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ABSTRACTS

Twenty-first Annual Meeting of the Association for Chemoreception Sciences

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1. Givaudan-Roure Lecture: How hearing happens: transduction, tuning and transmission by hair cells of the internal ear

A.J. Hudspeth

Howard Hughes Medical Institute and Laboratory of Sensory Neuroscience, The Rockefeller University, 1230 York Ave, New York, NY10021, USA

Hearing is remarkable for both its normal efficacy and its vulnerability. Humans detect sound at frequencies as high as 20 kHz; their ears measure vibrations as small as 0.3nm. Ten percent of us experience significant hearing problems, however, primarily as a result of damage to hair cells, the sensory receptors of the internal ear.

This lecture will consider the mechanisms by which a hair cell carries out its three principal tasks: transduction of mechanical inputs into electrical responses, frequency tuning and synaptic transmission. Hearing commences when sound vibrations displace the hair bundle, a cluster of mechanically sensitive processes emanating from a hair cell's apical surface, thereby directly opening transduction channels. When a hair bundle is exposed to protracted stimulation, the cell adapts; myosin molecules reset the elastic gating springs that sense hair-bundle deflection. The hair bundle may also participate in mechanical amplification, a process that enhances the sensitivity of human hearing 100-fold.

The basolateral membrane surface of a hair cell contains ion channels of several varieties, including voltage-activated Ca^{2+} channels and Ca^{2+}-activated K^+ channels. These molecules cluster at presynaptic active zones, where the high local Ca^{2+} concentration promotes the rapid release of neurotransmitter. The interplay of Ca^{2+} and K^+ currents also underlies membrane oscillations that tune each hair cell to a specific frequency of mechanical stimulation. Tuning is effected by variations in the number of ion channels and in the kinetics of K^+-channel activation, the latter adjustment brought about by alternative splicing of the mRNA encoding the K^+ channels.

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Symposium: Adaptation in Vision and Olfaction

10. Partitioning light adaptation in salamander rod photoreceptors

E.N. Pugh Jr and S.S. Nikonov

Department of Psychology & Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104, USA

Vertebrate rod photoreceptors light adapt; that is, they adjust their dynamic range and sensitivity to the ambient level of illumination. The mechanisms underlying rod light adaptation can be conceptually divided into two distinct but overlapping categories: (1) those that serve to extend the dynamic range of response to steady illumination; (2) those that diminish flash sensitivity. This talk willexamine the biochemical mechanisms thought to underlie rodlight adaptation, and in particular the hypothesis that all light adaptation is effected by mechanisms that sense the decline in calcium that accompanies the response to illumination. It will be shown that the calcium-dependent processes best understood at the molecular level—the activation of guanylyl cyclase (GC) by calcium-sensing GCAPs and the CaCM-dependent shift in the $K_{1/2}$ of the cGMP-gated channels—only contribute to category (1), and in fact serve only to increase, rather than decrease, absolute flash sensitivity. What, then, underlies category (2) adaptation, the decline in flash sensitivity? One extant hypothesis is that (2) is effected by a calcium-dependent decrease in the rate with which photoactivated rhodopsin (R*) activates G-protein; evidence rejecting this hypothesis will be presented. Evidence and analysis will then be presented that two other processes effect most of the ~20-fold decline in normalized flash sensitivity: one is the steady-phosphodiesterase activity, which contributes a 'time constant' that declines almost 20-fold (in a calcium-independent manner) over the rod operating range; the second is a calcium-dependent change in the lifetime of an early transduction intermediate, most likely R*.

11. Psychophysical and behavioral characteristics of olfactory adaptation

P. Dalton

Monell Chemical Senses Center, Philadelphia, PA 19104, USA

Sensory adaptation allows organisms to reach behavioral equilibrium with the ambient environment and respond primarily to changes in stimulation. Given its functional significance, it is not surprising that adaptation in the olfactory system exhibits many of the same characteristics as adaptation in other sensory systems, including vision. Repeated or prolonged exposure to an odorant typically leads to stimulus-specific decreases in olfactory sensitivity to that odorant, but sensitivity recovers over time in the absence of further exposure. Psychophysical analysis shows that olfactory adaptation results in elevations in odor thresholds and in reduced responsiveness to suprathreshold stimulation. Further, the magnitude of the decrease and the time course of adaptation and recovery are dependent on the concentration of the odor and on the duration of exposure.

It is generally agreed that olfactory adaptation can occur at multiple levels in the olfactory system and can involve both

peripheral (receptor level) and more central (post-receptor) components. Evidence for peripheral and central involvement comes from studies showing that monorhinal stimulation results inadaptation in both the ipsilateral and contralateral nostril, although the degree of adaptation in the ipsilateral nostril is more profound and recovery is slower. Additional evidence for central involvement comes from studies that have found relatively small decreases in peripheral response following repeated stimulation despite substantial reductions in perceived intensity. In most studies of adaptation, however, the peripheral and central processes are rarely differentiated. Although relatively few in number, studies of the parametric features of olfactory adaptation in both vertebrate (e.g. rat) and invertebrate (e.g. *Drosophila, Caenorhabditis elegans*) animal models appear to replicate the findings in psychophysical studies of adult humans.

Despite the broad overall similarity of olfactory adaptation to adaptation in other sensory systems, olfactory adaptation exhibits some significant differences. Adaptation in olfaction has been shown to be very long-lasting in some cases and may be modulated by the contribution of preneural events and physicochemical properties of the odorant molecules that govern diffusion to receptor sites and post-receptor clearance.

12. Cellular and molecular basis of odorant adaptation

F. Zufall

Department of Anatomy and Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Adaptation is the process by which sensory neurons set the sensitivity and regulate the amplification (gain) of signal transduction at different stimulus intensities. This mechanism is essential to prevent saturation of the transduction machinery which would lead to a loss in discriminatory function. Here, I summarize recent progress utilizing acutely dissociated olfactory receptor neurons (ORNs) from salamander for the investigation of odor adaptation. Salamander ORNs are advantageous over other preparations because they enable the comparison of both electrophysiological responses to odors and Ca^{2+} responses in individual olfactory cilia, the site of odor recognition. The experimental results provide evidence to support several critical hypotheses: (1) adaptation in ORNs occurs by modulation of the primary odor transduction process that converts, within the olfactory cilia, odor stimuli intoelectrical signals. (2) Ca^{2+} entering the ORNs through transduction channels provides a key signal for triggering adaptation. The dynamics of ciliary Ca^{2+} changes determine the rate of recovery from odor adaptation. (3) Adaptation is, overall, arelatively complex process. There are multiple forms of odor adaptation in single ORNs and there is evidence that they depend on different sets of molecular mechanisms. Approaches to dissectthe mechanisms underlying these different forms of odor adaptation are summarized.

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13. Adaptation-induced changes in sensitivity in frog olfactory receptor cells

J. Reisert and H.R. Matthews

Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG, UK

In order to study adaptation in frog olfactory receptor cells (ORCs) odour-induced responses were recorded using the suction pipette technique. ORCs responded to a 1s odour test pulse of increasing concentration with graded increases in both suction current and action potential firing frequency, together with a reduction in the number of spikes fired in response to the stimulus (J. Reisert and H.R. Matthews, 1996, J.Physiol., 494: 14). Exposure of ORCs to a 4s adapting pre-pulse led to a reduction in the suction current response to a 1s test pulse in comparison with the response under control conditions, and to a progressive shift of the dose–response relationship to higher test-pulse concentrations as the pre-pulse concentration increased. Similarly, the dose–response relation for action potential firing was shifted to higher odour concentrations at low to intermediate pre-pulse concentrations, and the firing frequency saturated at a lower level. However, at high pre-pulse concentrations action potentials were no longer generated in response to the test pulse, probably reflecting the failure of the suction current to recover fully by the time of the test pulse, thereby depolarizing the cell sufficiently to inactivate the voltage-gated sodium conductance (D. Trotier, 1994, Semin. Cell Biol., 5: 47). The sensitivity of the suction current response decreased ~30-fold over a 10-fold increase in pre-pulse concentration. In comparison, sensitivity determined from the spiking responses fell even more steeply, the same 10-fold increase in pre-pulse concentration entirely abolishing the ability of the ORC to fire action potentials in response to the test pulse. These results illustrate the relatively poor ability of ORCs to adapt to a steady odour stimulus over this time scale.

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Transgenic Workshop

59. Transgenetic approaches to study taste: targeting a null mutation in α-gustducin and directing heterologous gene expression in taste cells with the gustducin promoter

G. Wong, L. Ruiz-Avila[1] and R.F. Margolskee[2]

Department of CNS/CV Discovery Research, Schering Plough Research Institute, Kenilworth, NJ, USA, [1]Almirallprodesfarma, Cardener 64, Barcelona, Spain and [2]Howard Hughes Medical Institute, Department of Physiology and Biophysics, The Mount Sinai School of Medicine, Box 1677, One Gustave L. Levy Place, New York, NY 10029, USA

Transgenic and knock-out mice have been useful for studying taste cell development and function. Applications of transgenetics in taste will be discussed, including the generation of α-gustducin deficient mice by targeted mutagenesis (Wong *et al.*, 1996, Nature, 381: 796). These data suggest that gustducin is a principal mediator of both bitter and sweet signal transduction.

Transgenetics has identified an 8.4 kb segment from the upstream region of the mouse α-gustducin gene which confers taste receptor cell (TRC) specific expression. Expression of a *lacZ* transgene driven by the 8.4 kb promoter and of endogenous

gustducin was coordinately lost after nerve section and simultaneously recovered upon reinnervation. Transgenic expression of rat α-gustducin restored responsiveness of gustducin null mice to both bitter and sweet compounds, demonstrating the functional utility of the gustducin promoter.

To gain insight into gustducin-mediated taste transduction pathways we have biochemically characterized a G352P-gustducin mutant *in vitro* and transgenically expressed it in TRCs. Compared with the WT transgene, the G352P mutant, which cannot be activated by seven transmembrane-helix receptors, did not restore responsiveness to bitter and sweet compounds. Rather, the mutant transgene acted as a dominant negative to further reduce the residual taste responses of the null mice.

Thus transgenetics, coupled to behavioral and electrophysiological analysis, can be highly informative to the study of the development of taste cells and the mechanisms underlying taste transduction.

60. Odorant receptors have dual roles

P. Feinstein, C. Zheng, A. Vassalli, T.C. Bozza and P. Mombaerts

The Rockefeller University, 1230 York Ave, New York, NY 10021, USA. e-mail: feinstp@rockvax.rockefeller.edu

In 1991 Buck and Axel identified a large family of genes that are believed to encode odorant receptors (ORs). Olfactory sensory neurons that express a particular OR project their axons to two glomeruli in each olfactory bulb, out of a possible choice of 1800. The location of these glomeruli is stereotyped, suggesting that thedevelopmental mechanisms underlying their formation are hardwired. Experiments involving genetic manipulation of OR genes have suggested that the OR itself controls this process that guides the sensory axons to their glomerular target.

We have continued to examine the role of ORs in axonal guidance. Through genetic manipulation of ORs in mice we have established a paradigm with two highly homologous ORs, M71 and M72, whose corresponding neuronal populations project axons to different glomerular targets. We have created multiple alleles of M71 and M72 that are marked with either GFP or lacZ. We have substituted the coding sequence of M71 with that of M72 (M72 → M71) and show that these neurons project their axons to the endogenous M72 glomeruli. This experiment shows for the first time that the OR itself is capable, at least in some instances, of instructing axons of OSNs to project to precise glomerular targets. We are in the process of identifying odorous ligands for the M71 and M72 ORs, in order to formally demonstrate that these gene products are receptors for odorants.

