slowly as a function of the increase of the logarithmic concentration. The subjects maintain a relative good coherence between them, but with a greater deviation at weaker concentrations.

(ii) The hedonic value of carvone is the same for the first four weak concentrations and diminishes a little at higher concentrations with greater deviation.

(iii) The hedonic value for linalool has no clear tendency as a function of the concentration and there is much deviation, especially at the higher concentrations. When the function between the perceived intensity and the hedonic value is traced for each individual, it appears that subjects use different strategies to give an hedonic value. For some subjects, the hedonic value changes as a function of the perceived intensity while the quality of the odorants is not involved. For some of the others, the hedonic value is completely independent of the perceived intensity. In this case, it is perhaps the quality of the odorant, rather than the intensity, which determinates the hedonic value.

74. Quantitative sensory profiling of oatmeal: development of the method and profiles of selected commercial samples

A. Lapveteläinen, H. Rannikko and H. Kallio

University of Turku, Department of Biochemistry and Food Chemistry, EIN-20014 Turku in co-operation with Melia Ltd, PO Box 101, 21201 Raisio, Finland

Optimization of rolled oats production necessitates that the quality of the end product can be characterized not only in physical and chemical, but also in sensory terms. The present study

was conducted to develop a suitable terminology for determining the odor, flavor, texture and appearance characteristics of rolled oats by means of quantitative profiling techniques. The study commenced with the analysis of oatmeal, since this is the primary use for rolled oats in Finland.

The sensory terminology was developed during eight sessions by 15 panelists. After the training sessions, the panelists also quantified the 12 main sensory characteristics of five commercial samples using a 10 cm line scale. The terminology was further developed using verbal definitions and reference substances in various intensity levels, where possible. The method was then applied to determining the effect of cooking conditions on the sensory characteristics of the oatmeal. For the development of terminology, the oatmeals were prepared according to the instructions given on the packages, e.g. by mixing 130 g of rolled oats with 1 l of boiling water and cooking for 10 min. The samples were kept on hot plates (80°C) until analyzed (10–15 min). When cooking conditions were tested, the rolled oats were added to both cold and boiling water.

The main sensory properties of oatmeal were included in the group of appearance and texture attributes. Odor and flavor were fairly weak, the most often used terms being toasted, sweet, cereal and chemical. The commercial samples differed in intensity of appearance attributes such as gelatinousness, stickiness to spoon and thickness, the last two attributes being determined while mixing the sample with a spoon. Distinctive differences were also found in oral texture attributes such as perception of swollen flake particles, average size of swollen flake particles, beadiness, slipperiness and coarseness. Varying the cooking conditions resulted in oatmeals which differed in most of the sensory characteristics quantified.

75. Defining odour descriptors with one or three standards. Effects on odour profiling performances

Isabelle Lesschaeve and Claire Sulmont[1]

INRA, Laboratoire de Recherches sur les Arômes, BV 1540, 21034 Dijon and [1]ENSBANA, campus universitaire, 1 Esplanade Erasme, 21000 Dijon, France

The training of a sensory descriptive panel is usually split in two parts: (i) development of a descriptive vocabulary and (ii) vocabulary alignment among panellists. The use of standards to improve agreement among panellists at each stage is recommended to obtain consistent results at the profiling task. O'Mahony and co-workers underlined the necessity to define each sensory characteristic with multiple standards to improve concept alignment. However, the impact of such a technique on profiling performances was little discussed. The aim of this work was to study the effect of one versus three standards, to define one odour attribute, on the odour profiling performances of a descriptive panel.

Two groups of 10 naive subjects were recruited to perform an incomplete odour profiling of 10 orange juices. Eight odour descriptors, selected from a previous study, were presented to the subjects as the odour characteristics chosen to be evaluated in the orange juices. The two groups were trained independently but with the same panel leaders. The training and profiling designs were the same for each group, except that each odour attribute was defined by one standard for group A and by three standards for group B. The aim of the eight training sessions was to teach subjects to identify each standard with the appropriate descriptor and train them to evaluate orange juices with this descriptive vocabulary. Four odour attributes were learnt by session: first, subjects learnt to name the four sets of standards with the correct labels by performing an identification task with immediate feedback. This was done three times. Then, to control subjects' learning, an identification test without feedback was performed on the eight sets of standards. Secondly, subjects learnt to identify the four attributes and to rate the perceived intensity in three orange juices by performing a profiling task. The agreement among panellists was recorded for each attribute and was considered as an index of the concept alignment.

The odour profiling of the 10 orange juices was then performed by both panels during six sessions (three replicates per juice). Profiling performances of the two groups were found to be different. The results are related to odour learning performances to comment on the adequacy of using multiple standards during the training of odour descriptive panel.

76. An olfactometric method for estimating annoyance due to combustion exhausts from road traffic

Edvard Lidén , Lennart Högman[1], Arne Ulander, Fuat Deniz and Anita Gidöf Gunnarsson

Department of Occupational and Environmental Medicine, Örebro Medical Centre Hospital, Örebro and [1]Department of Psychology, Stockholm University, Stockholm, Sweden

Exposure from combustion exhausts in urban areas poses a health risk for susceptible groups such as asthmatics and evokes annoyance in the general public. Symptoms and annoyance in an exposed group living in urban areas might, when systematically collected, serve as early warning signals of illness. When quantifying annoyance from combustion exhausts in different groups there is a need for valid measures. In the present study a well-known trigeminal irritant, pyridine, was used as a reference substance, simulating the irritating properties of NO_x (nitrogen oxides).

Twenty-five subjects (11 female, 14 male) with an average age of 33.4 years (range 17–55) participated in a field trial close to a road with heavy traffic (13 000 vehicles/day) in Örebro city. They were first trained to give sensory estimates with magnitude estimation and later to give estimates of their perceived intensity of annoyance when exposed to combustion exhausts and perceived intensity of annoyance in a considerably less polluted backyard. The different exposures were estimated in relation to the degree of annoyance from seven concentrations of pyridine (0.25, 0.5, 1, 2, 4, 8 and 16 p.p.m.) which were presented (latin square design) with a static olfactometer.

The intensity of annoyance (R) caused by the seven concentrations of pyridine (S) aligns to Steven's power function: $R = c \times S^n$, where c is a constant and n is the exponent equal to 0.6 (in good agreement with earlier studies). On a group level there was a good correlation ($r = 0.97$) between the estimated intensity of annoyance and the actual concentration of pyridine. The estimated intensity of annoyance from combustion exhausts could be

expressed as an equivalent of pyridine in p.p.m. Thus on a group level, the estimated intensity of exposure due to combustion exhausts from the heavily used road corresponds to an equivalent of 1.14 p.p.m. of pyridine, which is four times the perceived intensity of annoyance from the less polluted backyard.

When assessing the degree of annoyance from combustion exhausts the estimated intensity of annoyance can be expressed in equivalents of pyridine. This opens the possibility of valid and reproducible measures on different occasions and in different groups.

77. An intensity/time study of sugar alcohol mixtures

Atala Lopez Chavez and Gordon G. Birch[1]

Agriculture and Food Technology Department, ITESM-CQ, Apartado Postal 36, Queretaro, Mexico and [1]Department of Food Science & Technology, University of Reading, Reading RG6 6AP, UK

In this study the sweetness time/intensity of 10 binary mixtures of D-glucitol, D-mannitol, xylitol, lactitol and maltitol prepared in water at 6% w/v of total concentration and 1:1 ratio were obtained using the Sensory Measurement Unit for Recording Flux instrument (SMURF). Three models to predict the taste intensity of mixtures based on the psychophysical power function of the unmixed components were investigated: the addition model, which implies that the gustatory system processes chemical information from the two substances in the mixture separately and then adds together the results; the substitution model, which supposes that the gustatory system does not distinguish between different sources of sweetness information, but processes them together as a higher concentration of either one of them; and the equiratio mixture model, which assumes that the substances in a mixture are mutually dependent, i.e. both compete equally for the same taste receptor sites, and also assumes that the sensory quality of the substances in the mixture are identical. Lactitol binary mixtures showed lower sweetness intensity than those predicted by any model but, in general, the best model to predict the sweetness intensity of sugar–alcohol binary mixtures is the addition model. The apparent molar volume of the binary aqueous solutions were calculated from Young's rule (addition rule) as modified by Ward and Millero. A relationship between sweetness intensity and apparent molar volume of one of the compounds of the binary mixture was found: lactitol mixtures show the lowest sweetness intensities and the biggest increases in apparent molar volume of the other component of the mixture.

78. Variation of pain and odor thresholds in healthy young adults: circadian aspects

Jörn Lötsch, Steven Nordin[1], Thomas Hummel, Claire Murphy[1,2] and Gerd Kobal

Department of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nürnberg, Germany, [1]Department of Psychology, San Diego State University and [2]University of California Medical Centre, San Diego, CA, USA

Circadian changes of pain (CO_2) and odor (H_2S) thresholds were studied in five young healthy male volunteers. Four measurements each were taken at 24:00, 04:00, 08:00, 12:00, 16:00 and 20:00 h (real time) on different days. To avoid sequence effects, the 24 tests were randomized, with a minimum intersession interval of 18 h. Odor and pain thresholds were assessed separately for each nostril using a dynamic olfactometer (staircase method). Concentrations ranged between 30 and 60% v/v for CO_2 and between 0.1 and 2 p.p.m. for H_2S. A total of 12 logarithmic concentration steps were used; threshold measurements always started from the lowest concentrations.

Neither absolute values of pain thresholds nor those of odor thresholds showed a circadian rhythm. However, circadian variation was observed with regard to the interindividual variability of odor thresholds observed at different test times. The interindividual variability of odor thresholds was smallest at 04:00 h, with thresholds between 0.4 and 1.2 p.p.m. It increased continuously until 16:00 h, when thresholds ranged between 0.1 and 2 p.p.m. From this time onward the interindividual variability of odor thresholds decreased. In contrast, the variability of pain thresholds among the subjects remained relatively stable.

The data suggest that neither thresholds of pain induced in the nasal mucosa nor odor thresholds seem to follow a circadian rhythm when sequence effects are excluded. Additionally, it is hypothesized that the increase in variance of odor thresholds is mostly due to environmental influences, indicating that olfaction is more subject to environmental influences than nociception.

This work was supported by grants SFB 353C2 (G.K.) and NIH DC02064 (C.M.), and an MFR travel grant (S.N.).

79. Sensory evaluation of mixtures of maltitol, sucrose and an orange aroma

Denise F. Nahon, Jacques P. Roozen and Cees de Graaf[1]

Department of Food Science, Section Chemistry and Microbiology and [1]Department of Human Nutrition, Wageningen Agricultural University, Bomenweg 2, 6703 HD Wageningen, The Netherlands

The sweetness of a solution can be evaluated by Beidler's mixture equation when dependent taste receptors are involved. The suitability of Beidler's model for mixtures of sucrose and maltitol was examined in the presence of an orange aroma. Also the interactions between orange aroma and both bulk sweeteners were studied. Quantitative descriptive analysis of different solutions indicated that the flavour profiles of sucrose and maltitol did not differ significantly at a constant level of orange aroma. The mean scores for the attribute 'sweet' remained constant for each combination of sucrose and maltitol. Therefore, Beidler's mixture equation can be used to choose combinations of both bulk sweeteners giving the same sweetness. Addition of orange aroma changed the flavour profiles and provided the different solutions with a more distinct taste. The mean scores for the attributes 'orange', 'sour', 'fruity' and 'aftertaste' significantly increased for most of the solutions. Further characterization of the attribute 'aftertaste' showed similar terms for the different solutions, of which 'orange', 'chemical', 'sour', 'fruity' and 'bitter' were more often mentioned for solutions containing an orange aroma.

80. Evaluation of pleasant and unpleasant by the eyeblink reflex

Masashi Nakagawa, Hajime Nagai, Miyuki Nakamura and Fumio Yamada[1]

Suntory Ltd, Institute for Fundamental Research, Mishima-gun, Osaka 618 and [1]Osaka Prefectural College of Nursing, Habikino City, Osaka 583, Japan

Some sensory evaluation studies of foods have been carried out using a psychophysiological index. According to the study of Lang and his colleagues, the startle blink reflex produced by a sudden auditory stimulus could be augmented by aversive visual stimuli and inhibited by pleasant visual stimuli. Yamada *et al.* found that the magnitude of the startle eyeblink reflex is affected by the subjective feeling of pleasantness provoked by natural sound. The results were compatible with the experiments using visual stimuli. In this study we expanded the stimuli for inducing the affective state from visual or auditory stimuli to olfactory stimuli as represented by variety of different pleasant and unpleasant odors related to food smells.

Subjects were 24 volunteers whose mean age was 24.5 years, range 18–36. Subjects received the startle noise pulse during exposure to olfactory stimulation. Three categories of odors were utilized by the preference test in advance: pleasant, unpleasant or neutral odors were selected among 33 food-related olfactory samples. The startle pulse was presented 1.5–2 s after exposure to odors. An integrated EMG of the orbicularis oculi after the onset of startle stimulus was assessed for statistical analysis.

The mean magnitude of EMG activity of pleasant, unpleasant and neutral odor conditions were calculated respectively. The results showed that pleasant odors inhibited the blink reflexes and unpleasant odors augmented them. The relationship between the mean magnitude of the startle eyeblink reflex and the subjective preference had an extremely high negative correlation coefficient ($r = 0.959$). These results were compatible with the experiments using visual or auditory stimuli. It was confirmed that pleasant feelings inhibited startle responses and unpleasant feelings augmented them.

It is suggested that startle modulation of the eyeblink reflex is a useful index of the objective measurement of the affective state, e.g. pleasantness.

81. Ultradian variation of olfactory sensitivity

Bettina Pause, K.-Oliver Schulz, Bernfried Sojka, Gabriele Fehm-Wolfsdorf and Roman Ferstl

Institute of Psychology, University of Kiel, Olshausenstraße 62, D-24098 Kiel, Germany

Rhythmic and bilaterally reciprocal alternations of nasal airway patency have been reported to occur in ~80% of healthy adults. This phenomenon is called the 'nasal cycle' and is due to cyclic variations in the autonomic tonicity of the nasal mucosa. The periodicity has been described as ranging between 40 min and 5 h, and may influence unilateral olfactory sensitivity. However, ultradian variations in unilateral olfactory sensitivity have not been described so far and no evidence has been found that changes in the nasal resistance are responsible for variations in olfactory thresholds.

The aim of the present study was to investigate whether an ultradian periodicity for unilateral olfactory sensitivity can be described. As hormones of the adrenal cortex have been considered to modulate olfactory sensitivity, the ultradian variation of salivary cortisol was also determined and correlated with the olfactory sensitivity. Twelve healthy male subjects (aged 20–30 years) participated voluntarily in the study. All of them were dextrals and nonsmokers, and had a regular sleep–wake cycle. Olfactory thresholds for linalool were determined monorhinally using a three-alternative forced-choice staircase detection procedure. In two consecutive sessions (one session per nostril) the olfactory sensitivity was measured 13 times over 8.5 h. The thresholds were determined every 30 min and two breaks of 1 h were included. All threshold measurements were preceded by saliva samplings. For periodicity determination by Fourier analysis a curve with 64 data points was approximated to the 13 threshold and cortisol data from the two main sessions (Akima interpolation). To control for the biological relevance of the variations, each subject participated additionally in 12 control sessions. During the control sessions the threshold for only one nostril of each subject was detected once. The control sessions were carried out on different days but at the same time of day.

The ultradian variances in olfactory sensitivity were similar to the variances between the test days (F-test). Therefore the periodicities have to be interpreted cautiously. However, the results of the Fourier analyses show a main rhythm in olfactory sensitivity with a periodicity of 4 h for the left nostril and a periodicity of 2 h 40 min for the right nostril. The periodicity of 2 h 40 min was also found for the cortisol levels. Negative correlations between the saliva cortisol level and olfactory sensitivity were found for nine of the 12 subjects and were significant for three subjects.

82. The influence of the label on the perceived familiarity, complexity and liking of flavoured yoghurts

Christelle Porcherot and Sylvie Issanchou

INRA, Laboratoire de Recherches sur les Arômes, 17 Rue Sully, BV1540, 21034 Dijon, France

In sensory evaluation, blind tests are generally performed to determine liking. However, impact of information on acceptability has been shown in a number of studies. In a work conducted to investigate the impact of perceived familiarity and complexity on liking, experiments were conducted with the same products (10 flavoured yoghurts) and the same subjects (86 consumers) in two conditions, blind and labelled. In each condition, subjects rated for each product the intensity, the natural character, the perceived familiarity and complexity (three items: complexity, difficulty to describe, perceived number of different flavour notes) of the flavour, the appropriateness of the flavour for the yoghurt and their liking as well as sweetness and smoothness of the product.

The effect of the label on the different criteria differed between the flavours. Not surprisingly an increase in the perceived

familiarity was observed for nine of the 10 flavours when the label was given, but this increase was significant in only three cases. Perceived complexity and difficulty to describe decreased for seven of the products and the decrease was highly significant for vanilla, a very familiar flavour in our culture for dairy products. Perceived complexity and the perceived number of different flavour notes increased significantly for two of the three mixed flavours. The liking of the flavour was significantly affected by the label in only two cases: an increase was observed for two flavours that were amongst the least familiar and the least preferred in the blind condition. These data suggest, like those obtained previously by Tuorila *et al.* (1994), that in some cases information could reduce uncertainty and thus contribute to an increase in liking. We also observed that the label influenced sweetness and smoothness in some cases, revealing that a flavour is also associated with taste and texture characteristics and induced expectations about taste and texture. Appropriateness and naturalness were also affected by the label, with a significant decrease in one case and a significant increase for respectively four and three flavours. Changes in liking were correlated to changes in naturalness, appropriateness and sweetness.

From these results, it can be concluded that there are interactions between sensory and cognitive information. Thus, it appears very important to perform acceptability experiments in blind and labelled conditions in order to better understand the impact of both sensory and cognitive components and the interaction between them.

References

Tuorila *et al.* (1994) *Appetite*, **23**, 231–246.