Our findings provide the best evidence to date that ORs have dual roles: as receptors for odorants and as instructive guidance molecules.

Presidential Symposium

168. Olfactory glomeruli: intrinsic organization

C.A. Greer

Department of Neurosurgery and Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06520-8039, USA

The olfactory bulb (OB) glomerulus is the site of primary afferent olfactory receptor cell (ORC) axon convergence. Data from several labs suggest that the ORC axons converging on a single glomerulus homogeneously express the same odor receptor (e.g. Mombaerts *et al.*, 1996, Cell, 87: 675–686). Upon reaching the glomerulus, ORC axons arborize and establish primary afferent synapses with the dendrites of projection- and interneurons. The glomerular neuropil is further defined by the presence of modulatory reciprocal dendrodendritic synaptic circuits. Recent evidence, however, suggests that the intraglomerular neuropil may be heterogeneously organized and delineated into well-defined subcompartments (Kasowski *et al.*, 1999, J. Comp. Neurol., in press). For example, synaptic vesicle associated proteins found predominately in either ORC axon terminals or OB neuron dendrites have distinct and largely nonoverlapping subglomerular distributions. Similarly, electron microscopic analyses of synapse distribution in the glomerulus suggest that local circuit dendrodendritic synapses, found predominately in dendritic bundles, are isolated from primary afferent ORC synapses by glial processes. Complementary reports from Kosaka *et al.* (1997, Neurosci. Res., 23: 73–88) and Chao *et al.* (1997, J. Comp. Neurol., 388: 191–210) also provide evidence of a heterogeneous distribution of dendritic and glial processes in the glomerulus. Further data bearing uponthe heterogeneity of intraglomerular organization will be presented. The implications of mammalian subglomerular compartments for odor processing and the turnover of ORC axons will be discussed.

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169. Targeting olfaction

P. Mombaerts

The Rockefeller University, New York, NY 10021-6399, USA

Understanding the logic of the processing of information in the main and accessory olfactory systems requires knowledge about the functional anatomy of the neuronal connections. A first step is to describe the topography of projections of the sensory neurons to the olfactory bulb.We have taken a genetic approach to visualize wiring diagrams in the olfactory system. We have created a series of gene-targeted mice that carry mutations in odorant receptor (OR) or vomeronasal (VR) receptor genes. The design of the mutations allows for co-expression of axonal markers, such as *taulacZ* or *tauGFP*, with the OR or VR. In these mice, neurons that express a given OR or VR can be imaged along their entire length by virtue of their expression of either *taulacZ* or *tauGFP*. Additional mutations have the objective to determine the role of the OR or VR in the guidance process that underlies the development of the projection pattern. Olfactory sensory neurons that express a given OR project in each main olfactory bulb to two large glomeruli that reside at fixed locations. By contrast, vomeronasal sensory neurons that express a given VR project in each accessory olfactory bulb to multiple small glomeruli that occupy variable positions, even when the bulbs of the same individual are compared. In both cases, the OR and the VR are determinants of the guidance machinery because changes in the coding region alter the pattern of projections. The similarities and differences of the principles of connectivity between the main and accessory olfactory systems will be discussed.

170. Afferent influences on glomerular development

P.C. Brunjes

Department of Psychology, University of Virginia, Charlottesville, VA22903, USA

It has been well documented that sensory systems are tuned by the type and amount of stimulation experienced during early life. Indeed, it is easily argued that studies examining the role of afferent input (e.g. of monocular deprivation) were pivotal in our understanding of the development, organization and function of sensory pathways and, in turn, that these studies have provided the framework for our general view of brain maturation. In recent years it has been recognized that many early changes can be described by Hebbian rules: that is, that convergent stimulation fortifies (or potentiates) cells or synapses and that asynchronous inputs have a destabilizing (or depressing) influence. Furthermore, a potential cellular mechanism that could be responsible for the changes has been uncovered in coincidence-detecting, NMDA-type receptors. These postsynaptic receptors are found in most regions of the brain, but some of the highest concentrations are found in the olfactory bulb. The glomerulus is important not only because it is the point at which olfactory input is parsed into meaningful data streams, but also because it is the first site of synaptic processing. The intricate synaptic conversations that occur in the glomerular neuropil are thus the first opportunity in which Hebbian strategies can be employed to tune olfactory system function. One prediction is, therefore, that activity is important in determining the ultimate structure and function of glomeruli. This presentation will examine what is known about the role of afferent influences in glomerular development.

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171. Integrative properties of olfactory bulb neurons

M.T. Shipley

Department of Anatomy & Neurobiology, Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

The glomerular ensemble is a map of olfactory receptor activity rather than odors. Different odors are represented in neural space by different patterns of glomerular activity. We are investigating how the intrinsic and circuit properties of olfactory bulb neurons integrate patterns of glomerular activity.

Juxtaglomerular (JG) neurons are morphologically and neurochemically heterogeneous; they also have different intrinsic properties and differential responses to synaptic input. GABA and dopamine released from JG neurons attenuate transmission from olfactory nerve (ON) terminals. This may limit the magnitude or duration of transmission at the first synapse in the olfactory network. We are investigating how JG cells shape glomerular as well as interglomerular activity.

Mitral cells (MCs) have two novel integrative mechanisms: (1) bistability and (2) long-lasting depolarizations (LLDs). MCs alternate between two membrane potentials—bistability. In the 'up-state' MCs are more responsive to ON input than in the 'down-state'. Transition from the down- to the up-state is prolonged by hyperpolarizing events. Thus bistability may temporally amplify lateral inhibition. Bistability appears to be an intrinsic property of MCs. LLDs are generated by intraglomerular circuitry. LLDs may function to amplify and integrate the transfer of ON inputs to mitral cells. Together, bistability and LLDs regulate the sensitivity of MCs to sensory input and gate MC output. Preliminary studies indicate that tufted cells lack bistability.

The integrative properties of bulb neurons are unexpectedly diverse. The challenge is to determine how these cellular-network properties compute odor space from patterns of glomerular activity.

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172. Functional meaning of glomerular units and their spatial arrangement in the mammalian olfactory bulb

K. Mori, H. Nagao, H. Kashiwadani and Y. Yoshihara[1]

Laboratory for Neuronal Recognition Molecules and [1]Laboratory forNeurobiology of Synapse, Brain Science Institute RIKEN, Wako, Saitama 351-0198, Japan

The mammalian glomerulus can be viewed as a functional unit assembling information derived from olfactory sensory neurons that express the same odorant receptor and transmitting the information to mitral/tufted cells that innervate the glomerulus. This view suggests the working hypothesis that individual glomerulus is dedicated to handle information derived from a single odorant receptor. If a single odorant receptor can be activated by a range of odor molecules having similar molecular structure, the above view is in agreement with the results of our previous physiological study; in the rabbit olfactory bulb, single mitral/tufted cells innervating a single glomerulus showed excitatory spike responses to a range of odor molecules having similar molecular structure (Imamura *et al.*, 1992, J. Neurophysiol., 68: 1986; Katoh *et al.* 1993, J. Neurophysiol., 70: 2161). In this symposium, we describe how these glomerular units are spatially arranged in the olfactory bulb, reporting the physiological mappings of single unit responses to odor stimulation and immunohistochemical mappings of specific subsets of glomeruli. We discuss also the functional interaction via local interneurons between neighboring glomerular units, reporting the odor-induced lateral inhibition and synchronized oscillatory discharges of mitral/tufted cells associated with different glomeruli. The synchronized oscillatory discharges might function as a part of neuronal mechanisms for temporal combination of signals originated from different odorant receptors.

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SYMPOSIUM: Short Term Impact of Environmental Chemicals

226. The role of dust in concentrating and transporting odors from swine buildings

R.W. Bottcher

Department of Biological and Agricultural Engineering, North Carolina State University, NC, USA

Intensive swine production can cause odorous emissions from several sources, and airflow from the animal buildings can be a predominant source. Ventilation is provided to remove the heat and moisture produced by the animals and ensure a productive environment; the airflow rates are much greater than in most other types of housing. Numerous compounds contribute to the unique

odors from swine facilities, including fatty acids, amines, aromatics and sulfur compounds. Dust particles concentrate and adsorb odorants in swine facilities. Odorants which adsorb to the dust particles, such as mercaptans and phenols, can exist in much higher concentrations in the dust particles than in equivalent volumes of air. Thus, inhalation of odorous dust and deposition of the dust particles on the olfactory organ are believed to be partly responsible for odor perception by some swine farm neighbors. Since dust particles disperse differently than vapors, accurate prediction of odor transport and dispersion downwind of swine farms may require models of dust dispersion and correlation between dust and odorant levels. A further complication is that many current techniques of odor measurement, such as dynamic olfactometry and electronic noses, require filtering of dust before odors are sensed by humans or electronic sensors. If the dust-borne odorants are responsible for odor perceptions, methods which do not adequately account for the dust-borne odorant contributions may be inaccurate and prevent good evaluations of odor control methods. Hence, odor control research must involve measurement of odors and odorants from swine buildings in both the vapor and dust phases.

227. Odor annoyance: new approaches for assessment

B. Danuser

Institut für Hygiene und Arbeitsphysiologie, Swiss Federal Institute of Technology CH-8092 Zurich, Switzerland

Very low levels of environmental chemicals can be detected by the nose and categorized as pleasant or unpleasant. Especially unpleasant odors possess an arousing, unpleasant quality. Personal history, environmental context, psychological status and even stimulus administration may influence responses to an odor. To understand odor annoyance we have to understand how these influences affect subjective process(es). Odors are affective stimuli because they are experienced primarily in terms of their pleasantness or unpleasantness. Emotions manifest themselves in at least three components: verbal expressions, motoric expressive behavior and vegetative changes controlled by the autonomous nervous system.

Aversive affective stimuli produce increased startle reflex responses in humans and animals. Startle modulation is associated with changes in pleasantness–unpleasantness and might also depend on arousal, the other dimension of affect. The startle reflex is well suited to assess the motoric expressive behavior caused by the pleasantness–unpleasantness of a stimulus.

Alterations in mental activity affect breathing patterns. An increase in the level of arousal *per se* leads to a relatively stereotyped increase in the time components. In contrast, noxious visual and chemical irritant stimulation results in a modification of both time and volume components. The most striking feature of respiration, however, might be its close connection to subjective experience. Breathing pattern is therefore a unique tool to investigate the relationship between vegetative and subjective processes.

These two tools, together with appropriate verbal reports, should help us to investigate the affects induced by odor stimuli and may even elucidate some aspect of odor-related diseases.

228. Odor-associated health complaints: competing explanatory models

D.J. Shusterman

University of California, San Francisco, Campus Box 0843, San Francisco, CA 94143, USA

For many local air quality management districts, the most commonly received complaints concern environmental odor pollution. In addition to odor annoyance, complainants often report such acute, self-limited health symptoms as headache, nausea and eye, nose and throat irritation. When compounds having similar odor and irritant thresholds are involved, odor may simply be acting as a marker of a toxicologically significant exposure. On the other hand, when potent odorants (e.g. reduced sulfur gases) are involved, conventional toxicologic paradigms are frequently inadequate to explain the observed pattern of symptom reporting. Alternative (non-toxicologic) explanations for odor-associated health effects include potential innate odor aversions, exacerbation of pre-existing conditions (odor-triggered asthma, odor-related exacerbation of the nausea of pregnancy, pre-existing somatoform disorders), conditioning phenomena, odor-triggered, stress-related illness, possible pheromonal phenomena and epidemiologic artifact (recall/reporting bias). Classification of odor-related complaints as 'health effects' or 'non-health effects' involves implicit value judgements on the part of the observer.

229. Acceptability of perceived odor and irritation: a tool for measuring human discomfort and ventilation requirements

P. Wargocki

International Centre for Indoor Environment and Energy, Technical University of Denmark, DK-2800 Lyngby, Denmark

Occupants of indoor environments decide whether the air quality indoors is acceptable or not. For over a decade now, the acceptability has consequently been used as a measure of the perceived air quality. It is surmised that by assessing whether perceived air quality is acceptable or not, people, during sensory evaluations, assess both the perceived odor and the sensory irritation produced by air pollutants. This assumption is to some extent confirmed by a strong correlation between the perceived odor intensity and the acceptability ratings. Dichotomous and continuous acceptability scales are used for measuring the perceived air quality. To reach a sufficient resolution, the former requires large panels of subjects. The latter is characterized by a large between-subject variation. No drift of the acceptability votes with time has been observed. With transformation models, assessments of acceptability can be used to estimate the percentage of persons dissatisfied with the air quality. Both the acceptability ratings and the percentage of persons dissatisfied can furthermore be used to estimate ventilation rates for the prescribed perceived air quality indoors. Untrained impartial human subjects are used to assess whether perceived air quality is acceptable or not. Perceived air quality can also be assessed by trained subjects, comparing against a well-established reference exposure of 2-propanone. Sensory assessments of untrained and trained subjects, however, are not alike. Transfer models have thus been established to compare them. Advantages and disadvantages of

using the acceptability to rate the annoyance produced by odors and irritants indoors are discussed.

Poster and Slide Presentations

2. A novel family of seven-transmembrane proteins: candidate olfactory receptors in *Drosophila*

C. Warr, P. Clyne, M. Freeman, D. Lessing, J. Kim[1] and J.R. Carlson

Department of Molecular, Cellular and Developmental Biology and [1]Department Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, USA

Although insects have proven to be valuable models for exploring the function, organization and development of the olfactory system, the receptor molecules which interact with odorants have not been identified in any insect. We have developed a novel search algorithm, used it to search the *Drosophila* genomic sequence database and identified a large multigene family encoding putative G-protein-coupled odorant receptors. The family may contain on the order of 100 genes, and their sequences are highly divergent from those of previously identified genes.

Nearly all the DOR (*Drosophila* olfactory receptor) genes areexpressed in one or both of the olfactory organs: the third antennal segment and the maxillary palp. For a number of genes we have shown that expression is restricted to a subset of olfactory receptor neurons. Different genes initiate expression at different times during the development of the adult antenna, leaving open the possibility of a role for *Drosophila* olfactory receptors in axon guidance and glomerulus formation. We have also found evidence that different DOR genes are expressed at different levels within cells. Expression of a subset of these candidate receptor genes depends on the POU domain transcription factor Acj6, suggesting that the odor-specificity of a subset of olfactory receptor neurons is governed at least in part by the action of Acj6 on receptor gene expression.

3. High-resolution Ca^{2+} imaging of olfactory epithelium and vomeronasal organ in a novel mouse slice preparation

T. Leinders-Zufall, A.C. Puche, M.T. Shipley and F. Zufall

Department of Anatomy and Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

To overcome the critical gap between physiological and molecular biological studies in olfaction, we have developed methods by which stimulus-induced activity in individual mouse olfactory receptor neurons (ORNs) and neurons of the vomeronasal organ (VNO) can be monitored both spatially and temporally. Our approach is based on a combination of confocal microscopy-based imaging of Ca^{2+} signals and the use of tissue slices in which the epithelial cytoarchitecture remains preserved. This provides the opportunity to combine the functional analysis of odor-induced activity with the analysis of odor receptor gene expression *in situ*. First, we tested whether VNO neurons utilize similar signal transduction pathways for stimulus detection as ORNs by analyzing the Ca^{2+} signals in response to direct activation of the cyclic nucleotide second messenger system. ORNs showed robust Ca^{2+} elevations in response to application of 8-Br-cGMP or IBMX but such Ca^{2+} signals were absent in the VNO. When we assessed the viability of each preparation by brief pulses of KCl, we found that this test generated depolarization-induced Ca^{2+} transients in both ORNs and VNO neurons. Thuse, the machinery for signal transduction is fundamentally different between ORNs and VNO neurons. A second important difference between the two types of chemosensory tissue was that VNO neurons exhibited prominent spontaneous Ca^{2+} oscillations whereas ORNs did not. Studies are currently underway to investigate the molecular basis underlying these VNO Ca^{2+} waves, their dependence on the developmental state of the tissue and whether they can be modulated by natural stimuli.

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4. Electrophysiological characterization of odor responses of rat and mouse olfactory receptor neurons in isolated epithelial patches

M. Ma, W.R. Chen and G.M. Shepherd

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

To understand the coding mechanisms underlying olfactory discrimination and recognition, it is necessary to characterize odor response properties of different populations of olfactory receptor neurons (ORNs) as well as their distributions in epithelium and projections to the olfactory bulb. In contrast with rapid progress in molecular biology, there is little physiological data from rodent ORNs. We have developed a new preparation of isolated olfactory epithelial patches from both rat and mouse that permits monitoring odor responses by patch-clamping directly on the ORN dendritic knobs, a subcellular site very close to the locus of olfactory signal transduction. Our results show that rat and mouse ORNs had similar intrinsic membrane properties. Most cells fired spontaneously at a low frequency (f) and about one half fired repetitively in response to current (I) injection with a linear f/I relation. Similar to amphibian ORNs, odor responses and short-term adaptation in rodent ORNs were mediated mainly by the cAMP pathway. However, the dose–response curves obtained here had much lower Hill coefficients than those of dissociated amphibian ORNs and the odor response range of each ORN appeared to be narrower in rodents than in amphibians. Compared with dissociated rat ORNs, a higher percentage of ORNs in the isolated patch responded to odors and IBMX. The results suggest that the new preparation offers the advantage of approximately *in vivo* physiological conditions while furnishing an opportunity to map single neuron responses in the epithelium in a spatially defined manner.

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5. Targeted disruption of the OCNC-2 gene reveals a restricted pattern of expression

S.D. Munger and R.R. Reed

Howard Hughes Medical Institute, Department Molecular Biology and Genetics, Johns Hopkins Medical Institution, 725 N. Wolfe St, PCTB 818, Baltimore, MD 21205, USA

Three subunits of cyclic nucleotide-gated (CNG) channels have

been detected in olfactory epithelium: OCNC-1, OCNC-2 and a splice variant of the rod β-subunit. The native channel in olfactory receptor neurons (ORNs) may be composed of two or all three of these subunits. Our laboratory has been interested in examining the roles of the cNG channel subunits in activity-dependent developmental processes as well as ORN transduction. OCNC-1 has been implicated in both of these functions. We have created a line of mice containing a targeted disruption of the *OCNC-2* gene in which exons encoding the OCNC-2 protein, from the S4 transmembrane domain to the carboxyl tail, were replaced with a construct containing an IRES-tau-lacZ reporter. OCNC-2-driven reporter expression is observed in a subset of the ORNs of neonatal (P1) heterozygotes. Expression is restricted to a subset of cells in the dorsal recesses, dorsal septum and the dorsal IIa turbinate, reminiscent of the rostral aspects of receptor expression zone I. Labeled axons converge on a subset of glomeruli in the main olfactory bulb. Vomeronasal organ neurons are unlabeled. In adult mice, labeled ORNs are more widely distributed in the epithelium, though expression remains heterogeneous. The ability to tag OCNC-2-expressing cells and the availability of OCNC-2-deficient mice resulting from these experiments should permit the analysis of OCNC-2 function in ORNs and could potentially reveal transduction pathway variations reflecting the differential expression of CNG channel subunits.

Supported by the Howard Hughes Medical Institute.

6. Odor transduction in normal mice and mice deficient in subunit 1 of the olfactory CNG channel

R.J. Delay and D. Restrepo

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

A variety of different transduction pathways have been shown tomediate odor responses in olfactory receptor neurons (ORNs) depending on the species. Of these pathways, the best characterized in mammals is that involving up-regulation of cAMP leading to the opening of a cyclic nucleotide-gated (CNG) channelfollowed by depolarization. We have used loose-patch and perforated-patch configurations of the patch-clamp technique to examine the odor responses of isolated ORNs. Application of 50 μM Mix A (hedione, geraniol, PEA, citralva, citronellal, eugenol and menthone) often elicited a hyperpolarizing response as measured with loose-patch recordings. Similar responses were observed with perforated-patch recordings. Interestingly, application of IBMX, a cAMP phosphodiesterase inhibitor that stops breakdown of cAMP, can elicit either a depolarizing or hyperpolarizing current. In addition, stimulation of ORNs isolated frommice deficient for subunit 1 of the olfactory CNG channel (Brunet *et al.*, 1996, Neuron, 17: 681–693) with a variety of odor mixtures has elicited only hyperpolarizing responses to date. These results indicate that odors can either hyperpolarize or depolarize mouse ORNs, and suggest that multiple pathways are involved in olfactory transduction in this species.

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7. Functional cloning and reconstitution of an odorant receptor in single olfactory neurons

K. Touhara [1,2], S. Shintaro[3], K. Inaki[2], J. Hirono[4], T. Sato[4], H. Sakano[3] and H. Tatsuya[2]

[1]Department of Integrated Biosciences, Graduate School of Frontier Sciences, [2]Department of Neurochemistry, Faculty of Medicine, [3]Department of Biochemistry and Biophysics, Faculty of Science, University of Tokyo, Tokyo 113 and [4]Life Electronics Research Center, Electrotechnical Laboratory, Amagaski 661, Japan

The olfactory system is remarkable in its capacity to discriminate a wide range of odorants via a series of transduction events initiated in olfactory receptor neurons. Each olfactory neuron is expected to express a single odorant receptor gene that belongs to the G-protein-coupled receptor family. The ligand–receptor interaction, however, has not been clearly characterized. This study is the first to demonstrate the functional identification and reconstitution of olfactory receptor(s) for specific odorant(s) from single olfactory neurons by a combination of Ca^{2+}-imaging and RT-PCR analysis. First, a candidate odorant receptor was cloned from a single tissue-printed olfactory neuron that displayed odorant-induced Ca^{2+}-increase. Next, recombinant adenovirus-mediated expression of the isolated receptor gene was established in the olfactory epithelium using green fluorescent protein as a marker. The infected neurons elicited external Ca^{2+}-entry when exposed to odorant that was originally utilized to identify the receptor gene. Experiments performed to determine ligand specificity revealed that the odorant receptor recognized specific structural motifs within odorant molecules. The odorant receptor-mediated signal transduction appears to be reconstituted by this two-step approach: (1) the receptor screening for a given odorant(s) from single neurons and (2) the functional expression of the receptor via recombinant adenovirus. The present functional cloning approach will enable us to examine ligand specificity of an odorant receptor and to study receptor specificity and diversity for a particular odorant of interest.