83. Olfactory input facilitates trigeminal chemosensitivity

S. Roscher, P. Mohammadian, A. Schneider, J. Wendler[1], T. Hummel and G. Kobal

Department of Pharmacology and Toxicology, University of Erlangen-Nuremberg and [1]Department of Medicine III, Institute of Clinical Immunology and Rheumatology, University of Erlangen-Nuremberg, Nuremberg, Germany

The study aimed to evaluate the influence of odorous stimulation on the perception of trigeminal stimuli. Twenty healthy volunteers (10 male, 10 female, aged between 18 and 35 years) participated in two experimental sessions. During both experiments 20 series of five stimuli were applied (interseries interval 40 s, interstimulus interval 2 s). In half of these series the stimulus was CO_2 (51% v/v), in the other half the same concentration of CO_2 was mixed with vanillin (2 p.p.m.). The order of the two types of stimuli was randomized and administered by means of an olfactometer. In both experiments subjects were asked to focus their attention on the pain induced by CO_2. In the first session subjects rated the intensity of both stimuli on a visual analogue scale (VAS). In order to test for the 'halo-dumping effect', in the second session an additional scale was introduced where subjects rated the degree of unpleasantness of the perceived stimuli.

The mixture of CO_2 and vanillin was rated as both more intense ($t = -2.81$; $P = 0.006$) and more unpleasant ($t = -2.43$; $P = 0.016$) than CO_2 presented alone. The same facilitating effect of vanillin on CO_2 intensity estimates was observed when subjects additionally rated the unpleasantness of the stimuli by means of a second VAS.

The findings confirm previous results of Kobal and Hummel and Livermore *et al.*, who found that estimates of CO_2 stimuli increased when applied in mixtures with odorants. Since both conditions revealed the same increasing effect we were able to demonstrate that the intensity increase was not due to a halo-dumping effect. It is concluded that the ability of olfactory stimuli to increase the irritation produced by a trigeminal stimulus is a stable effect that is independent of scaling procedures.

84. Variability and invariants in early odour preferences: comparative data from children belonging to three cultures

B. Schaal, R. Soussignan[1], L. Marlier[2], F. Kontar[3], I.S. Karima[4] and R.E. Tremblay[5]

Laboratoire de Comportement, CNRS URA 1291, 37380 Nouzilly, [1]Psychobiologie du Developpement, EPHE, Paris, [2]Psychophysique Sensorielle, CNRS URA 1939, Strasbourg, France, [3]Psychology Department, University of Damascus, Damascus, Syria, [4]Centre Culturel Français, Bandung, Indonesia and [5]Département de Psychologie, Université de Montréal, Québec, Canada

Human cultures invariably organize their odour space along the hedonic dimension. The degree of variability of this unavoidable trait of odour perceptions has been little investigated across cultures, and the preferences exhibited by western societies are often taken as standards for the species. In the present study, an intercultural approach was used to assess how similar children were in judging the hedonic valence of a given set of odorants. Children 6–12 years old were studied in three cultural groups: French Canadians, Sundanese Indonesian and Syrian. The subjects were exposed to the same set of 14 synthetic odorants (mostly food and body-related odorants) selected for their contrasting hedonic tone and cultural representativeness. The subjects were required to assess and posit each stimulus on a hedonic scale ranging from 'very bad' to 'very good'. The ongoing analyses of the data indicate the following results:

(i) The subjects in all cultures agreed on the stimuli they assessed as being unpleasant. The most negative stimuli were those related to body malodours. However, the Indonesian sample was more tolerant of these odorants.

(ii) The inter-cultural agreement was lower for the stimuli judged as being pleasurable. Some odorants elicited similar responses in all three cultural groups, while some were positively evaluated in two or only one samples.

These results are discussed as evidence of both culturally invariant and variable hedonic responses. We suggest that the culturally invariant responses might result from inborn tendencies, while the culturally variable responses mostly depend on local exposure effects.

85. Investigation of trigeminal sensations by use of respiratory behaviour

Hilmar Gudziol and Mario Schubert

ENT Department of Friedrich-Schiller-University of Jena, Lessingstraße 2, 07740 Jena, Germany

Walker and Jennings reported an increased nasal irritation threshold in anosmics. Kobal *et al.* found that the amplitudes of chemo-somatosensory event-related potentials evoked by suprathreshold CO_2 stimulation were smaller in patients with hyposmia or anosmia than in normosmics. We investigated the respiratory behaviour during the stimulation by formic acid or menthol in patients with decreased smelling ability and in healthy persons. The respiratory pattern was recorded by the pressure sensor of a PC-rhinomanometer, represented and stored. The following results were found:

(i) The detection threshold for formic acid in anosmics is higher than in normosmics. Anosmics detect menthol only in a high concentration by a cooling sensation.

(ii) During stimulation with ascending concentrations of formic acid or menthol, respiratory sniffing patterns were found with lower concentrations than the individual detection threshold concentration.

The registration of respiration is a good method for the examination of naso-trigeminal function. By our objectifying method we found the same results as Walker and Jennings and Kobal *et al.*—that loss of olfactory function results in a decrease of trigeminal sensation. Differences by stimulation of different trigeminal receptors should be investigated.

86. The effect of KCl and fat content on perceived characteristics of cream cheese in PROP tasters and nontasters

H. Tuorila, N. Urala, L. Lähteenmäki and H. Rita[1]

Department of Food Technology and [1]Department of Forest Resource Management, University of Helsinki, PO Box 27 (Viikki B), FIN-00014 Helsinki, Finland

Research on the genetically determined ability to taste PROP (6-*n*-propylthiouracil) suggests that the tasters also perceive other bitter compounds intensely. For example, the bitter note of potassium chloride (KCl) has been reported to be particularly pronounced to those who find the taste of PROP extremely strong (so-called 'supertasters'). In the present experiment, we partially substituted KCl for NaCl in cream cheese and examined the effect of this substitution on the ratings of pleasantness, bitterness, sourness and saltiness in nontasters (NT, $n = 15$), 'low tasters' (LT, $n = 15$) and 'high tasters' (HT, $n = 11$). The taster grouping was based on individual responses on a labeled magnitude scale to a filter paper saturated with PROP (NT, scores up to 20; LT, scores 21–55; UT, scores 56–90). The cream cheese samples were prepared at two fat levels, 10% ('low-fat') and 40% ('high-fat'), and with four salt combinations. The basic recipe contained 0.6% NaCl ('100:0 proportion'); the corresponding saltiness was the target in samples in which NaCl:KCl mixtures were added in proportions of 75:25, 50:50 and 25:75. The added quantities were 0.67, 0.75 and 0.86% (w/w) respectively. The relative saltiness of KCl against NaCl, 0.60, was determined in a separate experiment and served as the basis for the added quantities. Overall, high-fat samples were rated as more pleasant, saltier and less bitter than low-fat samples. However, the PROP tasting status interacted with the fat level in all these ratings. The HT group rated the pleasantness of high-fat samples highest and the NT group rated their pleasantness lowest; the ratings of low-fat samples were fairly similar in all subgroups. The HT group gave the bitterness of high-fat samples the lowest and the NT group the highest ratings; on the other hand, the HT group rated the bitterness of low-fat samples somewhat higher that did the other two subgroups. Increasing the proportion of KCl decreased the pleasantness ratings and, particularly in the HT group, decreased the saltiness ratings. Sourness ratings remained unaffected by the manipulations. The results challenge our working hypothesis that PROP tasters would respond less favorably to KCl than nontasters, and would perceive its bitterness more strongly. A possible explanation for the unexpected findings is the suppressing effect of fat on perceived taste intensities in tasters compared with nontasters; another recent report suggests that creaminess perception is subject to taster status.

87. The relationship between field dependence/independence and the impact of a fine fragrance on first impressions

I. Freienstein, P. Castle[1], J. Jamieson[1] and S. Van Toller[1]

Institut für Psychologie, Georg-August-Universität, Göttingen, Germany and [1]Department of Psychology, University of Warwick, Coventry CV4 7AL, UK

Previous research has shown that odour exerts an influence on impression formation. When forming first impressions, people tend to assume that a person who has positive characteristics in one area also possesses qualities in other, unrelated areas. In this respect, personal attractiveness is of great importance. In line with the widespread belief that a pleasant scent enhances a person's attraction, there are grounds for believing in a potential impact of fine fragrances on first impressions.

Where previous studies sought to explore the effects of odour on interpersonal attraction in a dynamic, 'interview-type' context, we aimed to examine the effect of odour on interpersonal attraction in a static context, using a single photographic image. Moreover, we aimed to investigate the possible relationship between odour, first impressions and field dependence/independence (FDI).

Field-dependent and -independent people employ different perceptual strategies in assimilating incoming sensory information. Whilst field-dependent people use contextual cues as external frames of reference in processing incoming information, field-independent people actively participate in restructuring incoming information, combining it with internal frames of reference. Four hypotheses were tested: (i) the attractiveness ratings of field-dependent subjects will be positively influenced in the scent condition, compared with the control condition; (ii) there will be no significant difference between the scent condition and the control condition for the field-independent group; (iii) there will be no significant difference between the ratings of the field-dependent and -independent groups in the control condition;

and (iv) the attractiveness ratings of the field-dependent group will be more positive than the ratings of the field-independent group in the scent condition.

Subjects were sorted into the field-dependent or field-independent group after being tested using the rod and frame test. Subjects in each group were then randomly assigned to an 'odour' (fine fragrance) or a 'no odour' condition and were required to rate the photographic image of a 'neutral' female face (as determined in a pilot study) on various personality characteristics.

Findings are discussed in terms of the significance of fine fragrance on impression formation in 'static' contexts.

88. An investigation into the effect of odour on the evaluation of a retail product as a function of field dependence/independence

Paul Castle and Steve Van Toller

Department of Psychology, University of Warwick, Coventry CV4 7AL, UK

The deployment of odour into commercial environments is becoming an increasingly popular technique in an attempt to enhance buying behaviour. Previous research shows, however, that the scenting of environments does not necessarily increase sales or enhance product appeal in such a universal manner.

In an attempt to understand why odour does not appear to produce this effect, we explored the personality dimension of field dependence/independence (FDI). Field-dependent people tend to rely on information as given and are guided by contextual cues in their responses. In contrast, field-independent people tend to actively restructure information, relying on internal frames of reference. Three hypotheses were tested: (i) the presence of odour in the environment will lead to a significant difference in the way a product is evaluated; (ii) field-dependent subjects will evaluate the product more highly than field-independent subjects in the presence of a pleasant ambient odour than in the absence of odour; and (iii) there will be a significant difference between the way in which field-dependent subjects and field-independent subjects evaluate the product in a malodorous environment.

In the first experiment, using the rod and frame test, subjects were required to determine the point at which an adjustable rod was perceived to be vertical (a determinant of FDI). In the second experiment, subjects were randomly allocated into one of four conditions (i, pleasant/ congruent odour; ii, pleasant/incongruent odour; iii, unpleasant/congruent odour; iv, control). Subjects (n = 100) were required to watch a video of a journey into a shopping mall and eventually into a department store. After viewing the video, subjects were required to inspect and evaluate a product on various characteristics, using a computer-gene rated questionnaire.

Data analysis addressed hypothesis (i) using ANOVA. Here a significant difference among the means of the four groups was found, but only on the characteristic 'softness-to-touch' [$F(3,94)$ = 6.627, $P < 0.01$]. However, no other characteristics of the product were enhanced in the presence of these odours, suggesting, on this occasion, that odour fails to produce an environment in which buying behaviour is modified.

Preliminary analysis of the FDI data suggests that this particular personality dimension is effective in highlighting differences in product evaluation in the presence or absence of odour within the environment. However, further analysis is being undertaken to clarify the issue.

Findings are discussed in terms of the functional significance of field dependence/independence to environmental fragrancing.

89. Investigtions on sweetness of polyols in hard-boiled candies

David Schäppl, Elena Tresoldi, Ruth L. Genner-Ritzmann and Felix Escher

Swiss Federal Institute of Technology (ETH), Institute of Food Science, CH-8092 Zürich, Switzerland

Sugar alcohols present important bulk sweeteners for replacing sucrose in a variety of food products. Isomalt [an equimolar mixture of 1-*O*-(α-D-gluco-pyranosyl)-D-mannitol and 6-*O*-(α-D-glucopyranosyl)-D-sorbitol] is of interest in manufacturing hard-boiled candy as this polyol is also able to serve as a principle texturogen. In order to develop candy formulations, the present study was initiated to determine the relative sweetness of isomalt and the influence of acidity and volatile flavour on perceived sweetness of candies with isomalt as compared with candies with sucrose. Sensory evaluation was carried out on specially prepared samples using a nine-point category scale for judging overall sweetness and 10 cm line scales with and without reference points for judging intensity of sweetness, sourness and flavour in paired comparison and complete block design tests. Commercial samples were characterized using descriptive analysis, including judgement of sweetness.

Candies with sucrose were perceived to be sweeter than candies with isomalt (using acesullam K or aspartame as artificial sweetener) when judging overall sweetness and in complete block design tests. Citric acid decreased the intensity of sweetness significantly in candies with isomalt as well as in candies with sucrose, while citric flavour had no effect on perceived sweetness intensity in both type of candies. After increasing the amount of artificial sweetener in candies with isomalt for a paired comparison test, sweetness intensity was judged to be on the same level as sweetness intensity of candies with sucrose. Comparison of commercial samples showed that it is possible to produce candies with isomalt with a very similar flavour profile to that of candies with sucrose.

90. Measurement of olfactory evoked magnetic fields by a 64-channel whole-head SQUID system

Saho Ayabe-Kanamura[1,2], Hiroshi Endo[2], Tatsu Kobayakawa[2], Tsunehiro Takeda[2] and Sachiko Salto[2]

[1]Institute of Psychology, University of Tsukuba, Tsukuba and [2]National Institute of Bioscience and Human-Technology, Higashi 1-1, Tsukuba, Ibaraki, 305, Japan

The human olfactory projections to the neocortex and their functional organization for a long time had remained concealed.

Recently, however, Kettenmann *et al.* (1996), using magneto-encephalographic (MEG) recordings, found that odor stimuli evoked the activation of the human superior temporal sulci. They insisted that the temporal lobe was involved in human olfactory function.

In this experiment we aimed to confirm the localization of the cortical areas activated during odor recognition. We recorded olfactory evoked potentials (OEP) from Cz and magnetic fields (OEMf) after phenylethyl alcohol stimulation for 200 ms using a Kobal's olfactometer (Kobal, 1981). The 30 stimuli were presented once every 40 s; thus it took ~20 min for each experimental session. For recording, we employed a 64-channel whole-head SQUID system (CTF Systems Inc., Canada) in a magnetically shielded room. Four subjects participated in this experiment. At the present time, we have been able to localize equivalent current dipoles (ECD) in the response peaked at nearly 800 ms after stimulus onset bilaterally in the area around the superior temporal sulcus after separate stimulation of the two nostrils. These ECDs corresponded in latency to the P2 component (~700 ms after stimulus onset) of the OEP responses, which confirmed the results obtained by Kettenmann *et al.* (1996).

In the near future, we will also investigate the activated area in the neocortex related to the process of odor recognition, such as pleasantness and familiarity.

References

Kettenmann, B., Jousmäki, V., Portin, K., Salmelin, R., Kobal, U. and Hari, R. (1996) Odorants activate the human superior temporal sulcus. *Neurosci. Lett.*, **203**, 143–145.

Kobal, U. (1981) *Elektrophysiologische Untersuchung des menschlichen Geruchssinns*. Thieme, Stuttgart, p. 171.

91. Time-course of olfactory adaptation in freely breathing anaesthetized rats

M. Chalansonnet and M.A. Chaput

Physiologie Neurosensorielle, CNRS-Université Claude Bernard Lyon I, 69622 Villeurbanne, France

Most psychophysical studies presume that adaptation is due to central rather than peripheral processes. By contrast, electrophysiological studies lead to less obvious conclusions. Adaptation may occur at peripheral and/or bulbar levels, and may be partial at both levels. However, it must be noted that these studies have rarely been performed in freely breathing animals evne though respiration may play an important role and limit adaptation. In this context, it seems fundamental to study adaptation simultaneously at the peripheral and bulbar levels, to perform experiments in freely breathing animals, to deliver different odors at several concentrations and to perform relative long stimulations.

Peripheral responses were obtained by recording electro-olfactogram (EOG), and bulbar activity consisted of extracellularly recorded mitral cell activities. Five odors (camphor, cineole, isoamyl acetate, limonene and methylamyl ketone) were delivered at seven concentrations ranging from 2×10^{-4} to 1×10^{-1} of the saturated vapor pressure. Stimulations lasted 10 s or 3 min. Data analysis consisted in correlating EOG amplitude with spike frequency at each respiratory cycle. For this, we used raster plots and firing frequency histograms synchronized on the respiratory cycle and we classified bulbar responses in different types (Chaput *et al.*, 1992).

The EOG showed a pronounced reduction in amplitude at the beginning of the test period, which generally occurred between the first and second stimulations. The EOG amplitude thereafter decreased exponentially until the end of the stimulation. Amplitude decay is more obvious for odors such as methylamyl ketone and isoamyl acetate, and is largely increased by concentration.

At the bulbar level, most of the cells were found to maintain their activity synchronized on the respiratory cycle during the whole duration of 10 s stimulations and to show a high frequency burst of activity in the first cycles of the stimulation, i.e. in cycles corresponding to the position of the EOG_{max}. During the 3 min exposures, these cells could modify their type of response, their frequency in the burst or their phase-locking on the respiratory cycle. These changes cannot be explained by the EOG decrease observed at the periphery and must be ascribed to centrifigal influences.

References

Chaput *et al.* (1992) *Eur. J. Neurosci.*, **4**, 813–822.

92. Stability of olfactory bulb responsiveness to increasing odor intensities in freely breathing rats

M.A. Chaput and M. Chalansonnet

Physiologie Neurosensorielle, CNRS-Université Claude Bernard Lyon I, 69622 Villeurbanne, France

This study aimed at revisiting the effects of increasing odor intensity on olfactory bulb (OB) responsiveness. It was performed in anaesthetized but freely breathing rats, since respiration has been shown to be important for the expression of mitral/tufted (M/T) cell responses.

We recorded the single-unit responses of M/T cells to increasing odor intensities ranging from 2×10^{-4} to 1×10^{-1} of the saturated vapor pressure. Animals were first stimulated with five odorants delivered for 10 s at 2 min intervals at least at the medium concentration of 2×10^{-2} of the saturated vapor pressure. Then odorants that evoked a response were applied in ascending order of concentrations.

Olfactory stimulation produces a strong patterning of MIT single-unit activity which is highly correlated with respiration. Thus data were analyzed with the use of raster plots and firing frequency histograms synchronized on respiration. These representations were used to determine whether the cell responded to the stimulation and to locate the position of firing peaks and troughs within individual respiratory cycles. Cycle-triggered histograms built on stimulation periods were also assigned to the different types previously described by Chaput *et al.* (1992).

Of 149 M/T cells initially stimulated with the five odorants, 51 cells submitted to a complete stimulation protocol and 23 cells that were prematurely lost were included in the study. As already reported, their responsiveness was found to significantly increase with intensity, and to reach a plateau at the highest concentrations.

Similarity in their response pattern was also enhanced by increasing odor intensity: cells show significantly more often the same response pattern, and peaks and holes tend to occupy more often the same position on the respiratory cycle. Their mean firing frequencies measured during the peaks did not increase significantly with concentrations. Lastly, no cell was found to pass from an activation at low (2×10^{-4} and/or 6×10^{-4}) odor concentrations to a suppression at higher intensities. Only three cells out of 17 were suppressed at low intensities and activated at higher ones, and no cell was successively suppressed, activated and then suppressed again when odor intensity was increased.

We conclude that M/T cells recorded in freely breathing animals show a stability of their responses, which agrees with the notion of spatiotemporal coding of olfactory information.

References

Chaput *et al.* (1992) *Eur. J. Neurosci.*, **4**, 813–822.

93. Improvement in the definition and determination of solubility parameters of solutes. Possible QSAR applications in olfaction

Françoise Chauvin, Pascal Callegari and Paul Laffort

CNRS-UPR 9054, I.E.S.G.I.—Groupe Physiologie de la Chimioréception, ENSBANA, 1 Esplanade Erasme, 21000 Dijon, France

This study is based on the postulate that the intermolecular forces involved in the recognition of odorants by olfactory receptors are poorly specific, and could be determined experimentally with the help of the solute–solvent phenomena governing gas–liquid chromatography.

A computer program called POLY-STRUC (from POLYnomial STRUCtured analysis) was established at the laboratory with the aim of testing with experimental data theories involving multiplicative matrix models. This is the case in chromatographic phenomena, where, according to Rohrschneider, the retention indexes can be written in the form:

$$I - I_{CH4} = \alpha A + \omega O + \varepsilon E + \pi P + \beta B + \ldots$$

where α, ω, ε, π, β, ... are the solute factors and A, O, E, P, B, ... are the solvent factors.

Several sets have been proposed for this purpose: the first one was generated by Karger *et al.*, with parameters of dispersion, orientation, induction, acidity and basicity. This first approach was then improved by Laffort and Patte, who changed the induction parameter by a polarizability index independently of the size of the molecule. In several articles published since 1990, Abraham and co-workers, keeping this framing, have improved the definition of the five solute parameters, which they call solvatochiomics.

The present study was undertaken in this view of improvement by applying the POLY-STRUC program to chromatographic retention indexes recently published by Kovats and co-workers (112 solutes on 18 stationary phases) and by an initiation with several parameters already proposed (the acidity and basicity parameters of Abraham *et al.* and the orientation parameter of Laffort and Patte), plus the molar volume and the polarizability defined recently by Chauvin and Laffort.

The final choice, based on new and more accurate experimental data than previously, leads to a slightly more satisfactory version. That is justified by the criteria provided by the POLY-STRUC program, which are exposed and discussed.

94. Human gustatory cortex: a comparative study using fMRI and MEG

B. Cerf, T. Kobayakawa[1], P.F. Van de Moortele[2], H. Endo[1], S. Saito[1], D. Le Bihan[2], T. Takeda[1], H. Ogawa[3], P. MacLeod and A. Faurion

Laboratoire de Neurobiologie Sensorielle, E.P.H.E., 91305 Massy, France, [1]Human Informatics Department, MITI, Japan, [2]Service Hospitalier Frederic Joliot, C.E.A., 91304 Orsay, France and [3]Kumamoto University, Japan

Gustatory projections are still not well known in humans: there have been a few reports of lesions involving the surroundings of the Sylvian fissure; and a recent PET study revealed taste-related activation in the left insula and temporal lobe.

We performed an fMRI study of taste-related brain activity using a 3 Tesla whole-body MR scanner allowing echo-planar imaging. Six subjects, aged 20–25 years, participated in the study. The 5 min stimulation paradigm consisted of three resting periods of 90 s, during which subjects received tap water (as a 50 µl bolus delviered every 3 s) and two activation periods of 30 s during which they received the gustatory stimulus. Each subject performed three experiments with tastants and a control with only water in the same session. We used a finger-span recorded perception profile to extract correlated brain activity. Activations were found for taste stimuli and not for water control experiments at the foot of pre- and post-central gyri, the upper part of the temporal gyri which lines the fissure, the anterior insulae and the frontal operculae, among others. These results fall in accordance with taste projections found in the monkey with electrophysiological techniques. Within the insula, however, we found two levels of gustatory projections, one in the inferior part of the insula in the dominant hemisphere and the other one in the superior part of the insula of both hemispheres.

The collaborative Japanese MEG study used a 64-channel whole-head SQUID system to measure gustatory evoked magnetic fields (GEMs) elicited by sharp gustatory stimulations. Six subjects, aged 21–32 years, participated in the study. Two temporally distinct components of GEM were found in relation to taste stimulation. For NaCl the first component was elicited at ~150 ms and the second component appeared a few hundred milliseconds afterwards.

The question arising from these results concerns the putative relationship between spatially (fMRI) and temporally (MEG) distinct taste projections. We are currently in the process of testing which of the fMRI localizations could fit both the early and late GEM components.

95. Taste sensitivity in cancer patients treated with chemotherapy

A.M. Dalix, F. Bellisle, A.-M.César-d'Ornano[1], D. Khayat[1] and A. Faurion[2]

Comportement alimentaire, U 341, Hôtel Dieu, 1 place du parvis Notre Dame, 75 Paris, [1]Service d'Oncologie Medicale, Hôpital La Pitié-Salpétrière, 47 Boulevard de l'Hôpital, 75013 Paris and [2]Neurobiologie Sensorielle 1, avenue des Olympiades, 91305 Massy, France

Electrogustometry was used to assess cancer patients' sensitivity before, during and after chemotherapy with a variety of chemically different treatments. Electrogustometry consists of an iontophoretic stimulation of the sensory cells with the cations contained in saliva and taste pore substance. Thresholds are given in microamperes. A 5 mm^2 stimulation was obtained with a metallic spherical electrode applied at the tip of the tongue, at 5 mm behind the right and left eye-teeth. Forty-nine patients (59 ± 11 years old, mean + SD) had thresholds between 5 and 250 μA (35 ± 47 μA) at the tip of the tongue and 45 ± 65 μA on both sides of the tongue. A group of 70 aged and healthy controls (65 ± 10 years) gave significantly different thresholds between 5 and 160 μA (24 ± 30 μA). The bilateral Student's *t*-test comparing the two populations was significant at $P < 0.01$. No important difference in sensitivity was observed between younger ($n = 34$) and older ($n = 70$) controls up to 78 years old. In both patients and controls, a few discrepancies between right and left sides could be accounted for by teeth surgery. The mode of threshold distribution was 40 μA in cancer patients under treatment and 21 μA in controls. Taste thresholds in some individuals could be measured repeatedly before and after different treatments. Recovery was observed at 18 days (twice the half time of most taste cells) or sooner following treatments. It seems that some but not all treatments actually impair taste cell sensitivity. We hypothesize that some chemotherapies directly destroy taste sensory cells as turnover of these cells is comparable to tumoral cell turnover. An analysis of the correlation between treatments and taste threshold is currently under way.

96. Physicochemical properties of sweeteners in artificial saliva and the mechanism of sweetness response

F. Hutteau and M. Mathlouthi

Laboratoire de Chimie Physique Industrielle, Faculté des Sciences de Reims Champagne-Ardennes, BP 1039, 51687 Reims Cedex 2, France

Inasmuch as sweetness perception takes place in the buccal environment where obviously water is present, the role of the aqueous solvent should not be neglected. This role was investigated for sugars and polyols in pure water and in the presence of 1% NaCl, KCl and $MgCl_2$ (Mathlouthi *et al.*, 1996). As water structure is sensitive to the effect of all solutes, including the minor ones, it was decided to use artificial saliva as a solvent for the study of the physicochemical properties of sugars, polyols and intense sweeteners with relevance to the mechanism of sweetness response.

Artificial saliva was prepared according to Van Ruth *et al.* (1995). Three sugars, four polyols and four intense sweeteners were studied. Viscometric constants ([η] and k'), apparent specific volume, hydration number, surface tension and contact angle with a hydrophobic surface as well as partition coefficient were measured.

Comparison of results obtained in pure water and in artificial saliva showed only slight differences. Sugars and polyols exhibited a slight increase in intrinsic viscosity [η] and a decrease in Huggins coefficient k', whereas sodium saccharin and acesulfame K showed a significant augmentation of k'. Changes in ASV were moderate except for sodium saccharin, which showed a decrease. The ratio B_{str}/B showed no noticeable modification. Likewise hydration numbers remained stable, and the surface tension of the water increased in the presence of sugars and polyols and decreased in the presence of artificial sweeteners. These results are interpreted in terms of hydrophilicity and hydrophobicity of the solutes. They are useful to show the relative ease of accession to the receptor site and are used to support our three-step model of sweet taste chemoreception.

References

Mathiouthi, M., Hutteau, F. and Angiboust, J.F. (1996) *Food Chem.*, in press.
Van Ruth, S.M., Roozen, J.P. and Cozijnsen, J.L. (1995) *Food Chem.*, **53**, 15–22.

97. Differences in magnetoencephalographically identified sources of cortical olfactory activity after stimulation with different odorants

B. Kettenmann, H. Stefan[1] and G. Kobal

Department of Experimental and Clinical Pharmacology and [1]Department of Neurology, University of Erlangen-Nuremberg, 91054 Erlangen, Germany

In the last few years progress has been made in understanding the cerebral mechanisms of human olfaction. So far, no differences between different odorants with regard to their neuronal processing have be identified. Here we report on differences in neocortical olfactory representation identified with dual-sensor magnetoencephalographic (MEG) recordings during well-defined olfactory stimulation with two odorants.

Twelve healthy volunteers (six males, six females; aged 28–32 years) participated in the experiments. The olfactory stimuli (ethyl vanillin and eugenol) were delivered within a humidified and temperature-controlled constant airflow with two tubings, one to each nasal cavity, without altering the thermal conditions at the mucosa. The stimulus sequence consisted of 200 ms pulses once every 40 s. Chemosensory function was assessed by evaluating an odor identification-, threshold- and discrimination test (Sniffin' Sticks).

Cortical responses were recorded with a Magnes™ 74-channel neuromagnetometer. Signals coinciding with eye blinks were discarded from the average. To compare timing between magnetic and electric responses, olfactory event-related potentials were recorded simultaneously from the vertex (Cz/A1). The functional MEG information was combined with anatomical data from magnetic resonance images leading to magnetic source imaging.

The areas activated by the different odorants differed from

each other but the two hemispheres were activated symmetrically. In addition to the sources localized after stimulation with the odorants vanillin, hydrogen sulfide (in previous experiments) and ethyl vanillin, we observed bilateral activation of the hippocampus during the first 600 ms after olfactory stimulation with eugenol.

Our results emphasize the role of the hippocampus and the limbic system with regard to different olfactory stimuli.

This study was supported by Unilever Research Laboratorium Vlaardingen.

98. Monorhinical olfactory testing of patients with temporal lobe epilepsy

J. Lehrner, C. Baumgartner, W. Serles, A. Olbrich, G. Almer, E. Pataraia and J. Bacher

Neurological University Clinic, Vienna, Austria

Medial temporal lobe structures play a prominent part in olfactory information processing and lesions in this region have resulted in olfactory dysfunction. The olfactory system in humans has mainly uncrossed projections into the cerebral cortex. Thus, it appears that each hemisphere is capable of processing olfactory information independently. In this study we addressed the question to what extent temporal lobe epilepsy produces 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 as candidates for epilepsy surgery. All patients had continous video-EEG monitoring, magnetic resonance imaging, single photon emission CT and neuropsychological testing. The patients also underwent a standard sodium amobarbital procedure for lateralization of language and memory functions. The goals of the olfactory assessment were to establish the patients' presurgical olfactory capabilities, and consequently lateralization of the functional deficit zone as defined by olfactory parameters. The olfactory test battery assessed monorhinally the following subfunctions: olfactory threshold, odor quality discrimination, unprompted and prompted odor identification, and odor memory. We report our preliminary results in a population of temporal lobe patients who were evaluated preoperatively. The potential contributions of quantitative olfactory testing in the preoperative evaluation of epilepsy surgery candidates are discussed.

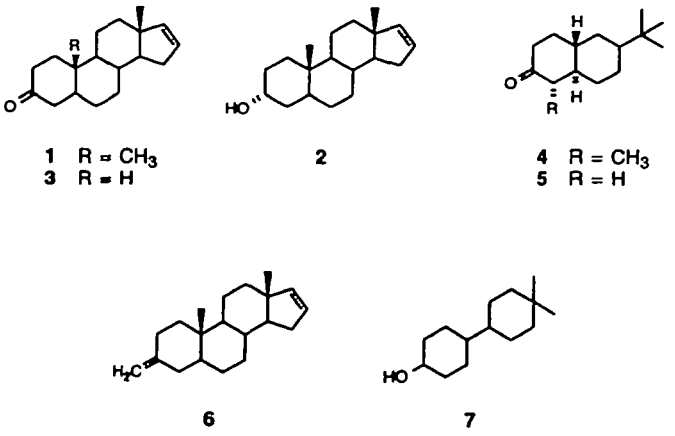
This research was supported by the Fonds zur Förderung der Wissenschaftlichen Forschung Österreichs (Projekt P10302 MED).

99. The odor of steroids: more enigmas?

C. Margot, C. Porcherot, D. Skuy, C.-A. Richard and M.-O. Portmann

Firmenich SA, Corporate Research Division, PO Box 239, CH-1211 Geneva 8, Switzerland

Androstenone **1** has been the focus of considerable interest since its discovery in Zurich by Prelog and Ruzicka more than half a century ago. It is the oxidation product of the pig pheromone



1 R = CH_3 **2** **4** R = CH_3
3 R = H **5** R = H

6 **7**

3α-androstenol **2** isolated from hog testes. The human perception of both compounds bears considerable interest because of the high incidence of specific hyposmias, the X-gene-linked bimodal sensitivity distribution of **1**, structure–odor correlations and anecdotal human pheromone reports.

In the ketone series, we present studies of the perception of the 18-nor steroid **3**, detected by almost 95% of our subjects, perceptual analogues **4** and **5**, and our findings about the *odorless* hydrocarbon **6**, previously described as having an odor similar to that of the parent androstenone **1**. Actually, **6** was found to be a slow release agent of a steroid-smelling oxidative degradation product under normal atmospheric conditions.

In the alcohol series, the almost odorless structural analogue **7** does not reduce the sensitivity to the sandalwood smelling androstenol **2**. Beyond that, a discussion of the possible perceptual links between the androstene alcohols and ketones is discussed.

100. Influence of somatostatin on odorant evoked potentials in the olfactory bulb of normal and staggerer mutant mice

Vincent Michel and François Math

Laboratoire de Neurosciences, Place Leclerc, 25000 Besançon, France

Among the various neuropeptides observed in the olfactory bulb, somatostatin (SOM) is one of the less studied in regard to its neuronal influence. Its inhibitory effect on neurons of the hypothalamus and amygdaloid nucleus has been described previously. From immunocytochemical demonstrations made in mammals, including man, it has been shown that SOM seems to be located at different olfactory bulb levels, though it is less abundant here than in other brain areas. Thus SOM has been observed mostly among the periglomerular cells, the short axon cells and nerve fibers originating from the anterior olfactory nucleus and anterior commissure. Despite these observations, there have not yet been any studies on the effect of SOM on olfactory bulb neurons, and the question of a modulatory influence on the olfactory message, perhaps during a period of hunger, for example, remain to be demonstrated.

In this first approach and using olfactory evoked potentials (OEP) in the olfactory bulb of normal mice, we have studied the influence of SOM on mitral cells and associative cells. This method

of investigation allowed a complete view of neuronal interactions during activation by odorant stimulations. Indeed, the characteristics of the OEPs have been largely analyzed and their components related to the different events which affect the mitral cell environment during activation. Our results demonstrate that SOM lengthened latency and induced a decrease of the OEPs' components, which implicates periglomerular or granular cells. This indicates that SOM plays a modulatory role on olfactory message transmission toward retrobulbar structures.

This study was also performed in staggerer (*sg*) mutant mice in which N-CAM gene alteration leads to both abnormal olfactory structural organization and to mitral and periglomerular cell degeneration. This neurodegenerative mutation, inducing smell alteration, might be a possible model for human genetic diseases associated with abnormal olfaction and accompanied by varia- tions in the amounts of brain or spinal fluid SOM. This is the case for Alzheimer's and Hungtington's diseases and schizophrenia. In *sg* mice, our observations have shown a low SOM influence on all parameters, except on the p2 component of OEP, which is related to the deepest associative cell control of mitral cell activity.