8. A central role for the Gβ subunit of heterotrimeric G-proteins in regulating lobster olfactory signalling

T.S. McClintock, F. Xu, B. Hollins and S.C. Bose

University of Kentucky College of Medicine, Lexington, KY 40536-0298, USA

We have isolated olfactory organ cDNA clones for a Gβ subunit ofheterotrimeric G-protein, a β isoform of phospholipase C (lobPLC-β), and a G-protein-coupled receptor kinase (lobGRK1). Immunoreactivity for all three proteins was present in the outer dendritic segments of the olfactory receptor neurons, the site ofolfactory transduction. This predicts that these proteins participate in olfactory signalling. Application of odorants or GTP-γ-S to homogenates of olfactory aesthetasc hairs caused the association of lobPLC-β with Gβ and with Gαq, and of lobGRK1 with Gβ. These associations are known to be direct interactions that result in activation of PLC-βs and of close homologs of lobGRK1. These results are consistent with the conclusions that lobPLC-β is the central enzyme in the primary olfactory transduction pathway of lobster ORNs, and that lobGRK1

mediates desensitization of lobster odor responses, hypotheses we plan to test further.

Gβ, Gαq, lobPLC-β and lobGRK1 were also expressed by neurons in the brain, most abundantly in neuropil. Stimulating brain homogenates with a mixture of neurotransmitters that are present in the olfactory lobe neuropil also caused the association of lobPLC-β with Gβ and with Gαq, as well as the association of lobGRK1 with Gβ. These results suggest that Gβ, Gαq, lobPLC-β and lobGRK1 function in neurotransmission in the brain. In lobsters, Gβg appears to participate in activation and desensitization of signalling pathways that both encode and process olfactory information.

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9. Cloning of TRP2, a candidate transduction channel for mammalian pheromone reception

E.R. Liman, C. Dulac[1] and D.P. Corey

Department of Neurobiology and Howard Hughes Medical Institute, Massachusetts General Hospital, Boston, MA 02114 and [1]Department of Molecular and Cellular Biology and Howard Hughes Medical Institute, Harvard University, Cambridge, MA 02138, USA

Our previous work has demonstrated that sensory transduction in the vomeronasal organ (VNO) is unrelated to that in the vertebrate olfactory system and is unlikely to be mediated by cyclic-nucleotide-gated channels. We hypothesized that sensory transduction in the VNO might instead involve an ion channel of the TRP (transient receptor potential) family, members of which mediate cyclic-nucleotide-independent sensory responses in *Drosophila melanogaster* and *Caenorhabditis elegans* and play unknown functions in mammals. Using degenerate RT-PCR we detected expression of a TRP channel in the VNO and obtained a full-length clone (TRP2) from a rat VNO cDNA library. TRP2 encodes a protein of 885 amino acids with 10–30% amino acid identity to previously identified TRP channels. Northern blot and *in situ* hybridization experiments showed that TRP2 is exclusively expressed in VNO sensory neurons. To determine the subcellular localization of TRP2, we generated a polyclonal against the 70 terminal amino acids of TRP2. On Western blots anti-TRP2 antibody recognized a protein of ~90 kDa in VNO but not in other tissues. Immunolabeling of sections of adult rat and mouse VNO showed a striking localization of the TRP2 protein at the luminal surface of the sensory epithelium. Immunolabeling of dissociated rat VNO neurons showed that the TRP2 protein is highly restricted to sensory microvilli, consistent with a role in sensory transduction. Interestingly, the human ortholog of TRP2 contains multiple premature stop codons and is a likely pseudogene, consistent with the notion that the human VNO is vestigial.

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14. Chemosignalling in rat: unique chemical structures and behavioral responses

M.V. Novotny, W. Ma, L. Zidek and J. Alberts[1]

Department of Chemistry, Indiana University, Bloomington, IN 47405 and [1]Department of Psychology, Indiana University, Bloomington, IN 47405, USA

Normal male rats excrete into their urine a series of volatile carbonyl compounds which are strongly androgen-dependent. They are either linear or 3-ethyl-substituted structures that bind to the urinary α2m-globulins, which is analogous to the way the mouse pheromones bind to their major urinary proteins. Recently, we found that normal male rats exhibit strong aversive reaction to the territory marked with an aqueous solution of the synthetic mixture of these carbonyl compounds. Additionally, their feeding site preference was significantly altered by the odor stimulus placed near their food dishes. Female rats feature the urinary volatile profiles that are distinctly different from males. Investigations with the estrogenized rats revealed the enhanced levels of several phenolic metabolites, two thioesters, and α- and β-farnesene in their voided urine. Some of these volatiles appear to originate in the clitoral gland. Male rats show preference for the farnesenes compared with other olfactory stimuli. Sensitivity for the farnesenes seems enhanced by the sexual experience of males.

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15. Repellent effects of capsaicin, denatonium, and vexar plastic mesh plant protectors on gnawing behavior of wild Norway rats

S.A. Shumake, R.T. Sterner and S.E. Gaddis

National Wildlife Research Center, 4101 LaPorte Ave, Fort Collins, CO80521, USA

Wild Norway rats can cause a great deal of damage to power and communications cables both within and outside of structures. A previous study with northern pocket gophers showed that a plastic sleeve covering containing chemical repellents and a polybutene carrier could be used to improve mucosal, chemosensory contact by gnawing rodents. Laboratory observations using time-lapse video recorders have indicated that rats will gnaw half-inch communications cables during 0.4–1.4 min gnawing bouts up to 170 times over nightly observation periods, completely severing the cables. In a recent study, 7-day cable exposure tests were conducted on groups of wild Norway rats with capsaicin and denatonium benzoate at 2.0% w/w concentrations within a plastic sleeve covering the cable samples; separate groups were also exposed for 7 days to cables that were treated with either a placebo carrier (polybutene) or a plastic mesh physical barrier material (Vexar). Measures of gnawing were cable weight loss, penetration depth gnawed, width and volume loss. Compared to placebo-treated cables, mean gnawing damage measures for capsaicin-, denatonium- and Vexar-treated cables were reduced by 60.0–79.9, 45.0–60.0 and 31.9–35.7% respectively. These levels of repellency, although substantial and reliable, could be enhanced by increasing the concentrations of the agents to obtain and maintain a higher, practical level of cable damage protection with this rodent species.

16. The effects of chorda tympani transection and regeneration on NaCl detection threshold

S.L. Kopka and A.C. Spector

University of Florida, Department of Psychology, Gainesville, FL 32611, USA

We have previously shown that chorda tympani (CT) regeneration in rats is the critical factor underlying recovery of function in a NaCl versus KCl discrimination task, after performance is impaired by chorda tympani transection (CTX). The present experiment focused on whether sensitivity to NaCl would return to normal, concomitant with CT regeneration. We determined NaCl detection threshold using a two-lever operant conditioning

paradigm in which rats were trained to discriminate between water and continually decreasing concentrations of NaCl. The geometric mean presurgical threshold for all animals was 0.008 M NaCl. Postsurgical thresholds in rats tested starting 62 days after CTX in which nerve regeneration was allowed (n = 6) and in two sham-operated groups which were tested beginning either 7 (n = 5) or 62 (n = 6) days after surgery returned virtually to their presurgical values. After amiloride treatment, however, these postsurgical thresholds increased by ~0.9 $\log_{10}$ units. Rats tested starting 62 days after CTX in which nerve regeneration was prevented (n = 6) and those tested beginning 7 days after CTX (n = 4) exhibited strikingly similar threshold increases of 1.12 and 1.33 $\log_{10}$ units respectively, indicating that compensatory phenomena following CTX cannot alone restore presurgical sensitivity. Amiloride treatment did not increase thresholds further in these two groups, suggesting that amiloride-sensitive receptors innervated by other gustatory nerves do not contribute significantly to NaCl detection threshold when CT input is absent. Taken with our prior results, gustatory sensibility to NaCl appears to completely recover upon CT regeneration.

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17. Effect of aging on bitter taste response and gene expression in rats

E.A. Hoy, T. Huque[1], C.N. Stewart, J.G. Brand[1,2,3] and S.A. Mackler[2,3]

Department Psychology, Franklin & Marshall College, Lancaster, PA 17604, [1]Monell Chemical Senses Center, Philadelphia, PA 19104, [2]University of Pennsylvania, Philadelphia, PA 19104 and [3]Veterans Affairs Medical Center, Philadelphia, PA 19104, USA

The effect of aging on bitter taste response was studied in rats of the Fisher 344 (NIA) strain. Younger rats (15) were 2 months old while older rats (15) were 22 months. The study lasted 3 months, 2 weeks after which animals were euthanized and taste and non-taste tissue removed from each animal and pooled according to age and tissue type. Preference testing (24 h) was conducted over 4days using quinine-HCl (0.1 mM), caffeine (0.1 mM), sucrose octaacetate (0.1 and 0.05 mM), tetrahydroisohumulone (IH) (0.1 mM) and benzyltriethyl ammonium chloride (1 mM) versus distilled water. Older rats drank significantly more caffeine (P < 0.02) but drank significantly less IH (P < 0.03). Variance in the data was large. To investigate age-related differences in gene expression between taste and non-taste tissues and between younger and older rats, differential display PCR (DDPCR) was used. Total RNA was extracted from each sample and diluted to 0.1 mg/ml RNA. DDPCR was performed using eight arbitrary primers, each in combination with three anchored primers in the presence of [^{33}P]dATP. Aliquots were analyzed by PAGE followed by autoradiography, yielding data on at least 1200 genes. Using older rats, 4–6 bands were seen in taste that were absent in non-taste tissue. Upon comparing taste tissue from younger versus older rats, at least 6–8 bands were present in older rats that were absent in younger rats. Identification of differentially expressed bands is in progress. Results indicate differences in gene expression and differences in responses to bitter compounds.

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18. Genetic variation in the syrian hamster: influence on intake of taste solutions

D.A. Blizard and M.E. Frank[1]

Center for Developmental Health Genetics, Pennsylvania State University, University Park, PA 16802 and [1]Department of BioStructure & Function, School of Dental Medicine, University of Connecticut Health Center, Farmington CT 06030, USA

Little is known about genetic variation that affects taste in Syrian hamsters (*Mesocricetus auratus*), an important experimental species for gustatory studies. Short-term, fluid intake was compared in outbred LVG hamsters (n = 20) from Charles River Labs and four closely related inbred strains: CHF-146, -147, -148 and H4 (n = 6 per strain) developed by Canadian Hybrid Farms (CHF). The intake test used takes advantage of hamster circadian rhythms and does not require water deprivation. Six-hour intakes of distilled water, 1 and 3 mM Na saccharin, 1 and 2 mM quinine-HCl, 100 mM sucrose, 50 mM D-phenylalanine, 100 mM maltose, 50 mM L-phenylalanine, 10 mM Na cyclamate and 1 mM caffeine were measured in all animals. As a group, CHF inbred strains exhibited increased intake of D-Phe, sucrose, 1 and 3 mM saccharin, maltose and L-Phe relative to water intake, but only showed a modest decrease in quinine intake in response to the 2 mM concentration. Outbred hamsters from Charles River exhibited increases in intake, relative to water, of sucrose and 3 mM saccharin solutions, and a large decrement in intake of both concentrations of quinine. The differences in response to quinine may reflect polymorphisms similar to those known to exist among inbred mouse strains. Although genetic origins of laboratory hamsters are narrow, the present observations suggest that variation present within existing stocks contains polymophisms relevant to gustatory processes. This variation may serve as a useful model for understanding the neurobiology of taste. Supported by UCHC.

19. NaCl preference is abolished by null mutation of the *isk* gene

R.B. Puchalski[1,2], E. Kelly[1], A.A. Bachmanov[1], M.G. Tordoff[1], S.P. Brazier[1], J. Kuang[1], I. Arrighi[3] and J. Barhanin[3]

[1]Monell Chemical Senses Center, Philadelphia, PA 19104, [2]Department of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104, USA and [3]Institut de Pharmacologie Moleculaire et Cellulaire, CNRS-UPR 411, Valbonne, France

Increased salt intake is a risk factor that promotes the development of hypertension. Our working hypothesis is that potassium channels are involved in the development or maintenance of hypertension. Potassium channels that affect salt intake or metabolism are candidates for study. To understand the role of one potassium channel, KCNQ1 (KvLQT1), in salt appetite, we measured consumption patterns of salt solutions in mice deficient for KCNQ1 channel currents. The mice have a null mutation of the *isk* gene, which encodes a regulator that potentiates KCNQ1 currents by coassembling with KCNQ1 channel proteins to form the native I_{Ks} cardiac potassium current. The channel is expressed in submandibular salivary glands, kidney, duodenum, uterus, T lymphocytes, heart, inner ear, retinal ganglion neurons and corneal epithelial cells. In two-bottle preference tests, *isk* –/– mice exhibited a preference of 50%, drinking equal amounts of water and 150 mM NaCl, whereas the wild type mice clearly preferred to

drink the salt solution, exhibiting a preference of 75%. Surprisingly, only female *isk* –/– mice exhibited the loss of appetite for NaCl. Female *isk* +/– mice, as well as male *isk* –/– and *isk* +/– mice, exhibited only small changes that were not statistically different from wild type controls. The *isk* –/– mice exhibited normal consumption patterns for KCl and saccharin. Conditioned taste aversion tests demonstrated that female *isk* –/– mice are able to taste NaCl. We hypothesize that the mice have a defect in their ability to metabolize salt, possibly through altered kidney or duodenum function.

20. NaCl preferences in 13 inbred mouse strains

A.A. Bachmanov, M.G. Tordoff and G.K. Beauchamp

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA

We have begun the first concerted examination of strain differences in NaCl acceptance by mice. Male mice from 13 strains (n = 11–12 per strain) were obtained from The Jackson Laboratory. Solutions of 75, 150, 300 and 450 mM NaCl were presented in increasing order of concentrations to individually housed mice using 48 h two-bottle tests with water as one choice. Three patterns of concentration-dependent responses to NaCl were observed. (i) Mice from the AKR/J, CBA/J and C3H/HeJ strains avoided all NaCl concentrations (i.e. consumed more water than NaCl), except for the nonsignificant avoidance of 75 mM NaCl by the AKR/J mice. (ii) Mice from the A/J, BALB/cByJ, C57BL/6J, C57L/J, DBA/2J, NOD/LtJ, PL/J, RIIIS/J and SWR/J strains weakly preferred or were neutral to 75 and 150 mM NaCl, and they avoided 300 and 450 mM NaCl. (iii) NZB/B1NJ mice preferred 75 and 150 mM NaCl, were neutral to 300 mM NaCl and avoided 450 mM NaCl. The NZB/B1NJ mice had the highest intakes of 300 and 450 mM NaCl. In subsequent studies, we plan to examine the contributions of sodium taste and metabolism to these strain variations.

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21. Sodium detectability in rats is not influenced by dietary NaCl exposure or size of anion

L.C. Geran and A.C. Spector

Department of Psychology, University of Florida, Gainesville, FL 32611, USA

Maternal NaCl intake can produce long-lasting effects on salt preference in offspring. However, it is unclear whether these results are due to alterations in taste function, post-ingestive factors or both. Using a two-lever operant paradigm, NaCl threshold was assessed for adult rats exposed to one of three sodium diets (NaCl 0.1% n = 7, 1.0% n = 7 or 6.0% n = 8) from embryonic day 1 (E1) through to the completion of testing. No differences in threshold were found among dietary groups ($P > 0.27$), extending previous results for rats exposed to test diets from E1 to postnatal day 30. Thus, salt preference differences are likely not the result of differences in NaCl sensitivity at the dietary levels tested. Detectability of sodium gluconate (NaG) was also assessed, and NaCl and NaG thresholds redetermined using a 100 μM amiloride solvent. NaG and NaCl thresholds did not differ significantly ($P > 0.88$), but NaCl plus amiloride increased threshold by 0.9 $\log_{10}$ units compared with NaCl alone ($P < 0.001$). This implies that normal sodium detectability is predominately mediated by the cation, and that the paracellular pathway of sodium transduction is not necessary for maintenance of normal detection. Blocking the epithelial channels, on the other hand, substantially impairs sodium detection. NaG plus amiloride raises threshold 1.3 $\log_{10}$ units higher than for NaG alone ($P < 0.0001$). NaG plus amiloride does not completely abolish competent performance, however, indicating that the animals were responsive to some chemical cue at the higher NaG concentrations.

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22. The effects of dietary salt exposure after weaning on adult salt intake in rats

D.J. Snyder, R.J. Contreras and J.C. Smith

Program in Neuroscience, Department of Psychology, The Florida State University, Tallahassee, FL 32306-1270, USA

We have shown that rats exposed perinatally to a high salt (NaCl) diet consume excessive amounts of NaCl during adulthood compared with rats with normal or basal salt experience. This effect is accompanied by significant physiological change and does not occur with later salt exposure. Thus, the perinatal period is critical for the development of salt appetite. To characterize more specifically the critical interval for long-term change, we are limiting salt exposure to smaller perinatal phases, beginning with the period after weaning. Male rat pups previously exposed to a 1.0% NaCl diet (similar to standard chow) were given *ad libitum* access to dH_2O and a 0.1, 1.0, 3.0 or 6.0% NaCl diet starting at weaning and continuing for 9 days, after which they were returned to standard chow. Significant differences were found for water but not food intake. In particular, animals in the 6.0% group drink a markedly elevated volume of water, which suggests that they are attempting to compensate for increased salt load. Compensatory effects may work against the development of differential salt behavior. Offspring began a series of 48 h two-bottle tests (dH_2O versus 0.03, 0.1, 0.2, 0.3 and 0.4 M NaCl) upon reaching ~120 days of age. NaCl intake did not produce any significant group differences. Associated water intake did produce a significant effect, but this was related to increased food intake in the 1.0% group alone and not to solution intake. In no case did adult salt behavior in rats with salt exposure after weaning resemble the effects we have seen previously with full perinatal salt exposure. We conclude that the period after weaning is not influential in the development of adult salt behavior.

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23. The effect of water deprivation and early developmental dietary NaCl exposure on taste reactivity of rats to NaCl and water

J.A. Couch, A. Markison, B.C. Sauer and A.C. Spector

Department of Psychology, University of Florida, Gainesville, FL 32611, USA

Many factors influence ingestive behavior, including physiological state, developmental dietary history and stimulus properties. We used the taste reactivity (TR) test, a method of quantifying oromotor responses to intraorally infused fluids, to examine

whether consummatory responses are affected by water deprivation and early developmental dietary NaCl exposure. Gravid Sprague–Dawley dams were presented with one of three diets that varied in NaCl content (basal: 0.10%, $n = 7$; intermediate: 1.0%, $n = 6$; high: 3.0%, $n = 5$) from conception through weaning. At postnatal day 30, offspring were placed on Purina Chow (~1.0% NaCl). Male offspring were tested for their TR to intraoral infusions of water and NaCl (0.03, 0.1, 0.3, 1.0 M) in either a 23 h water-deprived or nondeprived state. Water deprivation significantly increased ingestive consummatory responses of rats to water and all NaCl concentrations, even 1.0 M ($P < 0.05$). Deprived rats also exhibited significantly fewer aversive behaviors to 1.0 M than nondeprived rats ($P < 0.001$) and there was virtually no aversive behavior elicited by the other stimuli. Although total ingestive behavior was significantly increased in the basal group ($P < 0.04$), the magnitude of the difference was minimal and there were no statistically discernible effects on individual oromotor ingestive behaviors. There were no effects of maternal dietary treatment on aversive behaviors. Thus, early developmental dietary NaCl exposure does not appear to affect the palatability of NaCl as revealed by TR. It also appears that the frequency of ingestive reflex-like consummatory responses are increased by water deprivation regardless of the rehydrating potential of the fluid stimulus.

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24. Rapid and labile short-term conditioned taste aversion in free-licking rats

R. Nardos, J.C. Smith[1] and T.A. Houpt

Departments of Biological Science and [1]Psychology, Florida State University, Tallahassee FL 32306, USA

Previously, we demonstrated rapid and labile short-term memory in conditioned taste aversion (CTA) by pairing an intraoral infusion of 5% sucrose with low doses of LiCl. To determine if similar short-term CTA occurs in free-licking rats, we used lickometers to measure the frequency of licking sucrose for 6 h after pairing sucrose with different doses of LiCl. To control for unconditioned toxic effects, we measured licking of a familiar solution after LiCl.

Rats were habituated to a daily schedule of 17 h food deprivation and 30 min access to 2% corn oil for 10 days. On the test day, the rats were allowed 1000 licks of either a familiar 2% corn oil or a novel 5% sucrose. After 30 min, both the corn oil and the sucrose groups were injected with LiCl (0, 5, 19, 38, 78 mg/kg, $n = 4$–11). Immediately after injection, the corn oil group had access to corn oil and the sucrose group had access to sucrose. Licking was recorded continuously for 6 h.

The sucrose group showed decreased licking 1 h after all doses of LiCl, but at 6 h licking was decreased only after 19 mg/kg or higher. Thus, CTA was rapidly expressed at 1 h after pairing sucrose and LiCl, but was gone within 6 h at the lowest dose of LiCl. The rapid CTA was not due to the toxic effects of LiCl, because the corn oil group had higher intake at all times and doses compared with the sucrose group.

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25. Conditioned aversions and preferences for solid foods in rats

P.L. Smith and J.C. Smith

Program in Neuroscience, Department of Psychology, The Florida State University, Tallahassee, FL 32306-1270, USA

We have previously shown that a LiCl-induced taste aversion to a sucrose and corn oil solution cannot be conditioned in rats with bilateral PBN-lesions. These data led to the conclusion that the rats are responding to the taste of the sugar/oil mixture since there is evidence that animals with lesions to this area are able to develop a conditioned 'trigeminal' aversion. The data involving 'trigeminal' aversion are based on liquid ingestion of capsaicin or pure corn oil. In future research we want to see if rats with PBN lesions would develop an aversion to solid foods, where texture would become a salient feature of the taste stimulus. However, there is little evidence for the role of texture in conditioned aversions to solid foods in normal rats.

In a series of three experiments, preference and aversions to solid foods were examined in simultaneous two-choice feeding tests between Purina and Harlan diets. In a series of general preference tests (experiment 1), rats showed reliable preferences for one diet (Teklad) over the other diet (Purina) when texture was consistent between the two diets. Experiment 2 showed rats suppressed intake of the Harlan diet (paired with a LiCl injection) when both diets had similar textural consistencies, while experiment 3 showed that LiCl-injected rats suppressed intake of one textural form of a diet (wet mash) when two forms of the same diet were presented. These results imply that ingestive behavior of solid foods involves both gustatory and textural stimulation, and future tests using these stimuli will be conducted to see if rats with PBN lesions can acquire an aversion to a food on the basis of texture.