101. The Scandinavian odor-identification test: development, reliability and validity

Steven Nordin, Annika Brämerson[1], Edvard Lidén[2] and Mats Bende[1]

Department of Psychology, Umeå University, Umeå, [1]Department of Otorhinolaryngology, Central Hospital, Skövde and [2]Department of Occupational and Environmental Medicine, Örebro Medical Center Hospital, Örebro, Sweden

The present study addressed the need for a culturally valid odor-identification test for clinical use on the Scandinavian population as well as the need for such a test (i) with a good ability to generalize test performance to olfactory status by use of a wide range of aromatic qualities; (ii) that assesses olfactory and trigeminal function separately; (iii) that requires only limited cognitive demands; and (iv) that is fast, easy to administer and relatively inexpensive. The Scandinavian odor-identification test (SOIT) was developed by selecting 16 odorous test stimuli that were relatively identifiable and rated as relatively familiar, strong in perceived intensity and pleasant by 42 healthy participants of varying ages. Four response alternatives were then selected for each test stimulus based on a confusion matrix of identification rates obtained from 60 healthy participants of various ages, in a manner that controlled the degree of task difficulty of the SOIT. Final results on test–retest reliability and validity of the SOIT are presented, but preliminary results from patients whose chief complaint was some but not total loss of olfactory sensitivity (n = 6) and healthy persons of various ages (n = 84) showed satisfactory reliability (r = 0.85). Preliminary results from patients (n = 6) and healthy persons (n = 12) also showed satisfactory validity as suggested by correspondence with performance on the University of Pennsylvania smell identification test (r = 0.86) and on the Connecticut Chemosensory Clinical Research Center threshold test (r = 0.74). A computer-based version of the SOIT is available and appropriate for any person with very basic experience with computers.

102. Taste changes of D-glucono-1,5-lactone with time: water effects

Sneha A. Parke, Gordon G. Birch, Tim Astley and David A. Stevens[1]

Department of Food Science and Technology, University of Reading, Whiteknights Road, PO Box 226, Reading RG6 6AP, UK
[1]On leave from Frances L. Hiatt School of Psychology, Clark University, 950 Main Street, Worcester, MA 01610, USA

D-Glucono-1,5-lactone (GDL) is a multisapophoric molecule which autohydrolyses rapidly in water to D-gluconic acid. These chemical changes are accompanied by a resultant change in taste from sweet/bitter to increasingly sour as the reaction progresses. GDL has a pyranose structure and has an apparent specific volume (ASV) of 0.61 cm^3/g; it is therefore expected to taste sweet. Gluconic acid has both sweet and sour sapophores, but as it ionizes in solution (pK_a = 3.60), it tastes sour. The ASV of the acid is 0.51 cm^3/g, which places it in the sour range.

The chemical changes can be followed over the first 105 min of hydrolysis using solution property measurements. An increase in density and sound velocity is observed. Apparent molar volumes and ASVs fall from 109 to 104 cm^3/mol and from 0.609 to 0.579 cm^3/g respectively. Isentropic apparent molar compressibilities also fall from 4.06×10^{-3} to 3.72×10^{-3} $cm^3/mol.bar$, and on a weight basis from 2.28×10^{-5} to 2.07×10^{-5} $cm^3/g.bar$. The pH of a 10% solution also decreases exponentially from 3.82 to 2.49 from time 3 to 105 min.

These changes accompany a fall in lactone concentration from 100 to 92% of its initial value over the first 105 min, with the resulting gluconic acid partially dissociating in solution. The percentage acid dissociated falls from 62 to 7.2% over this period of time, but there are enough hydronium ions present to cause increased sourness. These measurements reflect the changes in the packing characteristics of the solute molecules within the hydrogen-bonded structure of water. As the number of ions in solution increases, compatibility of the solute with the water structure is altered. The water structure becomes increasingly disturbed by the hydrogen-bonded network around the solute, which increases solute–solvent interaction. This water of hydration is held tightly to the ions and the solute molecules become heavily hydrated. This may in turn explain the taste changes occurring at the level of the receptor, assuming the possibility that hydrated molecules become more easily and rapidly transported to the receptors. This also supports the theory that sour receptors lie deeper in the lingual epithelium than sweet and bitter receptors.

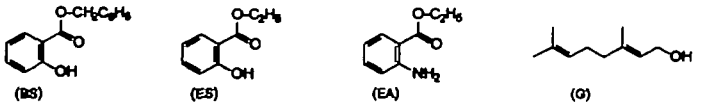
Computer models have shown that there is a high water molecule population around the oxygen atoms which constitute the 3,4-α glycol group of the GDL molecule. This shows that this part of the molecule is heavily hydrated and may indeed be the AH,B site.

103. Perceptual relations between similar odors: the intriguing behavior of two salicylic esters

Christelle Porcherot and Christian Margot

Firmenich SA, Corporate Research Division, PO Box 239, CH-1211 Geneva 8, Switzerland

Benzyl salicylate (BS) is a frequently used perfumery material. Surprisingly, threshold measurements demonstrated a widespread hyposmia ('specific anosmia') since 31% of a 61-subject panel could not detect the compound in the dilutions and most of them were unable to smell even the pure neat material. In contrast, the lighter ethyl salicylate (ES) is universally perceived. According to perfumery descriptions, most salicylates share a common green balsamic odor quality with various side notes. The chemically different but structurally related ethyl anthranilate (EA) bears an olfactory resemblance to ES and was also perceived by the entire panel. Individual detection thresholds of ES and EA were correlated ($r = 0.47$, $P = 0.03$).



Puzzled by hyposmia to BS despite considerable olfactory homology to ES and EA, we investigated adaptation/cross-adaptation effects on identification thresholds, adding geraniol (G) to the set as a perceptually and structurally distinct control. Among the panel tested for threshold we chose 10 subjects with good sensitivity towards these four compounds and 10 subjects anosmic to BS but otherwise sensitive to the other three.

Self-adaptation was significant for all substances, with an average threshold shift of 3–4 quaternary dilution steps (64–256 factor), but ES was far less efficient, with an average shift of <2 quaternary steps. ES–EA and ES–BS were often confused at almost any concentration, but not BS–EA.

Non-reciprocal, modest cross-adaptation was observed only on the identification of BS and EA when ES was the adapting stimulus. As self-adaptation to ES was rather modest, cross-adaptation values were also quite low. Thus, despite the olfactory similarities of BS–ES and EA–ES, cross-adaptation was surprisingly low and unreciprocal. It is possible that ES is a trigeminal stimulus and future investigation should clarify whether this component obscured the cross-adaptation.

104. Pictures 'smell' differently for the left and right hemisphere

Christina-Maria Röhrenbach and Marianne Regard

University Hospital Zürich, Neurological Clinic, Neuropsychological Unit, CH-809 1 Zurich, Switzerland

We are interested in the brain–behaviour relationship of emotions. Our experimental study focuses on the following two questions: are 'smelling' visual stimuli more powerful elicitors of emotional reactions than 'not-smelling'? Are there differences in processing mechanisms between the left and right hemisphere? In a series of experiments with tachistoscopically lateralized stimuli (ambiguous figures, faces, architectural shapes) we found hemispheric asymmetries in 'like–dislike' judgements. Subjects prefer stimuli presented to the right visual field (RVF) while they refuse stimuli of the left visual field (LVF). Using the same forced-choice preference paradigm, we applied visual stimuli that were more or less associated with odours. Colour photographs were presented pairwise to the left and right visual fields for 140 ms. By pressing a response key either on the left or right side, subjects had to indicate which of the two stimuli they 'like more' (preference) and in a second condition which they 'like less' (refusal). Stimuli were selected and categorized by pre-ratings. The answers were analysed according to the pre-rated stimulus categories (pleasant & smelling, pleasant & not-smelling, unpleasant & smelling, unpleasant & not-smelling), side of stimulation and choice condition. Generally, left hemispheric processing led to more preference judgements and right hemispheric processing to more refusal judgements. 'Smelling stimuli' (pleasant and unpleasant) were preferred, while 'not-smelling stimuli' were more frequently refused. 'Smelling & pleasant' stimuli were strongly preferred when presented to the left hemisphere. The results show that 'smelling' pictures are strong triggers of emotional judgements, especially pleasant ones, but, as we found for other visual stimuli, judgements depend on the hemisphere stimulated. The right hemisphere seems to be 'dominant' for avoidance behaviour and the left hemisphere for approach.

105. Odour description and intensity evaluation of 4-methylnonanoic acid in solution

S. Rousset-Akrim, F. Ruiz de Huidobro[1] and J.L. Berdagué

Instiut National de la Recherche Agronomique, Station de Recherches sur la Viande, Theix, 63122 Saint-Genès-Champanelle, France and [1]Comunidad de Madrid, Subdireccion General de Investigacion Agraria, Finca 'El Encin', Carretera N-II, km. 38,200, Apartado 127, 28800 Alcala de Henares, Spain

In a previous experiment about the chemical and sensory analysis of rendered fat of sheep, we showed that 4-methylnonanoic acid was a contributor to sheepmeat odour. The aim of this study was to characterize the sensory odour profile and determine the intensity of this pure compound in solution at different concentrations by 20 assessors. Seven concentrations of 4-methylnonanoic acid (control S0 = 0%, S1 = 0.0001%, S2 = 0.001%, S3 = 0.01%, S4 = 0.1%, S5 = 1% and S6 = 10%) were prepared by mixing different quantities of 4-methyl nonanoic acid in melting deodorized lard at 50°C. The products at 45°C were smelt. During training, the assessors received simultaneously seven flasks with different concentrations of 4-methylnonanoic acid, and described their odours. Eleven attributes were frequently quoted: fatty, rancid, roast, cheese, ham, sheepfold, mouldy, plastic, vegetable, smoked and lamb leg. During the last two sessions, assessors first received flask S5 coded as the reference of X odour. We asked them to memorize this odour. Five minutes later they received successively the seven flasks coded with random

three-digit number in the order: S2, S1, S5, S0, S4, S6 and S3 for the first replicate and S1, S0, S3, S5, S2, S6 and S4 for the second replicate. For each flask, assessors had to evaluate the intensity of the X odour and of the 11 attributes on non-structured scales. A two-way analysis of variance (concentration, session) was performed for each subject to assess the number of significant attributes which discriminated the concentrations of 4-methylnonanoic acid. Principal component analysis and cluster analysis were carried out to determine those groups of assessors which had similar scoring for X odour. Curves of intensity perception against concentration of 4-methylnonanoic acid were plotted. Results showed that X odour was a discriminant attribute for 16 assessors out of the 20. Rancid and cheese were discriminatory for half of the panel. The other attributes were less frequently discriminatory. Correlation coefficients calculated with the mean data of the panel were highly significant (0.97) between X odour and the attributes rancid, cheese, sheepfold and mouldy. To a lesser extent, the X odour was associated with roast and lamb leg (0.89). PCA and cluster analysis showed three major groups composed of three, three and 14 assessors. The intensity curves fitted for each group indicated that the first group of three assessors did not distinguish any concentration from the control S0. Their perception threshold was >10% 4-methylnonanoic acid in the lard solution. The second group of three people discriminated only S6 from the control. Moreover, the particularity of their scorings was to give a high intensity to S6 and also to S2 and S1. These concentrations were scored highly perhaps because they were smelt just after the X flask (identical to S5) and could be associated to a residual perception. The last and the greatest group was composed of people who perceived S6, S5 and rarely S4 as having a more intense perception of the X odour than S0. Only one person distinguished S3 and S2 from S0 and nobody perceived 4-methylnonanoic acid in S1. We found a 10 000-fold difference in threshold between assessors. This compound contributed to sheepmeat odour and even more so to other odours such as rancid, cheese or animal.

106. Effects of odours on attentional processes and mood in schizophrenia and depression

C. Rupp, J. Ilmberger[2], H. Oberbauer, A. Scholtz[1], C. Wanko and H. Hinterhuber

Departments of Psychiatry and [1]Otorhinolaryngology, University Hospitals of Innsbruck, Anichstraße 35, A-6020 Innsbruck, Austria and [2]Clinic for Physical Medicine, Klinikum Großhadern, Ludwig-Maximilians University of Munich, Munich, Germany

Various studies have shown the influence of olfactory input on different types of behavior related to central nervous system activity. The sedative and stimulative properties of lavender oil and the oil of jasmine have been shown in studies using neurophysiological methods and animal research. Based on these findings we examined in an earlier study the effects of these two odours on attentional processes in healthy subjects. Results showed a decrease in performance while inhaling lavender and an increase in performance while inhaling jasmine. These opposing effects were found in tasks requiring visual vigilance and selective attention.

One basic problem in patients with schizophrenic and depressive disorders, although due to different underlying mechanisms, is specific attention deficits, in particular vigilance, selective attention and focusing of attention. The aim of the present study was to investigate the influence of lavender and jasmine on different disturbed attentional processes in schizophrenic and depressive disorders, and their effect on mood during test situation. By using the 'test battery of attentional performance' we examine five different attentional processes: (i) alertness; (ii) incompatibility; (iii) go/no-go; (iv) covert shifts of attention; and (v) visual vigilance. These five functions of attention are known to be influenced by inhaling those essential oils and/or impaired in these psychiatric disorders. To investigate the mood profiles we used the 'multidimensional mood questionnaire' including three mood dimensions—'pleasant–unpleasant', 'awake–sleepy' and 'calm–restless'—and study subjects were required to answer questions concerning different subjective ratings of the smell. All patients had to meet ICD-10 and DSM-IV criteria for schizophrenia and depression and to be rated by a psychiatrist on different rating scales: the brief psychiatric rating scale, the positive and negative syndrome scale or the Hamilton depression scale. Study subjects were also examined by an otorhinolaryngologist and participated in a study of olfactory functions.

Results based on an analysis of attentional processes and mood profiles under these odours are presented.

107. Fitting comparison of the Γ and Γ-vectorial models apllied to experimental data in olfactory, gustatory and pharmacological mixtures

Nathalie Serée and Paul Laffort

CNRS-UPR 9054, I.E.S.G.I.—Groupe physiologie de la Chimioréception, ENSBANA, 1 Esplanade Erasme, 21000 Dijon, France

Presently, among the published models on olfactory mixtures, only two are characterized by an interaction index which satisfies the conditions: (i) constant reference value when a substance is added to itself; and (ii) invariance, for a given pair of odorants, at all concentrations. These two models are called Γ and Γ-vectorial. They are respectively based on previously published models, which only satisfy the second condition above mentioned: the U model and the vectorial model.

In the present study, the Γ and Γ-vectorial models are comparatively tested on the basis of several published data in olfaction, taste and pharmacology. Three criteria have been applied to test these two models: (i) the standard deviation of the index; (ii) the variation of the index versus the response level (in olfaction, the odorous perceived intensity) to the mixture; and (iii) the variation of the index versus the proportion of the response to one of the two components of the binary mixture, called *tau*.

The results concerning the Γ-vectorial model have been disappointing in all cases. The Γ model, by contrast, appears satisfactory on the basis of these three criteria, for several published data, in olfaction and taste as well as in pharmacology. In some cases, however, a significant variation of the Γ index versus the *tau* proportion is observed, whereas an invariance is

generally observed of the Γ index versus the level of response (low or high).

An improvement of the Γ model, called Γ', is also proposed. In this version, the scattering of the index is the same than the one found for cosαU (the index of the U model), whereas it is often slightly greater for the Γ index.

108. The sweetness anomaly of D- and L-arabinose

Rachel W. Siertsema, Gordon G. Birch, Tim Astley and David A. Stevens

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

Experiments have shown that there is no significant difference in sweetness between the D- and L- enantiomers of arabinose. This equality in sweetness cannot be explained by the usual AH, B theory, since the fit of the stimulus molecule on the receptor must be different for each arabinose enantiomer. The partial molar volume (93.0 cm^3/mol) and the isentropic partial molar compressibility (-2.1×10^{-3} cm^3/mol/bar) are, however, identical for each enantiomer, as would be expected.

α-D-Arabinose is unusual in that it is the only simple D-sugar that is most stable in the $_4C^1$ conformation. β-Arabinose might appear to be equally stable in both the $^4C_1$ and $_4C^1$ conformations. Minimized energy calculations, however, reveal that β-arabinose prefers the $_4C^1$ conformation, where the hydroxyl groups at positions 1 and 4 are axial and those at positions 2 and 3 equatorial.

Variations in the extent of hydration and hydrogen bond density around the solute molecule give indications of its compatability with the three-dimensional stmcture of water. They may also reveal a possible explanation for this sweetness anomaly.

Molecular dynamics simulations in a 20 × 20 × 20 Å box, containing 250 water molecules, indicate that the 3,4 α-glycol group in arabinose is a centre of heavy hydration. If the D- and L-enantiomers are aligned with the receptor in the same way topologically, the D-sugar appears to have a more favourable configuration, like D-glucose.The 3,4 centre of hydration, however, fortifies the glucophore in the L- enantiomer and not in the D-enantiomer. This may explain their similar sweetness potencies.

This research was funded by EC grant PL-94-2107.

109. The effect of menthone and menthol on cortical EEG activity and a concentration task

James Jamieson and Steve Van Toller

Department of Psychology, University of Warwick, Coventry CV4 7AL, UK

Observation of cortical EEG activity, from 28 scalp electrodes (Van Toller *et al.*, 1993), was used to study the effect of a range of odours on the electrical activity of the cortex. We focused on the effect of an odour thought to stimulate the trigeminal (fifth cranial) nerve. The odour, peppermint, was shown to increase EEG activity levels in subjects during sleep (Badia *et al.*, 1990). Peppermint was chosen as one of seven odours to investigate the effects of odour on EEG maps (W.R. Klemm and S. Warrenburg, unpublished data) in a study of arousal, intensity and pleasantness. Peppermint was also used as a stimulus in an investigation into stress and performance (Warm *et al.*, 1990). These studies indicated that the performance of subjects was improved by the peppermint odour. They attributed the results to arousal, rather than a property conferred by the peppermint.

Menthol, menthone and its corresponding isomer are major constituents of peppermint oil. Menthone is a clear, colourless, volatile liquid, which can produce the stinging, unpleasant sensation redolent of a trigeminal stimulus.

The aims of this experiment were threefold. In the first instance, we are studying whether or not there are significant differences in the EEG output after the introduction of the trigeminal stimulus. If so, we will ascertain which electrodes the increased activity is centred on. Moreover, we intend to correlate the brain activity during the odorous stimulation (menthol and menthone) and a no odour control with the results of a vigilance task. We hope to show that the results from the task are improved in the menthone condition relative to the menthol or control conditions. The subjects gave hedonic ratings to each odour and these will be used to determine whether the menthone is more stimulating than the menthol. Differences in the task condition results indicate that the menthone appears to have a significant effect on performance over the menthol odour.