26. The role of stimulus intensity in conditioned taste aversion

B.K. Formaker, M.E. Frank and B.I. MacKinnon

Department of BioStructure & Function, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT 06030-3705, USA

In order to examine the role of stimulus intensity in the expression of conditioned taste aversions we analyzed the effects of test stimulus concentration on single-taste quality aversions in 40 adult male golden hamsters (*Mesocricetus auratus*). In one experiment, animals were tested with 10, 30, 100 and 300 mM sucrose after conditioning to either 100 mM sucrose ($n = 10$) or distilled water ($n = 10$). In another experiment, animals were tested with 0.1, 0.3, 1 and 3 mM quinine-HCl (QHCl) after conditioning to either 1 mM QHCl ($n = 10$) or distilled water ($n = 10$). Animals conditioned to distilled water served as controls. In both experiments one conditioning trial was employed and aversion testing was repeated four times after conditioning. Results indicated that for both chemical compounds drinking suppression increased monotonically as a function of test stimulus concentration. Behavioral asymptote occurred at 100 mM for sucrose but was not evident for QHCl at the concentrations tested. Relative to controls, experimental drinking behavior remained significantly suppressed for both chemical compounds through all four testing replications. The current results indicate that under single-taste quality conditions, test stimulus intensities greater than the conditioning

stimulus result in greater or equal suppressive drinking behavior. Thus, drinking suppression to a classically conditioned single-taste quality is a positive monotonic function of test stimulus concentration that does *not* peak around the conditioning concentration.

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27. The taste of linoleic acid to the male albino rat

J.C. Smith and E.M. Fisher

Department of Psychology, The Florida State University, Tallahassee, FL32306-1270, USA

Smith *et al.* have shown that when a rat is given a LiCl injection following intake of an emulsion of sucrose and corn oil, the subsequent conditioned aversion to the sucrose–fat mixture generalizes to the fat more than to the sucrose component. Since this generalization has been shown in short-term taste tests lasting only a few seconds, it is hypothesized that there is a taste component to the corn oil. Recent evidence shows that some of thebreakdown of oils into their fatty acid components occurs inthe oral cavity. Approximately 60% of the corn oil is composed of the polyunsaturated fatty acid, linoleic acid. The present experiments were designed to see if the rats could distinguish lowconcentrations of linoleic acid when mixed with sweetened solutions bygeneralizing to the acid when a conditioned aversion was established to the sweet–acid mixture. The CS taste solution was amixture of glucose, saccharin and a 22 μM concentration of linoleic acid. An i.p. injection of LiCl was administered following the initial ingestion of the G + S + L mixture. An aversion was conditioned to the mixture as measured in both long- and short-term taste tests, and it was shown that the aversion generalized to the linoleic acid. Further tests were run by conditioning an aversion to 22 μM linoleic acid in water and testing for the aversion at lower concentrations as low as 3 μM. The resultsof further tests with other oils and fatty acids will be presented.

28. Taste synergy between imp and glutamate ligands

E.R. Delay and S.D. Roper[1]

Regis University, Denver, CO 80221 and [1]University of Miami Medical School, Miami, FL, USA

Synergy between monosodium glutamate (MSG) and monophosphate nucleotides such as 5′-inosine monophosphate (IMP) is a characteristic attribute of glutamate taste, called 'umami'. We have adapted a model (Rifkin and Bartoshuk, 1980) to detect synergism between taste stimuli in rats using taste preference procedures. This method measures lick rates of nondeprived rats during 30 s trials in an MS80 Davis lickometer. In earlier reports (Harbaugh *et al.*, 1995), we showed synergism between MSG and IMP, but not between MSG and sucrose. This report is a summary of tests for synergism between glutamate agonists and IMP on taste preference in rats.

Rats were tested for interactions between IMP and either NMDA, KA or L-AP4 (all at neutral pH) using an ABA design. During A, a baseline lick rate (LR) for each concentration of a tastant was established to compute model preference scores (i.e. simple summation) that represent no synergism (cf. Rifkin and Bartoshuk, 1980). During B, LRs to mixtures of NMDA/IMP, KA/IMP or L-AP4/IMP were measured and converted to behavioral preference scores. When model scores were compared with behavioral scores with ANOVA procedures, no evidence of synergism between IMP and NMDA or IMP and KA was found. However, like the results for MSG and IMP, LRs for mixtures of L-AP4 and IMP showed synergism. These data support previous research suggesting that L-AP4, a specific ligand for class III mGluR receptors, may be involved in transduction for glutamate taste.

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29. Taste preferences of brown trout juveniles from different sea basins

S.S. Sidorov

Department of Ichthyology, Faculty of Biology, Moscow State University, Moscow, 119899 Russia

Experiments were carried out on brown trout juveniles (body length 5–6 cm) from population of Baltic Sea (Luga River), WhiteSea (Kandalaksha Bay) and Caspian Sea (Terek River). Taste perception was estimated using the method based on fish taste responses to pellets containing taste substances. Classical taste substances and 21 free amino acids (L-isomers) were used astaste stimuli (0.1–0.001 M). It was found that brown trout juveniles from all three populations revealed a strong preference forpellets containing the same substances; isoleucine had the strongest deterrent effect; sodium chloride, calcium chloride, sucrose and many of the free amino acids were indifferent taste stimuli. In all experiments the pellets were gulped down after the first grasping. The time spent by the fish determining the gustatory qualities of a pellet clearly correlated with the level of consumption of pellets; the less attractive pellets were in taste, the shorter the period for pellet testing. The results of this study show that the palatability of classical taste substances and free amino acids is the same in brown trout specimens from the different sea basins. By this means the gustatory spectra have no significant intraspecies specificity in fishes.

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30. Preferences of European starlings to mixtures of natural plant products

L. Clark

USDA, National Wildlife Research Center, 4101 La Porte Ave, Fort Collins, CO 80521, USA

Studies that consider multiple compounds in mixture are useful inthat they address the complexity of physiological and behavioralmechanisms about how animals process and perceive cues associated within complex foods. Such studies are important because, in an ecological context, the dynamic changes that occur in fruits influence the timing of frugivory, and by implication, the optimal timing of seed dispersal by vertebrates, and hence the evolutionary fitness of the plant. I characterized the preference and aversions of a potential avian seed disperser, the European starling, as it is confronted with mixtures of natural plant products from two different functional classes of trigeminal irritants, methyl anthranilate (MA, a pungent compound to birds) and tannic acid(TA, an astringent), and for fructose (F), a sweet taste highly

preferred by birds. Simple solutions of F are preferred as a function of increasing concentration. Simple solutions of MA or TA are avoided as a function of increasing concentration. Starlings avoid mixtures of MA + TA as if they were attending to the most unpalatable concentration of a single component, contrary to expectations based on averaging models. When in binary mixture, F overshadows the unpalatable properties of MA or TA. However, in trinary mixture, using concentrations of MA and TA that starlings are indifferent to otherwise, the preference for F can be completely eliminated. This observation is not predicted on the basis of responses to any of the simple and binary solutions. These results are important in understanding how plants may be able to manipulate the behavior of seed dispersers and the prevention of untimely presentation.

31. Two discrete learning events in the discrimination of binary mixtures by catfish

T. Valentincic

Department of Biology, University of Ljubljana, Vecna pot 111, 1000 Ljubljana, Slovenia. e-mail: tine.valentincic@uni-lj.si

Catfish learn to detect two attributes of a binary mixture: (1) the more stimulatory component and (2), with additional discrimination training, the difference between the more stimulatory component and the mixture. The more stimulatory component is determined experimentally by the component that evokes the larger electro-olfactogram (EOG) response when tested alone. In the initial learning step during the conditioning trials, catfish recognized the binary mixture as its more stimulatory component. This learned state persisted through 30–200 conditioning and testtrials. If, at any point, the conditioned catfish were given the opportunity to compare successively (at least five times) the more stimulatory component and the conditioned mixture, they started to discriminate between the more stimulatory component and the mixture. To facilitate understanding of how catfish learn olfactory stimuli, a comparison with artificial nerve networks is applied. Inan artificial network, the first layer contains coded sensory information, the second layer contains attributes such as parts ofthe 'whole' and the third layer contains the 'whole'. Learning the attributes, such as the detection of a mixture as its more stimulatory component, can theoretically occur in layer two of the olfactory network—in the olfactory bulb. During discrimination training, the one attribute in the second layer is subdivided into two attributes, i.e. one derived from the action of the more stimulatory component and the second derived from the difference between the more stimulatory component alone and the conditioned mixture. To link these two attributes, the third layer of the olfactory network is required.

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32. Feeding on fructose and glucose is greater by *Drosophila* exposed to fructose than to glucose in the rearing medium

G.C.H. Chua, J.H. Hwang and L.M. Kennedy

Neuroscience Laboratory, Biology Department, Clark University, Worcester, MA 01610, USA

Behavioral and electrophysiological data from *Drosophila* species and human psychophysical data suggest separate receptor cell mechanisms for fructose and glucose taste (L.M. Kennedy *et al.*, 1997, Food Chem., 60: 311). The sensitivities for these sugars may involve experience-inducible taste mechanisms in the humans (S. Eylam and L.M. Kennedy, 1998, Ann. NY Acad. Sci., 855: 170; 1998, Chem. Senses, 23: 588). Here we investigated potential inducible mechanisms in *D. melanogaster.* Flies were raised in separate media, containing 139 mM fructose or 555 mM glucose, and then tested for their sensitivities to 8, 16, 32, 64, 128 mM fructose or glucose. After food-deprivation (in 1% agar) for 24 h in the dark, groups of flies were given a choice between sugars in 1% agar (red) or 1% agar (blue) for 2 h (in dark), and the numbers of flies having fed were counted according to their abdominal colors. The percentages (± SE) choosing fructose were higher for fructose-raised flies than for glucose-raised flies at all concentrations except 8 and 16 mM, for which they were similar. The percentages choosing glucose also were higher for fructose-raised flies at all concentrations except 8 and 128 mM, for which they were similar. The percentages choosing agar was ≤0.007% for any sugar concentration, but control tests showed that flies did not prefer red over blue solutions. These data support the proposal of experience-induced changes in sugar sensitivities, and indicate thatexperience with fructose leads to greater sensitivities than experience with glucose.

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33. Feeding behavior of the adult barnacle *Balanus amphitrite* (Darwin) is masked in the absence of water flow

M. McClary

Bloomfield College, Bloomfield, NJ 07003, USA

Adult barnacles have two basic feeding behaviors. When barnacles are in flowing water, they display a behavior called extension. During extension barnacles extend their cirri up and into the current and hold them there for several seconds before sweeping them down and back into their shells. When there is no water flow barnacles display another behavior called normal beat. During normal beat they extend their cirri up and into the water but do not hold them there as for extension behavior. Instead they sweep them immediately down and back into their shells. Although it is known that the presence of food increases the frequency of cirral sweeping of extension behavior, it is unknown if the presence of food will increase the frequency of cirral sweeping of normal beat behavior. The frequency of cirral sweeping was calculated for 20 adult barnacles, *Balanus amphitrite*, with and without the presence of food and in the absence of water flow to induce normal beat behavior. The presence of food did not change the frequency of cirral sweeping of normal beat behavior. These results suggest that the frequency of cirral sweeping of normal beat behavior of adult *B. amphitrite* occurs close to its maximum rate whether food is present or not. It is concluded that flowing water should be usedwhen conducting feeding behavior experiments on adult *B.amphitrite*. Future studies will determine if this is also true forflow-dependent barnacles such as *Semibalanus balanoides* (Linnaeus).