References

Badia, P. *et al* (1990) Responsiveness to olfactory stimuli presented in sleep. *Physiol. Behav.*, **48**, 87–90.

Van Toller, S. *et al.* (1993) An analysis of spontaneous human cortical E.E.G. activity to odours. *Chem. Senses*, **18**, 1–16.

Warm, J.S. *et al.* (1990) Effects of fragrances on vigilance performance and stress. *Perfum. Flav.*, **15**, 15–18.

110. A molecular dynamics investigation into the water structuring properties of simple monosaccharides with different taste properties

Gareth R.H. Wilden, Gordon G. Birch, Michael G.B. Drew, P. Mark Rodger and Tim Astley

Departments of Chemistry and Food Science & Technology, University of Reading, Whiteknights, Reading RG6 6AD, UK

The aim of this project was to relate the structures of simple monosaccharides and the effect of their substituents on local solvent structure to the known taste characteristics of these compounds. For many simple monosaccharides, the simple change from α to β by anomerization at C1 produces a marked difference in taste, from sweet to non-sweet, or even from sweet to bitter. The likely mechanism of the interaction with the receptor site is through hydrogen bonding, so an examination of the effect of each sugar on the water structure was instigated.

Molecular mechanics and dynamics calculations have been used to produce the data. The selected compounds were first examined using the QUANTA and CERIUS[2] packages, and molecular dynamics simulations were carried out using the CHARMm forcefield and the DL-POLY molecular dynamics simulation software. After an initial testing phase to verify the performance of the software and determine correct parameters, production runs

were carried out on the α and β forms, $^4C_1$ and $^1C_4$ configurations, of all of the simple hexose monosaccharides. Results have been processed using in-house software. For the simulations, the degree of hydrogen bonding has been calculated, in addition to radial and angular distributions for each hydroxyl group of the sugar.

A relationship between the degree of hydrogen bonding and the radial distribution function can be shown, and this can also be related to the taste properties of the molecules.

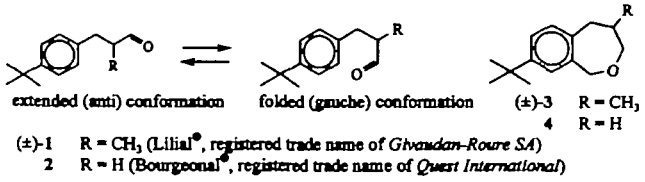
This work is funded by the EC (AIR3 CT94 2107) under the title 'The Mechanistic Understanding of the Sweetness Response'.

111. Synthesis and properties of conformationally restricted analogues of floral-type odorants

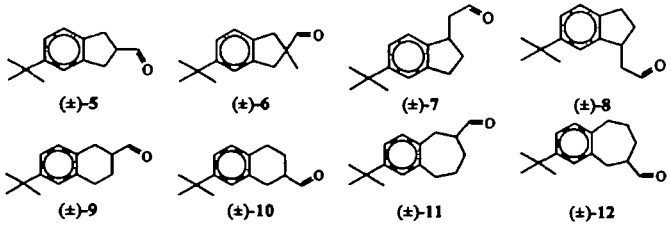
Serge Lamboley, Cédric Morel, André F. Boschung, Nigel G. Richards and Béat Winter

Firmenich SA, Corporate Research Division, PO Box 239, CH-1211 Geneva 8, Switzerland

The floral (lily-of-the-valley) type odorants **1** and **2** have two favored side-chain conformations—extended (anti) or folded (gauche)—and the population equilibrium is dependent on the substituent R and on the environment. In order to determine the 'bioactive' conformation of **1** and **2**, the analogues **3** and **4**, 'locked' in the folded conformations, have been recently prepared and shown to be odour-inactive (Skouroumounis and Winter, 1996).



(±)-1 R = CH_3 (Lilial®, registered trade name of *Givaudan-Roure SA*)
2 R = H (Bourgeonal®, registered trade name of *Quest International*)



We have now designed and synthesized the conformationally restricted analogues **5–12**, which mimic the extended conformation of **1** and **2** in various slightly different orientations, also depending on the conformation of the additional ring system. Compound **5** (Winter *et al.*, 1996) has been found to reproduce most closely the odoriferous activity of **2**, and this result adds support to the assumption that the 'bioactive' conformation of **1** and **2** is the extended one.

References

Skouroumounis, G. and Winter, B. (1996) *Helv. Chim. Acta*, **79**, 1095.

Winter, B., Blanc, P.-A. and Lamboley, S. (1996) European Patent Application EP 685,444, priority 31 May 1994 (CA: 1996, 124, 155 725t).

112. Chemotopical pattern formation in the olfactory system

Jörg Strotmann, Sidonie Konzelmann, Isabella Andreini and Heinz Breer

University of Stuttgart-Hohenheim, Institute of Zoophysiology, Garbenstraße 30, 70599 Stuttgart, Germany

Olfactory reception is mediated by several millions of sensory neurons in the nasal neuroepithelium and it has been assumed that spatial segregation of the olfactory input may be used to encode olfactory information. This notion was supported by the observation that individual odorants elicit distinct patterns of reactivity in the olfactory epithelium, indicative of the spatial segregation of sensory neurons responding to similar odorants. The response spectrum of individual olfactory neurons seems to be based on their distinct receptor types. *In situ* hybridization studies using receptor-specific probes have revealed that olfactory neurons expressing particular receptor subtypes are in fact segregated in distinct rostro-caudal zones and clusters within the nasal epithelium. Within their zones cells expressing a distinct receptor type are preferentially located in a characteristic laminar layer and arranged in an orderly fashion; they are positioned at well-defined horizontal distances. These observations suggest that the topographic and laminar localization of sensory neurons is under stringent control. Exploring the onset of receptor expression during ontogenesis may provide some clues towards an understanding of the underlying mechanisms. At prenatal stage E18, when the olfactory axon has synapsed onto secondary neurons in the bulb, many receptor-expressing neurons were found, already displaying a characterisitic spatial distribution. At E16 small groups of reactive cells were detectable. The first signs of receptor-expressing cells were observed between stages E12 and E14. During the prenatal stage until birth the number of receptor-expressing cells increased exponentially and a pattern of segregation resembling that of adults was established very early. Elucidating the correlation between the onset of receptor expression and synaptogenesis of sensory neurons is of fundamental importance to understanding the mechanisms governing the formation and maintainance of chemotopic patterns in olfactory systems.

This work was supported by the Deutsche Forschungsgemeinschaft.

113. Two classes of olfactory receptors in vertebrates

Joachim Freitag, Jürgen Krieger, Mario Mezler and Heinz Breer

University of Stuttgart-Hohenheim, Institute of Zoophysiology, Garbenstraße 30, 70599 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 extremly large, consisting of as many as a thousand different subtypes, whereas in fish the number of receptor types appears to be considerably smaller. This difference in receptor diversity between mammals

and fish may reflect a phylogenetic trend of augmenting the receptor repertoire via gene duplication, followed by mutations and recombinations, eventually leading to the large variety of receptor types in mammals. However, it is also conceivable that the small repertoire of receptor types in fish may reflect the difference in odor complexity in aquatic versus terrestrial environments; in fact, the number of odorants in water is much more limited than the volatile airborne odorants. To gain some insight into the evolution and functional implication of olfactory receptor diversity, receptor genes were studied in amphibia (*Xenopus laevis*), which are not only ranked at an intermediate position between fish and mammals on a phylogenetic scale but are also adapted to aquatic and terrestrial life, and are thus capable of smelling airborne as well as water-soluble odorants. It was found that *X. laevis* possess a gene repertoire encoding two distinct classes of olfactory receptors: one class related to receptors of fish and one class similar to receptors of mammals. Exploring their topographical distribution in the nose, it was found that the fish-like receptors are exclusively expressed in the lateral diverticulum, specialized for detecting water-soluble odorants, whereas mammalian-like receptors are expressed in sensory neurons of the main diverticulum, responsible for the reception of volatile odors. Sequence comparison revealed that the fish-like receptors represent closely related members of only two subfamilies, whereas mammalian-like receptors are more distantly related, most of them representing a different subfamily; thus, although originating from a common ancestor, both receptor classes apparently evolved differently. Exploring the olfactory receptor genes from the 'living fossil' *Latimeria chalumnae* may contribute to unravelling the phylogenetic origin and evolutionary divergence of the multigene family.

This work was supported by the Deutsche Forschungsgemeinschaft.

114. Mechanisms regulating the responsiveness of olfactory neurons

Ingrid Boekoff, Christine Kroner, Johannes Noe and Heinz Breer

University of Stuttgart-Hohenheim, Institute of Zoophysiology, Garbenstraße 30, 70599 Stuttgart, Germany

The olfactory system responds precisely to iterative stimulation and thus can continuously monitor changes in the odorous environment. This is due to the phasic responses of receptor cells. The basis for this characteristic feature of olfactory neurons is a rapid termination of the odor-induced primary reaction via a negativ feedback reaction controlled by phosphorylation of odorant receptors mediated by specific kinases. Phosphatase inhibitors, like okadaic acid, reduce the responsiveness of olfactory cilia; this result, together with data using specific antibodies, strongly suggests that protein phosphatase 2A catalyses the dephosphorylation of odorant receptors. This notion is supported by the finding that activators of phosphatase 2A significantly increase the responsiveness of olfactory cilia. Thus, a cycle of phosphorylation and dephosphorylation may control the inactivation and reactivation of olfactory receptor proteins in response to odor stimulation.

Odor stimulation leads to a transient increase in the intracellular [Ca^{2+}] due to an influx of Ca^{2+} ions. Recent studies suggest that the intracellular level of Ca^{2+} ions may modulate the reactivity of olfactory sensory cells. We have found that increased levels of [Ca^{2+}] attenuate the responsiveness of olfactory cilia; the odor-induced cAMP-signal in isolated olfactory cilia was diminished by elevated calcium concentrations in a dose-dependent manner. Stimulation of olfactory cilia preparations with *high* odor doses elicited a *slow* and *sustained* elevation of the cGMP level. The observation that a detectable increase in cGMP concentration only occurred in response to strong stimuli, as well as the slow kinetics of the cGMP responses, imply that cGMP may not be involved directly in the transduction process but rather may regulate the responsiveness of sensory neurons. In fact, it has been found that an elevated level of cGMP diminishes the responsiveness of olfactory cilia to odor stimulation via cGMP-dependent kinases. These results suggest that Ca^{2+} and cGMP may play a central role in adaptation of olfactory neurons.

This work was supported by the Deutsche Forschungsgemeinschaft.

115. Odorant binding proteins: comparative studies

Jürgen Krieger, Dietrich Lōbel, Jürgen Strobel, Marina Mameli, Isabella Andreini and Heinz Breer

University of Stuttgart-Hohenheim, Institute of Zoophysiology, Garbenstraße 30, 70599 Stuttgart, Germany

The reception of pheromones and odorants is mediated by olfactory sensory neurons in both the antennae of insects and the nasal neuroepithelium of vertebrates. The receptive structures of the cells are bathed in a characteristic protecting fluid. In terrestrial animals, which smell volatile, primarily lipophilic molecules, the airborne odorant and pheromone molecules have to cross the aqueous milieu before reaching the chemosensory membrane. Low-molecular-weight proteins are found in rather high concentrations in the sensillar fluid of insect antennae as well as in the mucus layer of the vertebrate nose. It has been suggested that these odorant binding proteins (OBP) facilitate the transfer of hydrophobic odorous molecules towards the sensory neurons. The acquisition of OBPs may thus represent one of the molecular adaptations that animals have evolved to deal with a terrestrial life style. Employing molecular biological approaches, numerous OBPs from different insect and vertebrate species have been cloned and their primary structures deciphered. In insects two major classes of OBPs have been identified: pheromone binding proteins (PBP) and general odorant binding proteins (GOBP). In fact, several species express two subtypes of each class of binding protein. Moreover, a third class of candidate binding proteins has recently been discovered in *Drosophila* and *Bombyx*. The diversity of OBPs in insects suggests that structurally distinct OBPs may recognize and bind separate classes of odorous molecules and thus act as selective signal filters. The phylogenetic relationship of the insect binding proteins is presently unclear but, based on the degree of sequence conservation, it can be assumed that all binding protems are derived from a common ancestral precursor. However, they display no significant homology to the OBPs identified in several vertebrate species, which appear to be members of a large family of proteins that bind small ligands,

including retinol and cholesterol binding proteins. This observation favors the view that insects and vertebrates evolved binding proteins for odorants independently, i.e. OBPs in vertebrates and insects represent evolutionary convergence.

This work was supported by the Deutsche Forschungsgemeinschaft.

116. Olfactory receptor gene subfamily expressed in clustered sensory neurons

Stefan Kubick, Jörg Strotmann and Heinz Breer

University of Stuttgart-Hohenheim, Institute of Zoophysiology, Garbenstraße 30, 70599 Stuttgart, Germany

Spatial segregation of sensory cells and their projection into the brain is used by the visual, auditory and somatosensory systems to define the stimulus location in the external environment. In contrast, the olfactory system does not encode information about spatial cues of the stimulus. Accordingly, odorant receptor types are expressed in rather broad zones and neurons expressing the same receptor type are randomly distributed within a zone. This dispersed distribution of receptor-expressing neurons in large zones is difficult to reconcile with the local 'hot spots' of physiological responsiveness. It has recently been discovered that in the rat nasal neuroepithelium one particular olfactory receptor gene exhibits a unique expression pattern: it is expressed only in neurons which are clustered in a small region of the epithelium. In comparative studies OR37-related genes were found to be expressed in a cluster of cells located at a very similar position within the nasal cavity in other rodent species, as well as in the opposum. Southern blot analyses indicated that OR37-related genes are not present in the genome of non-mammalian species. Alignment of receptor sequences revealed that the receptor protein encoded by the OR37 gene exhibits some unique structural features, notably an extension of the third extracellular loop by an insertion of 6–8 charged amino acid residues. Molecular cloning approaches led to the discovery of several OR37-related genes which apparently form a small gene family. The encoded isoforms of OR37 show remarkable differences in the charged amino acids in their third extracellular loop; these structural differences may correspond to important functional variants. Introducing the OR37 gene into a mammalian cell line (LLC-PK_1) led to an expression of receptor proteins which were glycosylated and inserted into the plasma membrane; the specific ligands for this receptor type are not yet known. Genomic analysis revealed that there are at least three OR37-related genes in the rat which appear to be located on the same chromosome. Deciphering the complete structure of the OR37 genes, especially the genomic motifs in the promotor region, may provide some insight into the mechanisms governing the unique spatial expression of the OR37 receptors.

This work was supported by the Deutsche Forschungsgemeinschaft.

117. Energetic processes in vertebrates' olfactory epithelium

Olga Gladysheva, Vera Troitskaya and Tatjana Semka

Institute of Educational Development, Lobachevski State University, Nizhny Novgorod, Russia

Electrophysiological experiments have shown that it is possible to record the EOG from isolated preparations of olfactory organs of different species of vertebrates for longer periods of time. These preparations remain physiologically active at low temperature (1 day for mammals, several days for amphibians). This stability of physiological activity is possible if the olfactory epithelium is able to maintain its energetic metabolism in isolated preparations. Measurements of the ATP level showed that if isolated preparations of olfactory organs are put in an atmosphere without oxygen the contents of macroerge decreased. After putting the preparations in an oxygen-containing atmosphere, the ATP level returned to the previous value. These results prove that it is possible to maintain a definite level of energetic metabolism using a mechanism of apical respiration and exogenous oxygen. EOG response to odor stimulation in isolated olfactory organ is accompanied by reduction of ATP and stops in an oxygen-free atmosphere or after the addition of respiratory inhibitors, which block the oxidative phosphorylation on the olfactory epithelium. The surface of isolated olfactory organs of various vertebrates is able to instantaneously hydrolize ATP in the incubation solution. Investigations of the nature of hydrolytic enzymes by methods of light and electron microscopic histochemistry and electrophoresis in polyacrylamide gel enabled us to conclude that various phosphohydrolase enzymes (ATPases, nonspecific alkaline phosphatases) are present on the surface structures of the olfactory epithelium (external membrane of olfactory cilia, glicocalix, deep levels of olfactory mucus, external membrane of olfactory bulbs). These surface phosphohydrolases can take part in the energetic support of receptor processes. But their role in more intimate processes of transformations of receptor proteins during the interaction with odorants is also possible.

118. Patch clamp study of histamine-activated potassium currents on rabbit olfactory bulb neurons

H. Hatt, K. Jahn and H.L. Haas[1]

Department of Cell Physiology, Ruhr-University Bochum, Universitätsstraße 150, D-44780 Bochum and [1]Department of Physiology II, Heinrich-Heine-University, Universitätsstraße 1, D-40225 Düsseldorf, Germany

The effects of histamine, and histamine agonists and antagonists on steady state current in principal neurons and interneurons were investigated in thin slices from the olfactory bulb of newborn rabbits with the nystatin perforated patch-clamp technique and local pipette application. No change in steady state current was observed in mitral cells. In most of the periglomerular, juxtaglomerular and granular cells, however, H_1-receptor activation caused an outward current; a similar effect, but mostly not on the

same neurons, was elicited by 8-bromo-cyclic AMP. These currents were reversed at the potassium equilibrium potential and blocked by apamin and therefore probably represent calcium-sensitive potassium currents. H_2-receptor activation caused an inward current which also reversed at the potassium equilibrium potential, indicating the blockage of a potassium current. Specific H_3-receptor activation and cyclic GMP were ineffective. Histamine usually caused a combined effect beginning with an inward current.

Histamine responses of olfactory bulb interneurons (*n* number of cells tested)

	%	(Absolute)	*n*
Histamine response	78	(162)	207
H_1	43	(88)	207
H_2	78	(160)	207
H_1 and H_2	42	(86)	207
H_1 only	1	(2)	207
H_2 only	36	(74)	207
8-bromo cAMP	59	(57)	97
8-bromo cGMP	0	(0)	12

Histaminergic neurons fire with changes in behavioural state. Our data give evidence that naturally transmitted histamine can markedly influence signal processing in the olfactory bulb via binding to specific receptors on interneurons. The mixed effect on interneurons and the effect on mitral cells after hyperpolarization allow a complex interaction with the olfactory network. By the described actions, the histaminergic system could influence central olfactory perception dependent on behavioural parameters.

This work was supported by the Deutsche Forschungsgemeinschaft.

119. Influence of different benzodiazepines on the $GABA_A$ current of rabbit olfactory bulb periglomerular cells

H. Hatt, J. Buffler[1] and K. Jahn

Lehrstuhl für Zellphysiologie, Ruhr-Universität Bochum, Universitätsstraße 150, D-44780 Bochum and [1]Neurologische Klinik der Technischen Universität München, Möhlstraße 28, D-81675 München, Germany

Using a thin slice preparation of rabbit (day 0–7) olfactory bulb, the effect of different benzodiazepines on the $GABA_A$ current of periglomerular cells was investigated. Patch clamp recordings (whole cell configuration) were made on visually identified neurons.

GABA-activated chloride currents could be recorded from all tested periglomerular cells ($n = 79$). The current could be mimicked by the $GABA_A$ agonist muscimol (100 μM) and was blocked completely by the selective antagonist bicuculline (1 μM). Benzodiazepine receptor agonists, inverse agonists and antagonists were applied together with GABA (100 μM).

Agonists: diazepam (10 μM) decreased the current amplitude to 61 ± 4% ($n = 9$) of the control and shortened the desensitization time constant compared with the current activated by GABA alone. The fast transient chloride current was followed by a steady-state current (reversal potential –60 mV). This second component is assumed to be the result of a changed nonspecific cation conductance and was only seen with diazepam. Zolpidem (10 μM) had no effect ($n = 27$) whereas 2-oxoquazepam (10 μM) reduced the time constant of decay without influencing the amplitude of the $GABA_A$ current ($n = 23$). The substance Cl 218–872 (10 μM) increased the current amplitude to 131 ± 12% ($n = 37$) of the control.

Inverse agonists: Ro 15–4513 (10 μM) diminished the current amplitude to 58 ± 13% ($n = 33$) of the control. The β-carboline β-CCM (10 μM) blocked the $GABA_A$ current completely ($n = 25$). The benzodiazepine antagonist Ro 15–1788 (10 μM) blocked the effects of Cl 218–872, 2-oxoquazepam, Ro 15–4513 and β-CCM when applied together with the benzodiazepine agonist or inverse agonist and GABA, but had no effect by itself. The substances PK 111–95 ($n = 17$) and Ro 5–6864 ($n = 8$) did not influence the $GABA_A$ current of periglomerular cells.

On GABA receptors in other tissue (spinal cord, hippocampus) we found pronounced differences in the effect of the benzodiazepines in comparison with the data presented here. The molecular basis for such differences could be another subunit composition of the GABA receptor protein.

This work was supported by the Deutsche Forschungsgemeinschaft.

120. Receptor cell subgroups in human olfactory epithelium

H. Hatt, M. Gjuric[1], G. Kobal[2] and N. Thürauf

Department of Cell Physiology, Ruhr-Universität Bochum, 44780 Bochum, [1]ENT Clinic, University of Erlangen-Nürnberg, 91054 Erlangen and [3]Department of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nürnberg, 91054 Erlangen, Germany

Patch clamp recordings revealed the presence of a non-desensitizing cyclic nucleotide-gated channel on human olfactory receptor neurons and a fast desensitizing non-specific cation channel activated by nucleotides on human supporting cells. Cyclic nucleotide-gated channels on olfactory receptor neurons showed a selective channel activation by cAMP ($K_{1/2}$ = 5 pM) and cGMP ($K_{1/2}$ = 2 pM), a unitary conductance of ~20 pS, a reversal potential of single channel currents close to 0 mV, a linear *I–V* relationship over the range of –80 to +80 mV and a strong extracellular but only a weaker intracellular blocking effect of Ca^{2+}. The channel activity outlasted the cyclic nucleotide pulses for hundreds of ms when higher agonist concentrations (>50 pM cAMP) were applied. The duration of the response was longer than in cyclic nucleotide-gated channels from other species studied so far. The plateau duration and the decay remained constant for pulses with a length of 50–150 ms, whereas pulses >50 ms successively reduced the time required by shortening the plateau phase. A higher difference for the $K_{1/2}$ of cAMP ($K_{1/2}$ = 22 pM)

and cGMP ($K_{1/2}$ = 2.5 pM) could be found for a small group (n = 3) of the cyclic nucleotide-gated channels. The $K_{1/2}$s of cAMP and cGMP found in this subgroup of ORNs with a different concentration dependency are very similar to the data of the cloned channel when the α-subunit is expressed exclusively. Morphological differences of these groups of cells could not be observed. It remains to determine whether a specific spatial distribution exists in the nasal cavity for ORNs expressing the α-subunit only.

121. Cyclic nucleotide- and inositol phosphate-gated ion channels in lobster olfactory receptor neurons

H. Hatt and B.W. Ache[1]

Department of Cell Physiology, Ruhr-University Bochum, Universitätsstraße 150, 44780 Bochum, Germany and [1]Whitney Laboratory and Department of Zoology and Neuroscience, University of Florida, St Augustine, FL 32086, USA

The idea of having two second messenger pathways in olfaction, one mediated by cAMP and the other by inositol 1,4,5-trisphosphate, is supported by evidence that both second messengers directly activate distinct ion channels in the outer dendrite of lobster olfactory receptor neurons. On inside out patches from dendritic membranes of olfactory cells we could identify two different types of IP_3 directly gated channels. The smaller conductance channel (27 pS) showed longer openings with characteristic flickering behaviour, a linear I–V relationship between –70 and +70 mV and a strong voltage-dependent open probability, with the channels having the highest probability of being open (0.78) at +70 mV, decaying to almost 0 at –70 mV. The larger conductance channel (64 ps) also showed long openings, a pronounced flickering behaviour and a linear I–V relationship, but the open probability was much less voltage dependent. Both types of IP_3-gated channels were unspecifically permeable for cations. Heparin at 25 μg/ml completely blocked the activation of the larger conductance channel. IP_4 (2 μM) activated another type of channel with an extremely large conductance (200 pS at +90 mV) and a low open probability (0.15). A third type of ligand-gated channel, activated by cAMP, could be identified. The channel had a conductance of 28 pS, a strong rectification at positive potentials (consistent with the channel being K^+-selective) and a voltage-independent open probability. cAMP- and IP_3-activated channels could co-localize to the same piece of membrane. Evidence that both types of second messenger-gated channels can occur in the same patch of membrane suggests that channels of both types can be expressed in one neuron. Evidence of more than one type of inositol phosphate-gated channel in this highly specialized region of the neuron furthers the idea that the output of individual olfactory receptor cells is regulated through multiple effectors and allows that effector diversity may contribute to functional diversity among olfactory receptor cells.

Supported by the DFG and SFB 220.

122. Imaging of olfactory dendrites of Antheraea in the scanning electron microscope (SEM) and atomic force microscope (AFM)

George Louis Kumar, Rainer Eschrich[1], Reinhard Guckenberge[1] and Thomas Axel Keil

Max-Planck-Institut für Verhaltensphysiologie, D-823 19 Seewiesen and [1]Max-Planck-Institut für Biochemie, D-82152 Martinsried, Germany

Olfactory transduction is thought to occur in the outer dendritic membrane of insect olfactory receptor neurons (ORNs). It is also considered, from electrophysiological studies, that the outer dendritic membrane has non-specific cation channels and IP_3-dependent Ca^{2+} channels (Zufall and Hall, 1991; Stengl, 1994). The presence of such channels is further supported by the observation that pheromone-stimulated dendrites take up cobalt (Kumar *et al.*, unpublished observations; Nagy *et al*, 1994). But to date, there has been no structural evidence regarding the existence of these channels. Therefore, in order to search for putative ion channels and receptor molecules, we imaged the olfactory dendrites in scanning electron microscope (SEM) and atomic force microscope (AFM), after extruding them out of the olfactory hairs and fixing them on plastic coverslips. With the aid of SEM, we could see the beaded structure of the dendrite as previously observed (Williams, 1988; Keil, 1993), but no fine structural details, as the membrane was sputtered with gold. Therefore, in order to avoid this problem, as well as to image the dendrite at atmospheric pressure in air and, more importantly, without using any fixative, we used an AFM. We imaged the dendrites in the contact mode of AFM after making four different sample preparations: (i) fixing them with (2.5%) glutaraldehyde and dehydrating them with graded concentrations of ethanol; (ii) in insect receptor lymph ringer with 0.1% glutaraldehyde; (iii) after treatment with Triton-X 100; and (iv) without fixation. Except for the second preparation, which we imaged in solution, all the other preparations were imaged in air. Using the first two methods, we could see the beaded structure of the dendrite but not any other structural details. With the third, we could mainly observe 1–2 microtubules (25–50 nm in diameter) as a result of the detergent treatment, which disrupts the membrane, and with the fourth method, we could see 'pores' on the membrane that were deeper than 3 nm, with a diameter in the range of 15 nm.

The density of the pores were ~20 pores/m^2 or ~10 000 pores for the whole surface area of the thicker dendrite (Keil, 1984). These 'pores' are probably cation channels, but at present we cannot decide which of the above types of channel they might represent. In the future we plan to use antibodies against calcium channels to find out whether there is any binding to these pores.

Supported by DFG-Grant 'Ke 398/2-1'.

References

Keil, T.A. (1984) *Zoomorphology*, **104**, 147–156.
Keil, T.A. (1993) *Tissue Cell*, **25**, 573–587.
Nagy *et al.* (1994) *J. Comp. Neurol.*, **344**, 321–335.
Stengl, M. (1994) *J. Comp Physiol. A*, **174**, 187–194.
Williams, J.L.D. (1988) *Int. J. Insect. Morphol. Embryol.*, **17**, 145–151.
Zufall, F. and Hall, H. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 8520–8524.

123. Gα15-like protein, an α-subunit of GTP-binding protein, is expressed in taste buds to be possibly involved in bitter taste signal transduction

Yuko Kusakabe, Soichi Arai and Keiko Abe

Department of Applied Biological Chemistry, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo, Japan

A variety of mechanisms are involved in taste signal transduction. Physiological studies have shown that GTP-binding protein (G protein)-coupled, receptor-mediated production of the second messengers, cyclic AMP and inositol 1,4,5-triphosphate (IP_3), participate in sweet and bitter taste signal transduction. It has also been reported that gustducin, a G protein cloned from rat taste tissue, and transducin are expressed in taste cells. They are, however, of the Gαi type of G protein, which are not able to activate phospholipase C to generate IP_3. It is likely that the IP_3 increase is due to the Gαq or Gβg type protein that activates phospholipase C-β (PLCβ).

We carried out an experiment to discover a Gαq-type G protein expected to be involved in taste signal transduction, and cloned a Gα15-like protein which has 94% similarity to a mouse Gα15 protein. To study expression of the Gα15-like protein, we generated an anti-Gα15-like antibody that recognizes a 43–44 kDa protein occurring in the circumvallate papillae. Immunostaining with the antibody showed that the Gα15-like protein is localized in taste bud cells. Since the Gα15-like protein belongs to the Gq class, we examined whether it actually activates PLCβ. For this examination, we quantified IP_3 by transfecting Cos-1 cells with an active type of Gα15-like or Gαq protein which had been demonstrated to activate PLCβ. As a result, the IP_3 amount reached 35 pmol/10^6cells when an active type of Gα15-like was transfected and this level was almost comparable with the amount (45 pmol/10^6 cells) observed when Gαq was transfected, whereas a much smaller value (12 pmol/10^6 cells) resulted from transfecting the cells with the vector alone, which was used as the control. This result demonstrates that the Gα15-like protein as well as Gαq protein is able to activate PLCβ, suggesting that the Gα15-like protein expressed in taste buds may play a role in taste signal transduction.

Incidentally, we examined the taste substances that bind to GUST27, a G protein-coupled receptor expressed in taste cells [*J. Biol. Chem.*, 1993]. After adding some sweet and bitter substances to insect sf21 cells which expressed GUST27, Gα15-like protein together with Gβi and Gγ2, we measured the amount of IP_3 in the cells. Some bitter substances, e.g. naringin, increased the amount of IP_3 in the cells. This result suggests that GUST27 might be a bitter taste receptor.

124. Distinct populations of toad olfactory neurons revealed by prolonged depolarizing current pulses

Rodolfo Madrid, Gonzalo Ugarte and Juan Bacigalupo

Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago, Chile

In addition to the odorant-dependent ionic conductances, olfactory neurons (ON) from *C. caudiverbera* have four major conductances: an inactivating Na^+ conductance, a sustained Ca^{2+} conductance, a V-dependent K^+ conductance and a Ca^{2+}-activated K^+ conductance (Delgado and Labarca, 1993). These four conductances give shape to the firing pattern that accompanies the depolarizing receptor potentials induced by odorants. We have investigated such a firing pattern with prolonged depolarizing I-pulses under I-clamp, in the absence of odorants. This study revealed that ON differ widely in their firing patterns; this may have important implications on olfactory coding. Of a total of 40 cells examined, 21 (53%) responded with a sustained increase in action potential firing (tonic cells, TC), 13 (32%) responded with a short burst (usually <4) of action potentials (phasic cells, PC) and 6 (15%) gave responses of intermediate characteristics. Action potential frequency was graded with the pulse amplitude. In PC the membrane remained depolarized after the burst.

We investigated the basis for the difference between TC and PC. The firing pattern of TC became transient upon removing extracellular Ca^{2+}, by supplementing normal Ringer with 0.1 mM Cd^{2+}, by replacing Sr^+ for Ca^{2+} in the external solution, even though Sr^+ permeated the Ca^{2+} conductance, indicating that an influx of Ca^{2+} is required for keeping a sustained firing rate. Under V-clamp we studied whether they differed in their V-dependent conductances. Normalized Na^+ and Ca^{2+} currents, studied using a Cs^+ internal solution, were virtually identical in TC and PC. Resting potential was larger in TC (–83 ± 12, n = 21) than in PC (–63 ± 12, n = 13). TC exhibited a more prominent Ca^{2+}-activated K^+ conductance than PC. These two latter factors may determine the different behaviors of TC and PC to depolarizing current steps. In TC they would keep the V_m below threshold for Na^+ channel inactivation, allowing sustained firing. In contrast, in PC, the V_m gradually depolarizes until reaching the threshold for Na^+ channel inactivation, ceasing action potential firing.

This study was supported by FONDECYT 1960878 and T'WAS 94-463 RG/BIO/LA.

References

Delgado and Labarca (1993) *Am. J. Physiol.*, **264**, C1418.

125. Freeze-substitution and post-embedding immunocytochemistry on rat taste buds: G proteins, calcitonin gene-related peptide (CGRP) and choline acetyl transferase (ChAT)

B.Ph.M. Menco, M.P. Yankova and S.A. Simon[1]

Department of Neurobiology & Physiology, Hogan Hall, Northwestern University, IL 60208 and [1]Department of Neurobiology, Duke University, Durham, NC 27710, USA

The utilization of freeze-substitution combined with low temperature embedding was explored for the purpose of preserving the ultrastructure of rat taste buds in order to perform postembedding immunocytochemistry. A major difference in taste-bud cells that were rapidly frozen without prior chemical fixation and those that were fixed and cryoprotected before freezing was that electron-opaque dense-core granules were virtually absent in all unfixed rapidly frozen taste-bud cells. The antibodies used in these initial studies were calcitonin gene-related peptide (CGRP), a peptide commonly found in nociceptive neurons, α-subunits of two G proteins that are involved in bitter taste transduction, and choline acetyl transferase (ChAT), an enzyme involved in the synthesis of acetylcholine. Anti-CGRP immunolabeled a subpopulation of unmyelinated perigemmal neurons; anti-$G_{q\alpha}$ labeled a larger subpopulation of these neurons and microvilli of, most likely, type II vallate taste-bud cells. α-Gustducin was found in cytoplasm of type II and/or III cells and probably in microvilli of type I cells of vallate taste buds. The best labeling results were obtained with anti-ChAT, which immunoreacted with microvilli and lateral membranes of some type II vallate taste bud cells, and the cytoplasms of some other type II and/or III vallate cells. In addition, anti-ChAT labeled electron-opaque materials inside taste-bud pores of vallate papillae but, under the same conditions, not granules of type I cells and most of the vesicles in von Ebner's glands. These data suggest that we cannot assume *a priori* that the contents of the dense-core granules of type I cells, or even of those of von Ebner's glands, contain the precursors of the taste-bud pore dense substances.

Supported by the Smokeless Tobacco Research Council, Inc. (031304, S.A.S. and B.Ph.M.M.), Philip Morris Corp. (S.A.S.), NSF (IBN 9109851, B.Ph.M.M.) and the NIHNIDCD (B.Ph.M.M.: RO1 DC-02491; S.A.S.: ROl DC-01065).

126. Biogenic amines in the vomeronasal organ

C. Zancanaro, C. Mucignat[1], A. Bolner[2], A. Sbarbati, G.P. Nordera[2] and F. Osculati

Anatomy and Histology, University of Verona, [1]Human Physiology, University of Parma and [2]A. Benedetti Institute, Villa Margherita, Arcugnano, Italy

Previous work in the frog has shown that the taste organ contains adrenaline (A), noradrenaline (NA) and serotonin (5-MT). We now report on biogenic amine measurements in the vomeronasal organ (VNO), which is crucial for reproductive and sexual behaviour. The biogenic amines content was determined with HPLC-ECD in isolated VNO of frogs and mice and expressed as pg/μg protein. Results were analysed by means of analysis of variance.