34. Olfaction, extraoral and oral taste senses have different level of stability in fish phylogeny

A.O. Kasumyan

Department of Ichthyology, Faculty of Biology, Moscow State University, Moscow, 119899 Russia

The olfactory spectrum of free amino acids in closely related sturgeon species (Russian sturgeon *Acipenser gueldenstaedti*, stellate sturgeon *A. stellatus* and Siberian sturgeon *A. baeri*) is narrow and similar. Only glycine and L-alanine induced intensive behavioral response in all three sturgeon species, all other free amino acids being ineffective stimuli. Anosmic specimens showed no behavioral response. Twelve amino acids were highly effective as extraoral tastants for all three species, but had different positions in the range of effectiveness. A high correlation in the extraoral taste response for free amino acids was found in Russian and stellate sturgeon. Responses to oral taste stimuli were found toonly a few amino acids. There is no one amino acid which stimulated consumption of pellets in all three sturgeon species. There are no significant correlations between sturgeon species in the oral taste responses. According to the phylogenetic tree of sturgeons on the basis of their karyotypes, the species with 240–260 chromosomes, the Russian and Siberian sturgeons, evolved >80 million years ago. The stellate sturgeon, with 120 chromosomes, evolved significantly earlier. Based on sturgeon phylogeny, I suggest that olfaction is a much more evolutionarily stable chemosensory system than extraoral and, especially, oral taste sense.

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35. Olfactory responses to amino acids in two marine teleosts, red sea bream *Pagrus major* and black sea bream *Acanthopagrus schlegeli* of wild and cultured stocks

R.R. Mana, K. Anraku and G. Kawamura

Laboratory of Fish Ethology, Faculty of Fisheries, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890-0056, Japan

Red sea bream and black sea bream are not only major marine species for marine ranching in Japan; anatomical observation has also pointed to a well-developed olfactory system in the two fish, rendering them perfect models for the present investigations in ecological-system preservation. In fish the olfactory system is capable of mediating vital behaviors such as food search and social interactions between conspecifics and interspecifics via olfactory receptor neurons that connect directly to the brain. Previous investigations have shown that amino acids are a potent class of biologically meaningful odorants in fish, as indicated from electrophysiological (T.J. Hara, 1972, J. Fish. Res. Bd Can., 29: 1351) and behavioral studies (O.A. Kasumyan, 1994, Biophysics, 39: 519). The potency of free amino acids were tested by recording bioelectrical signals at the olfactory bulb (electroencephalogram) of red sea bream and black sea bream of wild and cultured stocks. Electrophysiological thresholds for potent amino acids were similar in wild and cultured stocks of both species, and there were no significant differences betwen the two species. The dose–response function of all amino acids tested were either exponential or sigmoidal in nature; however, the dose–response curves were not necessarily similar in both stocks of respective species within the concentration range tested (10^{-6}–10^{-1} M). Relative response effectiveness of four different stocks were not the same. The results suggested nonspecificity of amino acids in wild and cultured fish stocks of red and black sea bream, and between the two species.

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36. Chemical orientation of brown bullhead catfish and stonecats under different flow conditions

M.L. Sherman and P.A. Moore

Laboratory for Sensory Ecology, Bowling Green, OH 43403, USA

For catfish, chemical signals are important in behaviors such as orientation to food, attracting mates, predator avoidance or determining social status. During chemical orientation an animal must be able to extract distance and location information from thesignal. This can be done based on the structure of the signal, which varies spatially and temporally. This structural variation isinfluenced by the hydrodynamic conditions of the environment. Evolutionary theory would predict that organisms develop certain behaviors and morphological features that help them acquire and process directional information from these signals. Previous catfishorientation work has focused on species that typically live in little or no flow conditions. However, many species of catfish live in moderate to high flow conditions and are exposed to different structural qualities of a signal. Environmental differences provide an opportunity to study chemical orientation of closely related species under certain types of evolutionary constraints. Weused a comparative study with the brown bullhead (*Ictalurus nebulosus*) and the stonecat (*Noturus flavus*) under different flowregimes to investigate differences in chemical orientation behaviors. Stonecats and bullheads oriented differently to food sources.

Changes in their orientation behavior were altered by changes in flow.

37. The organization of serotonin-immunoreactive fibers in the olfactory nerve and in glomerular units of the larval sea lamprey

B.S. Zielinski and H.N. Hua

Department of Biological Sciences University of Windsor, Windsor, Canada N9B 3P4

Serotonin may mediate activity of olfactory receptor neurons in the agnathan vertebrate, *Petromyzon marinus*. In this study, serotonin-immunoreactive fibers were examined in the lamina propria of the olfactory mucosa, in the olfactory nerve and in theglomerular layer olfactory bulb of the larval sea lamprey. Serotonin-immunoreactive (-IR) fibers were seen within olfactory nerve fascicles within the lamina propria and within the olfactory nerve, where these fibers were parallel to the axons of olfactory receptor neurons. The absense of colocalization with the lectin *Giffonia miplicifolia* isolectin B4, an indicator of the axons olfactory receptor neurons in the larval lamprey (Tobet *et al.*, 1996, J. Comp. Neurol., 376: 97–111), shows that the serotonin-IR is not localized in olfactory receptor neurons. Some of these fibers were observed passing from the olfactory nerve into the olfactory bulb. In the glomerular layer, these fibers were concentrated at

periglomerular locations and terminals extended into glomerular units of olfactory receptor axons. This arrangement of the serotonin-IR fibers in the olfactory nerve and in the glomerular units suggests that sertonin may act presynaptically to modulate the responses of olfactory receptor neurons.

38. Labeling of olfactory ensheathing cells by the lectin *Phaseolus vulgaris*

B.W. Lipscomb, H.B. Treloar and C.A. Greer

Department of Neurosurgery and Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06520-8039, USA

In the olfactory system plant lectins (carbohydrate binding proteins) label subsets of olfactory receptor neuron (ORN) axonsthat are believed to express different carbohydrate epitopes (J. Plendl *et al.*, 1998, Acta. Anat., 161: 234–253). Moreover, an endogenous mammalian lectin, galectin-1, is found in the olfactory nerve on a specialized population of glia, ensheathing cells (J.Tenne-Brown *et al.*, 1998, Int. J. Devl Biol., 42:791–799). The presence of both carbohydrate epitopes and a carbohydrate binding protein in the olfactory pathway raises the possibility that carbohydrate–protein interactions play a role in ORN axon fasciculation and/or guidance. To pursue these possibilities further we have been screening the olfactory pathway for lectin binding. One lectin, *Phaseolus vulgaris* isolectin E (PHA-E), appeared to label ensheathing cells in neonatal mice and rats. To characterize PHA-E labeling we double-labeled with PHA-E and either $p75^{NGFR}$, an olfactory ensheathing cell marker, or laminin, an extracellular matrix (ECM) molecule secreted by ensheathing cells. PHA-E showed evidence of colocalization with $p75^{NGFR}$ and laminin, indicative of its association with ensheathing cells. To test for PHA-E expression in ORN axons, we double-labeled with PHA-E and GAP-43. PHA-E and GAP-43 did not colocalize, rather PHA-E labeling surrounded bundles of GAP-43 labeled ORN axons. Although further studies are required to determine the cell surface versus ECM localization of PHA-E, the current data are nevertheless consistent with the hypothesis that differential carbohydrate expression contributes to ORN axon fasciculation/guidance.

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39. Recovery of olfactory bulb laminar volumes following olfactory nerve regeneration

S. Karnik, D. Kanter, E. Kallwitz and E. Meisami

Department of Molecular & Integrative Physiology, University of Illinois, Urbana, IL 61801, USA

Olfactory receptor neurons (ORNs) of adult rodents are capable of regeneration following degeneration. Regeneration includes axon growth and reinnervation of the olfactory bulb (OB). However, the effects of nerve regeneration on OB plasticity and structural recovery are not well known. We induced degeneration of ORNs by unilateral irrigation of the nasal cavity in 25 day rats using 0.9% Triton X solution, known to cause degeneration of mature ORNs followed by their regeneration and repopulation of olfactory epithelium. We then followed changes in morphology and morphometry of OB laminae at weekly intervals for 12 weeks,using Nissl-stained complete frontal serial sections and determined volumes of whole OB, olfactory nerve layer (ONL), glomerular layer (GL) and external plexiform layer (EPL). Results showed that degeneration, as indicated by a decline in volumes of whole OB and layers on the treated side compared with contralateral control, was already evident after 1 week, with maximum degeneration occurring at 4 weeks post-irrigation. Degeneration was statistically significant and most evident in the ONL followed, in order, by GL and EPL. Granular layer was not studied. Regeneration, indicated by increased volumes of whole OB and itslaminae on the treated side (decline in interbulbar difference), was evident by week 7 and nearly completed by week 12 post-treatment. Results show remarkable structural plasticity of the OB and its ability to recover size and laminar organization during regeneration.

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40. Neuronal fate and afferent control of proliferating cells in the olfactory brain of adult decapod crustaceans

A. Hansen and M. Schmidt

Zoological Institute, University of Hamburg, D-20146 Hamburg, Germany

In *Carcinus maenas*, the number of olfactory projection neurons (OPN) increases throughout the lifetime in conjunction with ongoing proliferation in the lateral soma clusters (LC) comprised of OPN cell bodies (M. Schmidt, 1997, Brain Res., 762: 131–143). In adult decapods, proliferation occurs not only in the LC but alsoin the other soma clusters of the central olfactory pathway, the medial soma clusters (MC) and the soma clusters of the hemiellipsoid bodies (HBC). To prove the neuronal fate of the proliferating cells, we double-labeled brains of *Panulirus argus* with antibodies against BrdU and either Substance P or FMRFa, neuropeptides shown to label many neurons with somata in the MC. In each MC, several somata expressed BrdU as well as FMRFa-like immunoreactivity in animals tested after a survival time of 3 months, but none in animals tested after 2 weeks. Substance P–BrdU double-labeled cells did not occur. All BrdU–FMRFa double-labeled somata were displaced towards the periphery of the MC away from the proliferation zone. These results demonstrate that within 3 months, proliferating cells in theMC of the spiny lobster undergo neuronal maturation. Furthermore, we asked whether olfactory afferents have an influence on the rate of neurogenesis in the central olfactory pathway. We injected adult shore crabs with BrdU and after 1 week amputated one of the antennules housing the olfactory organ. One months later, immunostained brains revealed that in the LC and the HBC, the number of BrdU-positive somata was significantly reduced on the amputated versus the untreated side. Thus the rate of neurogenesis in the central olfactory system of adult shore crabs appears to be regulated by olfactory afferents.

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41. Possible functions of taurine in the primary olfactory pathway

I. Kratskin, O. Belluzzi[1], G. Smutzer, D. Ross[2] and L. Hastings

Smell and Taste Center, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA, [1]Dipartimento di Scienze Biomediche, Sez. di Fisiologia, Università di Modena, 41100 Modena, Italy and [2]Department of Otorhinolaryngology, University of Oklahoma Health Science Center, Oklahoma City, OK 73190, USA

Taurine is the most abundant neuroactive amino acid in the olfactory epithelium and olfactory bulb, and immunocytochemical studies suggest that taurine is present in olfactory sensory neurons. Whole-cell recordings in slices of the rat olfactory bulb showed that applications of 5 mM taurine decrease the amount of transmitter glutamate released from olfactory axons and reduce excitability of relay bulbar neurons via direct activation of somatic $GABA_A$ receptors followed by a shift of the membrane potential towards E_{Cl}. This indicates that taurine may modulate, at both pre- and postsynaptic levels, transmission in the first olfactory synapse.