Results show that, in the frog, the VNO contains significantly higher amounts of A and NA than the olfactory and respiratory mucosa ($P < 0.0005$); and the 5-HT content was higher in VNO in comparison with the olfactory ($P < 0.025$) but not the respiratory ($P < 0.1$) mucosa. The mean VNO content of A was significantly higher in female than in male frogs ($P < 0.025$). In male mice the VNO content of A, NA and 5-HT ranked pre-pubertal > adult > pubertal; the content of the three amines was significantly higher in pre-pubertal than in pubertal and adult animals (P values <0.05–<0.00001). Females showed a different pattern: in pubertal females the VNO content of A was higher than in pre-pubertal and adult animals ($P < 0.0001$ and 0.002 respectively), whereas 5-HT content was higher in adult than in pre-pubertal ($P < 0.00001$) and pubertal ($P < 0.01$) annnals. The NA content was similar at all ages. Comparison of male and female mice showed that in pre-pubertal animals the content of A, NA and 5-HT was significantly higher in males than in females ($P < 0.05$–< 0.0001). In pubertal animals, the A content was higher in females than in males ($P < 0.00001$). This was not found in adults, where the 5-HT content was higher in males ($P < 0.0005$). Urine of adult males contains pheromones, such as the major urinary protein, that act through the VNO. Acute exposure (10 times intranasally at 10 min intervals) of adult female mice to urine of adult males (20 μl) was associated with reduced VNO content of the three amines, which was significant for A and 5-HT ($P < 0.004$ and < 0.008 respectively). Results suggest that: (i) the VNO contains discrete amounts of biogenic amines in amphibian and mammal species; (ii) the amine content of VNO can change according to the age and sexual maturity of mice; and (iii) acute exposure to male pheromones possibly induces amine depletion in the VNO of sexually mature female mice. These results prompt the further characterization of the role of biogenic amines in the VNO, inclusive of morphological localization, to clarify their physiological finction(s) in the VNO.

127. Soluble proteins in the salivary glands and in the vomeronasal organ of the pig

Silvana Marchese, Daniela Pes and Paolo Pelosi

Istituto di Industrie Agrarie, Via S. Michele, 4-56124 Pisa, Italy

Small soluble proteins are involved in chemical communication in several species. They belong to the superfamily of lipocalins and act as odour and pheromone carriers. These proteins have been identified both in chemosensory organs and in sites of synthesis and delivery of chemical stimuli. They include odorant-binding proteins and major urinary proteins of the mouse and rat, hamster aphrodisin and salivary proteins.

Pheromaxein is a 15 kDa soluble protein, present in the saliva of male pigs, with binding affinity to 5α-androst-16-en-3-one, a specific sex pheromone; this protein has not been further characterized.

This work investigates the presence and the structure of soluble proteins involved in chemical communication, both in the male salivary glands and in the female vomeronasal organ. The electrophoretic pattern of crude extracts of submaxillary glands

has revealed the presence of abundant proteins of 15 and 19/21 kDa only in the male sample.

The 15 kDa (p15) protein has been purified by conventional ion-exchange chromatography. Its N-terminal sequence revealed a significant similarity with rat vomeromodulin, a 70 kDa glycosylated polypeptide of the vomeronasal organ; however, an internal fragment of sequence, obtained after cleavage with BNPS-skatole, did not revealed any similarity with vomeromodulin or with other proteins.

The same chromatographic fractionation also afforded a purified sample of another protein, present as a heterodimer with subunits of 19 and 21 kDa (p19/21). The N-terminal sequence of this second protein also revealed significant similarity with another region of rat vomeromodulin.

An extract of the female pig vomeronasal organ was shown to contain several proteins in the 20 kDa range. Fractionation by anion-exchange chromatography and binding assay, performed with a pheromone analogue, 5α-androstan-3-one, indicated some of these proteins to be putative OBPs. A 16 kDa band was purified to homogeneity and subjected to sequential degradation, but its amino-terminal was found to be blocked. Peptides have been prepared by selective cleavage with CNBr, suitable for the determination of the internal amino acid sequence.

128. Structural studies on pig OBP

Sara Paolini, Silvana Marchese, Andrea Scaloni[1] and Paolo Pelosi

Istituto di Industrie Agrarie, Via S. Michele, 4-56124 Pisa and [1]Mass Spectrometry Laboratory, CNR, Via Pansini, 5, 80131 Napoli, Italy

An odorant-binding protein (OBP) of apparent mol. wt of 22 kDa was purified from pig nasal mucosa few years ago in our laboratory. It binds radiolabelled 2-isobutyl-3-methoxypyrazine with a dissociation constant of 0.5 μM and several other hydrophobic molecules with comparable affinities. Because of its low isoelectric point (4.2), this protein can be purified easily and rapidly. This fact and the convenient accessibility of the biological material make this OBP particularly attractive for structural studies.

We have recently used the pig OBP in experiments aimed at: (i) the determination of the amino acid sequence; (ii) the identification of the ligand binding site; and (iii) the preparation of crystals for X-ray diffraction studies.

Amino acid sequence. Direct Edman degradation allowed the establishment of a short N-terminal segment. More information was obtained from sequencing peptides generated by enzymatic (endoprotease Glu-C and trypsin) and chemical (cyanogen bromide, NTCB, BNPS-skatole) cleavage. The denatured, reduced and carboxymethylated protein was hydrolized in different conditions, the peptide mixture separated by tricine–SDS electrophoresis and individual peptides isolated by electroelution. Sequential Edman degradation of the purified peptides allowed mapping of the first 120 amino acids, corresponding to ~80% of the total length of the protein, as estimated by electrospray–mass spectrometry (mol. wt = 17.7 kDa).

Binding site. Preliminary information on the ligand-binding region of the protein was obtained with the use of a fluorescent photoaffinity label, 1-azidoanthracene. This compounds binds the pig OBP reversibly with a dissociation constant lower than micromolar and becomes covalently attached to the protein upon irradiation with UV light. The OBP, thus derivatized, was partially hydrolized with enzymes and chemical reagents, and the peptides were separated by electrophoresis. The presence of fluorescence on the peptides, as detected under UV light, provided information on the region of the protein where the photoaffinity probe was bound.

Crystallization. Crystals of the pig OBP were grown in ammonium sulphate and in the presence of 2-isobutyl-3-methoxypyrazine. Samples of the protein kept in the same conditions, but in the absence of a ligand, failed to crystallize.

129. Soluble proteins in chemosensory organs of phasmids and other orders of insects

Marina Mameli, Andrea Tuccini, Mario Mazza[1], Gaia Monteforti[2] and Paolo Pelosi

Istituto di Industrie Agrarie, Via S. Michele, 4-56124 Pisa, [1]Dipartimento dell'Ambiente e del Territorio, University of Pisa and [2]Scuola Superiore Studi Universitari e Perfezionamento 'S. Anna', Pisa, Italy

Soluble proteins of low molecular weight were purified from chemosensory organs of insects belonging to several orders, such as Phasmatodea, Blattoidea, Orthoptera and Coleoptera. The most extensive and informative data were collected with phasmid species, while only preliminary information was obtained for proteins of the other orders.

Proteins in the range of 14–19 kDa were isolated from antennae, legs and palpi of five species of phasmids. On the basis of their N-terminal amino acid sequences, all except one were grouped into two classes. Polypeptides of 14 and 15 kDa, expressed in the antennae and legs of *Eurycantha calcarata* and *Extatosoma tiaratum*, as well as in the antennae of *Carausius morosus*, bear a close similarity (~45% identity) with a soluble protein associated with the sensilla coeloconica of *Drosophila melanogaster*. Two proteins of 19 and 18 kDa, isolated from the antennae and the ancillary palpi, respectively, of *Acrophylla wuelfingi* are 59 and 75% identical, in their N-terminal region, to a 19 kDa antennal protein of *C. morosus*. Similarity between members of the two classes is not significant, being limited to 2–3 identical amino acids in the most favorable cases. Finally, a 17 kDa protein specifically expressed in the antennae of *Sipyloidea sipylus* did not show any homology with the other proteins. The expression in sensory organs and the characteristics of these proteins may suggest a function in chemosensory transduction.

A 14 kDa protein, particularly abundant in the antennae of *Blatta orientalis*, has been purified and its N-terminal sequence has been determined. Highest similarity is with OS-D of *D. melanogaster* and with the 14 and 15 kDa proteins of the phasmids mentioned above.

Soluble proteins in the range 14–20 kDa, highly expressed in chemosensory organs, have also been purified from the following species: *Gronphadorrina portentosa* (Blattodea), *Eiprepocnemis plorans* (Orthoptera), *Ditiscus marginalis* and *Hydrophilus piceus* (Coleoptera).

Their chemical characterization and amino acid sequences are currently being investigated.

130. Odorant-binding proteins of the rabbit

Marina Garibotti, Ambretta Navarrini, Anna Maria Pisanelli[1] and Paolo Pelosi

Istituto di Industrie Agrarie, Via S. Michele, 4-56124 Pisa, Italy and [1]Department of Instrumentation and Analytical Science, UMIST, Manchester, UK

Following the purification of an odorant-binding protein (OBP) from rabbit nasal mucosa, we have identified, purified and partially characterized two additional OBPs from the nasal tissue of the same animal species.

The new proteins have been classified as OBPs based on their binding affinity to 2-isobutyl-3-methoxypyrazine, the odorant used as a radioactive probe in most of these studies. Their purification was easily accomplished by anion-exchange chromatography and gel filtration, along with standard protocols. OBP-II is a monomer of 21 kDa and isoelectric point 4.2; OBP-III is a dimer with subunits of 23 kDa and isoelectric point 4.8.

The partial amino acid sequences of the three OBPs, determined by Edman degradation, confirm that they are members of the OBP family, but reveal poor similarity between them. However, greater similarity is found between each OBP and other members of the lipocalin family. In particular, OBP-I is most similar to bovine OBP (55% identity in the N-terminal region); OBP-II is >50% identical, limited to its first 18 amino acids, to mouse OBP-I and porcupine OBP-II, while OBP-III shares 26 out of the its first 40 amino acids with MUP4, a mouse salivary protein.

The presence of three or more OBPs with different amino acid sequences in the same animal species may suggest a role of these proteins in the recognition of odorant molecules.

131. Membrane properties of the microvillous receptor neurons of the rat vomeronasal organ

Didier Trotier, Kjell B. Døving[1] and Camilla Shalchian-Tabrizi[1]

Laboratoire de Neurobiologie Sensorielle, Ecole Pratique des Hautes Etudes, 1 Avenue des Olympiades, F-91305 Massy, France and [1]Department of Biology, University of Oslo, Box 1051, N-0316 Oslo, Norway

The vomeronasal organ of rats was removed from its bone cavity and the sensory epithelium separated from the non-sensory tissue, placed in a Ca^{2+}-free solution and dissociated mechanically after treatment with papain. The suspension of isolated cells was placed in a recording chamber perfused with Ringer's solution in an upright microscope. The temperature of the recording chamber was kept between 32 and 35°C. Recordings were made with patch pipettes in conventional or gramicidin-perforated whole-cell mode. Isolated vomeronasal receptor neurons (VRNs) was of characteristic bipolar shape, with a long slender dendrite.

In voltage-clamp recordings depolarizing voltage pulses evoked a fast and transient inward current, probably I_{Na}, followed by a slowly inactivating outward current, probably I_K. Hyperpolarizing voltage pulses more negative than –80 mV activated an inward rectifying current I_h.

In current-clamp recordings many receptor neurons showed discrete depolarizations which could sometimes induce action potentials without any overt stimulation. Application of a Ringer's solution containing 4 μM K^+, to decrease the current caused by the Na,K-ATPase, induced depolarization and action potentials. At room temperature resting potentials were decreased. Both observations suggest that the sodium pump current contributed to set the resting membrane potential of rat VRNs. This finding has previously been demonstrated for frog VRNs, in which the sodium pump current commonly polarizes the membrane to potentials more negative than –80 mV (Trotier and Døving, 1996).

However, very negative resting potentials were not observed in rat VRNs. A likely explanation could be due to the seal resistance between the pipette and the cell membrane, which was much smaller than the seal resistance (>40 GΩ) obtained with frog VRNs. In this condition the hyperpolarizing sodium pump current passed through the seal resistance rather than the membrane resistance and cells became depolarized. Voltage-dependent channels activated by depolarization, therefore, mainly contribute to set both the observed membrane potentials and the membrane conductance. This artificial decrease in the contribution of the hyperpolarizing sodium pump current could explain why exposure of rat microvillous VRNs to ouabain or dihydro-ouabain (100 pM) hardly changed the observed membrane potentials. Alternatively, the Na,K-ATPase expressed in rat VRNs could be less sensitive to ouabain than the Na,K-ATPase expressed in frog VRNs.

This study indicates that microvillous receptor neurons isolated from rat vomeronasal organs are amenable to patch-clamping and present membrane currents similar to those observed in frog vomeronasal microvillous receptor neurons.

References

Trotier and Døving (1996) *J. Physiol.*, **490**, 611–621.

132. The G-protein γ-subunit, Gγ8, is expressed in the developing axons of olfactory and vomeronasal neurons

Roberto Tirindelli, Paolo Rovero[1] and Nicholas J.P. Ryba[2]

Istituto di Fisiologia Umana, Universitá di Parma, Via Gramsci 14, 43100 Parma, [1]Istituto di Mutagenesi e Differenziamento, CNR, Pisa, Italy and [2]Laboratory of Immunology, NIDR, National Institutes of Health, Bethesda, MD 20892, USA

The tissue localization of the G-protein γ-subunit, Gγ8 (Ryba and Tirindelli, 1995), which is specifically expressed in the olfactory and vomeronasal neurons, was studied in rats at different ages. Gγ8 appears to be a specific marker of the immature olfactory and vomeronasal neurons. Its distribution differs from that of Golfα (Jones and Reed, 1985), a G-protein α-subunit which is predominantly expressed in mature olfactory neurons. Gγ8 immunoreactivity indicates that an undifferentiated organization of the olfactory epithelium persists up to 3 weeks postnatally although neonates possess a functional sense of smell. Gγ8 accumulates at the highest levels in the axons of the developing olfactory neurons 2 weeks after birth (P14). Moreover, up to P14, Gγ8-positive neurons are present in the region of the olfactory and vomeronasal epithelium where they are not observed in later life.

In the olfactory epithelium and in the bulb, Gγ8 expression becomes weaker and patchy with increasing age, suggesting that the process of continuous regeneration of the olfactory neurons occurs in discrete areas. Gγ8-enhanced expression following axotomy indicates that this system is potentially active throughout life. Conversely, in the vomeronasal epithelium Gγ8 expression persists virtually unmodified in adult. This indicates that the degree of differentiation may differ between olfactory and vomeronasal neurons.

References

Ryba, N.J.P. and Tirindelli, R. (1995) *J. Biol. Chem.*, **270**, 6757–6767.
Jones, T.J. and Reed, R.R. (1985) *Science*, **244**, 790–795.

133. Proliferation density in the olfactory epithelium changes during postnatal development in the adult rat

Elke Weller and Albert I. Farbman

Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208, USA

In the vertebrate olfactory epithelium, receptor cells are continuously replaced throughout life by the division of basal cells and differentiation of the daughter cells into neurons. In the adult rat there is a continuous increase in the total surface area occupied by olfactory epithelium so that between 1 and 6 months of age the olfactory surface increases about three- to fourfold. Coincident with the increase in surface area there is an increase in the total number of olfactory neurons. In this study on rat olfactory epithelium we asked whether there is a change in the number of dividing cells with age, within the first 6 postnatal months.

In Sprague–Dawley rats aged 40, 66, 105 and 180 postnatal days we used BrdU to label dividing cells. We counted the number of proliferating cells on 10 μm paraffin sections of the olfactory epithelium on the septum and turbinates in different regions ranging from the anterior to the posterior part of the nasal cavity.

The density (number per mm) of proliferating cells decreased continuously with age:

Age (postnatal days)	P40	P66	P105	P180
Labeled basal cells per mm	36.8	24.2	16.8	10.2

Although there is a pronounced increase in total olfactory surface in the age range studied, the density of proliferating cells in P180 animals is less than one-third that seen in P40 animals. This means that the total number of dividing cells is relatively unchanged. This suggests that in the adult rat there is a relatively constant number of progenitor cells (perhaps related to odor specificity) to serve a constant number of glomeruli in the olfactory bulb. The increased area of olfactory epithelium with age indicates that more neurons are being produced than are dying, and the increase in total number reflects an increase in the number converging on each glomerulus.

Supported by NIH grant DC 00347.

134. Kin recognition in honey bees: genetic basis of cuticular hydrocarbon profile in natural conditions

Gérard Arnold, Benoit De Schepper, Brigitte Quenet and Claudine Masson

Laboratoire de Neurobiologie Comparée des Invertébrés, INRA-CNRS (URA 1190), 91440 Bures-sur-Yvette, France

In a honey bee colony, polyandry leads to the coexistence of several (7–20) subfamilies. Workers discriminate members of the same subfamily (full-sisters) from workers of other subfamilies (half-sisters) (Getz and Smith, 1983).

In this report we examined the cuticular hydrocarbon composition of honey bee workers from a colony with a naturally inseminated queen. The insects were reared under various conditions, in order to determine the respective parts of the genetic and environmental factors in their hydrocarbon profiles. Three sets of newly emerged workers (*Apis mellifera mellifera*), all daughters of the same queen, were established. In the first set ('hive bees') workers were marked for aging and replaced in the hive, in the second set ('grouped bees') they were placed in groups of 10 and in the third set ('isolated bees') they were reared individually. After 5 days, workers of each set (117, 77 and 117 bees respectively) were individually analyzed for cuticular hydrocarbons and assigned to their respective subfamily using two highly variable microsatellite loci (A76 and A107) (Estoup *et al.*, 1994). Sixteen different subfamilies were identified in the complete sample. The qualitative and quantitative analyses of the cuticular hydrocarbon composition showed 26 compounds, but for the statistical analyses 12 compounds were excluded because their concentration was too low to be measured reliably.

From the generalized linear model method we can conclude that there is a clear subfamily effect i.e. hydrocarbon profiles differ significantly between subfamilies. This demonstrates that these profiles conserve invariant properties even when altered by the rearing conditions. Our study demonstrates that cuticular hydrocarbons possess the necessary prerequisites of sufficient variability and genetic determinism to be used as labels for subfamily recognition. Using highly variable microsatellites, we could extend to natural conditions preliminary results obtained with colonies composed of only two distinct subfamilies (Page *et al.*, 1991).

References

Getz, W.M. and Smith, K.U. (1983) *Nature*, **302**, 147.
Estoup, A. *et al.* (1994) *Proc. R. Soc. Lond.* B, **258**, 1.
Page, R.E. *et al.* (1991) *J. Chem. Ecol.*, **17**, 745.

135. OBP-like proteins in the honey bee *Apis mellifera* L: purification, characterization and maturation

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

Laboratoire de Neurobiologie Comparée des Invertebrés, INRA-CNRS (URA 1190), BP23, Bures-sur-Yvette F91440 and [1]Unité de Recherches de Biochimie et Structure des Protéines, INRA, Domaine de Vilvert, Jouy-en-Josas F78352, France

Perireceptor olfactory events involve a family of abundant soluble proteins. The ability of these proteins to bind odor molecules suggests that they may play a role in the cascade of molecular mechanisms leading to odor transduction.

The so-called odorant-binding proteins (OBPs) and their pheromonal counterparts (PBPs) have been described in some species of insects and mammals (for review see Pelosi, 1994). In this presentation we report data related to the study of such proteins in the honey bee *Apis mellifera* L., a social insect which possesses a very rich olfactory repertoire (i.e. food aromas, social and sexual pheromones) sustaining behaviours crucial for both the individuals and the colony.

Worker OBP-like proteins were isolated on the bases of biochemical criteria and tissue specificity. Soluble proteins from adults and developing insects were fractionated by non-denaturing gel electrophoresis and HPLC on line ion-spray mass spectrometry. Next, several antennal-specific proteins were submitted to N-terminal sequencing. They presented electrophoretic properties, precise masses and signal peptide elimination commonly found in OBPs. Molecular cloning is in progress to establish or confirm sequence similarities with known OBPs or PBPs.

Interestingly the expression of these proteins during the bee development is closely related to the maturation of receptor cells (Masson and Arnold, 1984). Taking into account the crucial role of the sensory neurons in the development of the bee olfactory system (Gascuel and Masson, 1987, 1991), such results suggest that these proteins are implicated in bee olfaction.

References

Gascuel, J. and Masson, C. (1987) *Neurosci. Res. Commun.*, **1**, 173–180.
Gascuel, J. and Masson, C. (1991) *J. Neurobiol.*, **22**, 795–810.
Masson, C. and Arnold, G. (1984) *J. Insect Physiol.*, **30**, 7–14.
Pelosi, P. (1994) *Crit. Rev. Biochem. Mol. Biol.*, **29**, 199–228.

136. Olfactory deficit in staggerer mice: hyposmia for butanol and vanillin

V. Deiss, C. Féron and C. Baudoin

L.E.E.C. U.A. C.N.R.S. 2214, Université Paris XIII, 93430 Villetaneuse, France

Male staggerer mutant mice do not prefer the vaginal secretion odors of estrous females to the vaginal secretion odors of anestrous females. Nevertheless staggerer males prefer the female urine odors to the odors of vaginal secretions. Staggerer mice are not anosmic but a hyposmia may be a cause of these behaviors. This hypothesis was tested in this study. Two-choice tests (butanol or vanillin versus amyl acetate odors) were used in order to determine behavioral thresholds for butanol, an aversive odor, and for vanillin, an attractive odor. Two groups of C57BL/6 male mice (one non-mutant group and one mutant group) were studied using an olfactometer. Different concentrations of butanol were used: from 5.5×10^{-4} to 5.5×10^{-1} M. Vanillin at different concentrations, from 6.6×10^{-5} to 6.6×10^{-2} M, was presented during the tests after a 1 month period of familiarization.

Aversive and attractive behavioral thresholds of staggerer mice are higher than those of non-mutant mice. The staggerer mutation induces hyposmia in mice. This olfactory deficit could explain, at least partially, abnormalities in the social and sexual behaviors of staggerer mice.

137. Responses of chestnut moths to sex attractants and plant volatiles: electrophysiology and behaviour

C.J. Den Otter, A. De Cristofaro[1] and Karen E. Voskamp

Sensory Physiology Group, Department of Animal Physiology, University of Groningen, Kerklaan 30, 9751 NN Haren, The Netherlands and [1]Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, Via Cavour 50, 86100 Campobasso, Italy

EAG recordings were made from both males and females of *Cydia fagiglandana* (Zell.) and *C. splendana* (Hb.) on stimulation with the tortricid sex attractants (*E,E*)-8,10-dodecadien-1-yl acetate (E8E10–12:Ac), (*E,E*)-8,10-dodecadien-1-ol (E8E10–12:OH) and (*Z*)-8-dodecen-1-yl acetate (Z8–12:Ac). The dose–response curves of the various attractants were almost identical for males of both species. Females were much less sensitive to the sex attractants. Recordings from single antennal olfactory cells of *C. fagiglandana* males showed responses of one or two cells on stimulation with the sex attractants and with volatiles from chestnut leaves.

The diel spontaneous locomotor activities of both species as measured in actographs ran almost concurrently. Most moths were active during the first 4–6 h and the last 2.5 h of the night. A correlation appeared to exist between the locomotor activities and behavioural responsiveness to attractive odours.

In a wind-tube behavioural responses to upwind attractants only occurred during a few minutes after the airstream had been switched off. It is proposed that either (i) attractive odours in combination with an airstream may evoke an 'internal excitatory state' that finds active expression in standing air or (ii) intermittent stimulation with odours, which may occur in the unsteady air shortly after switching off the airflow, is indispensable for inducing upwind displacement.

Males as well as females were attracted to E8E10–12:Ac, to calling conspecific females and to the smear of abdomen tips cut off from calling conspecific females. The studies suggest that E8E10–12:Ac is a main component of the female sex pheromone of both *Cydia* species. With three conspecific females present in the tube, the females lined up ~20 cm from each other and started to call. This suggests that sensitivity of the females to their own sex attractants may permit them to detect the presence of other calling females, leading to settling and synchronization of pheromone production, and avoidance of pheromone-releasing females. As a

result, calling females may distribute evenly throughout the environment.

138. Olfactory deficit in staggerer mice: role of social factors

C. Féron, V. Deiss and C. Baudoin

L.E.E.C. U.A. C.N.R.S. 2214, Université Paris XIII, 93430 Villetaneuse, France

The staggerer mutation is known to affect mainly the cerebellum. Consequently the mutant mice exhibit obvious motor deficiencies explaining some of their behavioural deficits. The staggerer males generally do not mate (~5% mate) and do not adapt their behaviour to the state of sexual receptivity of the females they meet. Tested for their olfactory preferences toward odours of urine and vaginal secretions of sexually receptive and unreceptive female, staggerer males are not attracted by odours of oestrus females while non-mutant males are. However, they are not anosmic.

After mating the staggerer males are attracted by the odours of sexually receptive females. Therefore, the mutation does not prevent the olfactory detection of female sexual receptivity. Social isolation is known to increase the sexual activity in male mice. After social deprivation most of the staggerer males display mounts and 33% of them reproduce. In olfactory tests before having any social contact with a female, isolated staggerer males show a clear attraction to odours of sexually receptive females. So a period of social isolation clearly improves the behavioural performances of staggerer mice in olfactory tests.

139. Comparative analysis of taste and olfactory behavioral responses to free amino acids in the White Sea cod, *Gadus morhua marisalbi*

Alexander Kasumyan, Sergej Sidoroy and Eugeny Marusov

Department of Ichthyology, Biological Faculty, Moscow State University, Vorobjevi gory, Moscow, 119899 Russia

The experiments were performed on cod, 17–23 cm in length, which were captured in the White Sea 1–2 weeks before the start of trials. Free amino acids (L-isomers) and water extracts of food organisms (freshwater shrimp, *Gammarus* sp.; lugworm, *Arenicola marina*; or mussel, *Mytilus edulis*) were used as chemical stimuli. The taste tests were performed by offering the fish single agar–agar pellets containing one of the substances tested. Fish olfactory responses were examined by adding into aquarium water solutions of free amino adds or extracts of food organisms.

It was found that seven amino acids from 21 tested—phenylalanine, norvaline, tryptophan, leucine, isoleucine, alanine and methionine (0.1–0.01 M)—were highly palatable; three amino acids—proline, arginine and lysine (0.1 M)—were aversive tastants in the cod. There were negative correlations between the palatability of free amino acids and the time of pellet retention by the fish ($r = -0.70$; $P < 0.001$) and the number of pellets grasped ($r = -0.73$; $P < 0.0001$). The most palatable pellets were swallowed by fish mainly after the first grasp, the time retention continuing up to 2–3 s. The threshold concentration for phenylalanine in agar–agar was close to 10^{-4} M, or <1.0 μg per pellet.

Solutions of only two amino acids, alanine and glycine, 10^{-4} M, induced good search feeding behavior in cod, less than to shrimp or lugworm extracts but stronger than to mussel extract. Serine, cysteine, asparagine, aspartic acid, glutamine, glutamic acid, histidine and arginine at the same concentration were less attractive, whilst the other 11 free amino acids did not influenced fish behaviour. No significant relationships between taste and olfactory behavioral responses to free amino acids was found ($r = 0.04$; $P > 0.05$).

This study was supported in part by the Russian Foundation for Basic Research, grant 96-04-48218.

140. The role of olfaction in home stream selection in hime salmon (landlocked sockeye salmon)

Shoji Kitamura, Kazumasa Ikuta, Masafumi Amano, Arimune Munakata[1], Masaaki Yaita[2], Sakae Kikuyama[2] and Katsumi Aida[1]

Nikko Branch, National Research Institute of Aquaculture, Nikko, Tochigi, [1]Department of Aquatic Bioscience, The University of Tokyo, Tokyo and [2]Department of Biology, School of Education, Waseda University, Tokyo, Japan

Olfaction is known to play a dominant role in salmon when they return to the home stream in which they spawn. Most of the evidence for this phenomenon has been shown by displacement and recapture experiments using sensorily impaired fish that had returned to their home streams. To confirm the role of olfaction in natal stream selection, we observed the upstream behavior and home stream selection of hime salmon (landlocked sockeye salmon, *Oncorhynchus nerka*) in an artificial two-choice stream, and examined the olfactory responses of the salmon to home streams and non-home streams electrophysiologically.

Mature hime salmon showed upstream behavior and 73.3% select the home stream water in the two-choice stream. Olfactory impairment deprived the fish of selectivity of the home stream. Electrophysiological experiments showed that the salmon could discriminate streams by olfaction. Thus, a decisive role of olfaction in home stream selection was confirmed in this study.

141. Role of the N-terminus of major urinary proteins in mouse pheromonal communication

C. Mucignat-Caretta, A. Caretta and A. Cavaggioni[1]

Institute of Human Physiology, University of Parma and [1]Institute of Human Physiology, University of Padova, Italy

In mammals, most pheromonal effects are elicited by volatile odorant substances, which act through the main olfactory system. Beside this, the vomeronasal organ and accessory olfactory system is also sensible to non-volatiles, and has been demonstrated to be involved in pheromonal communication. It is thus conceivable that chemical signals are represented by large molecules. It has long

been known that exposure of prepubertal female mice to pheromones contained in urine from adult males results in acceleration of the onset of puberty. In the urine of adult male mice, production and excretion of several substances are androgen-dependent, including the large amount of protein excreted in male urine which consists of the major urinary protein complex (MUP), a lipocalin whose structure resembles an eight β-sheet barrel surrounding a hydrophobic pocket, which binds odorant molecules. When given to prepubertal females, MUP can induce the acceleration of puberty in its natural conformation, namely with natural ligands bound in the hydrophobic pocket, and also when no ligand is present, whereas the ligands alone, without the protein, have no such effect. Moreover, when all ligands are displaced in the protein by only one non-natural ligand, the protein is still able to induce acceleration of puberty. When the protein is denatured by boiling, no effect on puberty onset is observed. These data point to a role for the mature protein in pheromonal communication. Different isoforms of MUP are coded by several genes, and there are also some MUP genes which code for only the N-terminal hexapeptide. When mice are given the synthetic hexapeptide corresponding to the deduced amino acid sequence, acceleration of puberty can still be observed. These data fit with earlier reports of pheromonal activity due to low mass urinary peptides. Since there was no evidence of the transcription of the genes which code for the hexapeptide, we purified the native hexapeptide from male urine by subsequent steps of molecular sieve and reverse phase chromatography, and identified it by RF in thin layer chromatography and mass spectroscopy, identical to the synthetic standard.

142. Quantal substructure of the elementary receptor currents in olfactory cells of the moth *Bombyx mori*

A. Redkozubov

Institute of Physiologically Active Substances, Russian Academy of Sciences, Chernogolovka, Russia

The antennal olfactory sensilla of the moth *Bombyx mori* were studied *in situ* using the voltage-clamp technique. The amplitude parameters of the elementary receptor currents (ERC) eliciting one action potential in the bombykol and bombykal receptor cells were analysed. The ERC amplitude varied within a range of 0.4–7 pA. The amplitude histograms of the ERCs recorded from both bombykal and bombykol receptor cells showed a series of peaks and indicated thereby discrete amplitude levels. The levels were multiples of 0.65 pA for the bombykol cells and 0.5 pA for the bombykal cells, suggesting quantal events underlying the ERC. The levels show a Poisson-fashion distribution with an average number of 2.5 events per ERC for the bombykol cell. In the bombykal cell the Poisson-like distribution of the levels indicated two populations of ERCs with means of 4.1 and 8.8 quantal events repectively. Thus, the ERC eliciting one action potential in a receptor cell, a putative response to a single pheromone molecule, is suggested to be due to the opening of less than three ion channels for the bombykol cell and of less than four or nine ion channels for the bombykal cell.

143. Delayed functional recovery for pheromone recognition after bilateral olfactory nerve axotomy in goldfish

Christian von Rekowski and Hans Peter Zippel

Physiol. Inst. d. Univ. Göttingen, Humboldtallee 23, 37073 Göttingen, Germany

The use of chemical signals (pheromones) for social interactions is well known for a variety of animals. Like many other fish species, goldfish (*Carassius auratus*) use pheromones for synchronization of spawning behavior between the genders. The preovulatory pheromone 17α,20β-dihydroxy-4-pregnen-3-one (17α,20β-P) is assumed to 'prepare' the male goldfish physiology for the coming spawning. Physiological studies have shown the extreme sensitivity of the olfactory epithelium to 17α,20β-P and, furthermore, a few recent studies have demonstrated the behavioral impact on mature males, i.e. increasing of aggressivity and locomotor activity. To find out more specific information about the goldfish performance and limitations in pheromone recognition in comparison to amino acid (AA) and food odor recognition, we performed the present behavioral experiments. The experimental paradigm made use of the responsiveness to17α,20β-P and a bile acid (taurolithocholic acid sulfoethylamide: TLC-S) in the context of feeding. During subsequent discrimination training (17α,20β-P reinforced with *Tubifex* worms, TLC-S not reinforced) nine groups of two goldfish each learned to prefer the reinforced pheromone. After 30 training sessions, however, the stability of behavioral responses was less balanced than during AA discrimination training (Zippel *et al.*, 1993; van Rekowski *et al.*, 1994). We therefore tested the fish with Arg (10^{-6} M, reinforced) versus Lys (10^{-6} M, not reinforced), and recorded stabile and discriminative responses after 20 sessions. After bilateral axotomy five groups of two goldfish each were anosmic and no reactions to any olfactory stimuli were recordable. Two weeks after axotomy a functional recovery was evident during application of AAs at 10^{-6} M concentration, as was described in earlier studies (van Rekowski and Zippel, 1993; Zippel, 1993). During application of the pheromone (10^{-8} M) versus tap water, however, no significant preference was recorded in the axotomized fish, whereas a preference was evident in the four intact control groups. A slight preference for 17α,20β-P was first recorded 7 weeks after axotomy. Nine weeks after operation the behavioral responses of axotomized goldfish to olfactory pheromone stimuli were similar to that of control groups. From the present experiments it is evident that, in contrast to a rapid (14 days) functional recovery for AAs and food odor, the response to pheromones and bile acids is drastically (65 days) delayed.

Supported by DFG (Zi 112/3-2).

References

van Rekowski and Zippel (1993) *Brain Res.*, **618**, 338–340.

van Rekowski *et al.* (1994) *Adv. Biosci.*, **93**, 491–496.

Zippel (1993) *J. Himforsch.*, **34**, 207–229.

Zippel *et al.* (1993) *J. Comp. Physiol. A*, **172**, 81–90.

144. Detection and quantification of plant damage using a biosensor based on insect antennae

Stefan Schütz, Bernhard Weißbecker and Hans E. Hummel

Biologischer und Biotechnischer Pflanzenschutz, Institut für Phytopathologie und Angewandte Zoologie, Ludwigstraße 21b, Justus Liebig Universität, D-35390 Gießen, Germany

The detection and quantification of plant damage can be useful in several fields of application. In the field of plant protection it is of crucial importance to know if damage thresholds are surpassed in order to apply timely plant protection measures. Moreover, in the field of vegetable and fruit storage it is important to know if the stored products are damaged or not. This approach will facilitate the classification of their quality and shelf life.

Colorado potato beetles are known for being attracted to damaged potato plants releasing damage-specific volatiles such as *Z*-3-hexen-1-ol. The sensitivity of the antenna of the Colorado potato beetle towards the components of this so-called 'green leaf odour' is known to be high, but correlation between plant damage intensity and quantities of released green leaf odour is not yet known.

Therefore, we examined the possibility of detecting and quantifying damage on potato plants *(Solanum tuberosum* L.) by using the antenna of the Colorado potato beetle *(Leptinotarsa decemlineata* Say) as a biosensor in an integrated electro-antennographic (EAG) system.

The quality of volatile compounds released upon mechanical damage of potato leaves was examined by GC–MS. Green leaf odour compounds were found to be released locally and immediately upon damage by the damaged leaf for ~1 h. Systemic response upon leaf damage occurred 24 h later and contained only smaller amounts of these volatiles.

In order to quantify the green leaf volatiles released by damaged potato plants, the plant emissions were examined by GC-FID and EAG. A quantitative calibration curve of the biosensor based on a modified EAG set-up and on *Z*-3-hexen-1-ol as calibration standard could be established.

A correlation of plant damage intensity with quantities of released green leaf odour and EAG response was found. Moreover, the influence of damage geometry on green leaf odour emission was investigated. The detailed time course of this damage-induced volatile emission could be obtained using the biosensor based on Colorado potato beetle antennae.