Preliminary experiments were carried out in rats using long-term intranasal administration of an antisense oligonucleotide to a segment of the mRNA of the rate-limiting enzyme of taurine synthesis. The purpose of antisense RNA administration was to specifically inhibit translation of this enzyme and to subsequently reduce taurine biosynthesis in the olfactory epithelium. In those rats, but not in rats treated with the sense and random sequence oligonucleotides, taurine content of olfactory tissue was selectively decreased by ~25%, expression of olfactory marker protein in the epithelium and bulb was diminished and olfactory function, as determined by an odor mixture discrimination task, was compromised. Our working hypothesis is that taurine is required for maturation of continuously replaced sensory neurons.

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42. Subsets of LHRH-ir cells in bonnethead shark nervus terminalis ganglion may differ in cholinergic function

J.F. Moeller and M. Meredith

Program in Neuroscience, Florida State University, Tallahassee, FL32306-0434, USA

Electrophysiological and immunohistochemical evidence suggests two distinct cell classes in the nervus terminalis (NT) ganglion of elasmobranchs. One class is LHRH-immunoreactive (ir) while the other is immunoreactive for FMRFamide-like peptides, including LPLRFamide. The LPLRFa-ir cells were strongly labeled with cholinesterase histochemistry, indicating they were potentially cholinergic cells. LHRH-ir cells were only lightly labeled. In current studies, sections of NT ganglion from bonnethead sharks were tested with an antiserum to choline acetyltransferase (ChAT), a more reliable marker for cholinergic cells. Sections were double-labeled with antibodies to LHRH or FMRFamide, and were later examined with a Zeiss confocal microscope. Levels of colocalization were measured with the Zeiss LSM 410 software. Surprisingly, the ChAT-ir cells colocalized strongly with the immunoreactivity of a broad-spectrum LHRH antiserum, GF-5, but not with that from the FMRFa antiserum. Moreover, there was no colocalization with immunoreactivity to a narrow-spectrum LHRH antiserum, 7CR-10, which shows high affinity towards the chicken-II LHRH isoform. This antiserum labels a subset of LHRH-ir structures including 'club-like' structures thought to be contact points between LHRH cells and blood vessels (Moeller *et al.*, 1997, Neurosci. Abstr., 23: 143). The difference in ChAT-ir between cells with differential immunoreactivity to LHRH antisera supports the hypothesis that the two classes of LHRH cells in the NT ganglion that express different LHRH isoforms may have different functions.

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43. Olfactory receptor neuronal activity determines mitral cell dendritic arbor during development and regeneration

A.C. Puche, S.D. Munger[1], R.R. Reed[1], F.L. Margolis and M.T.Shipley

Department Anatomy and Neurobiology, Program in Neuroscience, University of Maryland, Baltimore, MD 21201 and [1]Howard Hughes Medical Institute, Department Molecular Biology and Genetics, Johns Hopkins Medical Institute, Baltimore, MD 21205, USA

The dendritic arbor of many neurons is more extensive during development than in adult. With maturation, dendrites becomes progressively restricted to the stable adult morphology. In the olfactory bulb of new born mice (P0), mitral cells (MC) have several apical dendrites which span multiple glomeruli. However, by ~P4 MCs have a single apical dendrite that is restricted to one glomerulus. This apical dendrite forms a 'tuft' of secondary processes throughout the glomerulus. Malun and Brunjes (1996) hypothesized that this developmental re-sculpting of MC dendrites was influenced by afferent neuron (ORN) activity. To test this hypothesis we examined apical dendrites of MCs in mice containing targeted disruptions to the X-linked olfactory cyclic nucleotide-gated channel subunit-1 (OCNC-1). OCNC-1 null mutants are functionally anosmic. Stochastic X-inactivation in heterozygotes results in 50% OCNC-1 'null' and 50% wild type ORNs in early development. Preliminary data suggests that apical dendrites of P4 heterozygote mice are not restricted to a single glomerulus. Studies are underway to also examine dendritic remodeling in naris occluded animals, which presumably have unilaterally reduced afferent input. In adults, ORN turnover predicts significant ORN-MC synaptic plasticity. To examine dendritic rearrangements during regeneration adult mice were subjected to permanent epithelial lesion. Long-term loss of ORNs resulted in reduced MC apical tufts, there was no evidence that MC dendrites recapitulated development by expanding their apical dendritic field. These results suggest that MC dendrites are developmentally shaped by ORN activity, and MCs require ORN axons to maintain their adult morphology.

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44. Determination of mRNA for low voltage activated calcium channels in the developing rat olfactory bulb

C. Brown, P. Best and E. Meisami

Department of Molecular & Integrative Physiology, University of Illinois, Urbana, IL 61801, USA

Expression, density and activity of low voltage activated (LVA) calcium channels are increased in some tissues undergoing growth and development, e.g. cardiac atrial and ventricular cells. Since this channel has been found in brain tissue and because olfactory tissue shows profound cell proliferation and growth in the postnatal rat, we investigated (1) the occurrence and tissue localization of LVA mRNA in the rat olfactory bulb and (2) whether there are changes in expression of LVA mRNA during periods of rapid tissue development. Preliminary studies in the olfactory bulbs of 21 day postnatal rats, using reverse transcriptase PCR, revealed the presence of mRNA for the pore-forming α1 subunit of the E type channel (α1E). This was shown by the presence of robust DNA bands on agarose gels, using the same primer successfully utilized in atrial tissue experiments. Results indicate that olfactory bulb tissue contains and expresses the same genes for the E form of LVA calcium channels as found in the growing cardiac atrial tissue. Experiments are in progress to determine whether mRNA levels for this channel are up-regulated during earlier ages when bulb cells undergo major phases of proliferation, differentiation and growth. We are also testing for the presence of the genes for other LVA calcium channels (α1G and α1H) in the bulb, and plan to use an antibody raised against this channel in our laboratory to test the tissue localization and expression of the α1E channel protein molecules by immunocytochemistry.

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45. Localization of protein kinases in adult mouse olfactory bulb

N. Liu, R.A. Berlin, H. Chang and H. Baker

Cornell University Medical College at The Burke Medical Research Institute, White Plains, NY 10605, USA

Protein phosphorylation plays an important role in mediating the cascade of intracellular responses to external stimuli. To establish a role for specific protein kinases in signal processing and gene regulation in the mammalian olfactory bulb (OB), the present study investigated the cellular distribution of PKAα, PKCα, CaMKII and CaMKIV in mouse OB. Adult mice subjected to unilateral naris closure were processed for immunostaining with specific antibodies. In control OB, PKAα localized to all layers, including olfactory nerve, glomerular (GL), external plexiform (EPL), mitral cell (MCL), internal plexiform (IPL), and granule cell layers (GCL). The strongest PKAα immunostaining occurred in periglomerular and granule cells. Robust PKCα immunostaining was found in the plasma membrane of granule cells and inprocesses within the EPL. CaMKIIα was localized to glia andneurons located in GL, EPL, MCL, IPL and GCL. CaMKIIα antibody strongly stained soma of periglomerular, mitral and granule cells. CaMKIIα immunostaining was strong in the EPL and IPL, but very weak within the glomerular neuropil. CaMKIIβ immunostaining occurred prominently in glia and lightly in mitral and periglomerular cells. CaMKIV was found primarily in the nuclei of granule cells with weak staining in their ascending dendrites. In the OB ipsilateral to naris closure, CaMKIIα decreased in MCL and CaMKIV increased in GCL. These data demonstrate that the major protein kinases vary in laminar and cellular distribution in the mouse olfactory bulb and suggest that they participate in different aspects of olfactory signal processing and gene regulation.

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46. Cloning and characterization of a cyclic nucleotide hosphodiesterase expressed in the olfactory system of *Manduca sexta*

M.B. Stoker, N.J. Gibson and A. Nighorn

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

We are interested in understanding the role of the intracellular messenger cGMP in mediating the processing of olfactory information in the insect *Manduca sexta*. Previous studies have focused on understanding the synthesis of cGMP through the characterization of guanylyl cyclases. This study focuses on the enzymes that degrade both cGMP and cAMP, cyclic nucleotide phosphodiesterases (PDEs). We have used RT-PCR with degenerate oligos designed against amino acids in the conserved domain (Charbonneau *et al*., 1986, Proc. Natl Acad. Sci. USA, 83: 9308–9312) to isolate PDEs from the *Manduca* olfactory system. Here we report the cloning of a *Manduca* PDE expressed in both the antennae and antennal lobe. We describe its relationship to other known PDEs and its localization within the olfactory system.

47. Ultrastructure of tyrosine hydroxylase-immunoreactive neuronal profiles in the glomerular layer of the salamander olfactory bulb

D.M. Allen and K.A. Hamilton

Department of Cellular Biology and Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130-3932, USA

Expression of tyrosine hydroxylase (TH) by the dopaminergic neurons of the olfactory bulb is regulated by the olfactory nerve. In mammals, populations of periglomerular cells and external tufted cells express TH. The TH^+ periglomerular cell bodies occur in the glomerular layer and the TH^+ external tufted cell bodies occur in the adjacent portion of the external plexiform layer. In amphibians, the neurons of the glomerular layer do not express TH. Although amphibians have TH^+ neurons that innervate the glomeruli, the cell bodies of the neurons occur in the deeper layers of the olfactory bulb, not in the periglomerular region.

In the present study, vibratomed sections of adult salamander olfactory bulbs were incubated overnight with monoclonal mouse anti-TH (Incstar) at 4°C. They were subsequently reacted with biotinylated horse anti-mouse IgG (Vector, 1:200) followed by avidin–biotin complex (Vector, ABC-Elite kit). Immunoreactivity for TH was revealed using diaminobenzidine HCl (0.05%, + 0.05% H_2O_2) as the chromagen. The sections were postfixed with 2%OsO_4, stained *en bloc* with 1% uranyl acetate (aqueous), dehydrated in ethanol series, flat-embedded in Polybed 812 and

examined using light microscopy to locate stained processes in the glomerular layer. Thin sections were then obtained and examined using a Philips CM-10 transmission electron microscope. The results show that single TH-immunoreactive profiles of the glomerular layer receive multiple type 1 (asymmetrical) synapses from olfactory nerve axons that, as in mammals, could regulate TH expression.

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48. Comparison of immunoreactivity for the gap junction protein connexin 43 in the rat and salamander olfactory bulbs

K.A. Hamilton

Department of Cellular Biology and Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130-3932, USA

Immunohistochemical staining has provided evidence that the gap junctions of mammalian olfactory bulb neurons are formed by connexin 43 (Cx43). In this study, polyclonal antisera against C-terminus Cx43 peptides were used to compare the distribution of Cx43-like proteins in rat and salamander olfactory bulb sections. High-pH fixation was used with immunoperoxidase and immunofluorescence methods that yield punctate labeling of rat heart gap junctions. With these methods, optimal punctate labeling was seen in both heart and bulb sections with an antiserum against BSA-conjugated Cx43 amino acids 346–360 (#18A, from E.L. Hertzberg, Albert Einstein College of Medicine) that labels phosphorylated and unphosphorylated Cx43 in rat brain. Because *Xenopus* Cx43 exhibits high homology with rat Cx43, the Cx43/18A antiserum presumably labels similar Cx43-like proteins in the salamander.

In the rat bulb sections, olfactory nerve fascicles were outlined by large puncta and they could be followed into the glomerular layer. The labeling was densest at the nerve–glomerular junction and surrounding the glomeruli. Large puncta were non-uniformly distributed throughout the glomeruli and they outlined scattered large profiles. Smaller puncta were uniformly distributed throughout the external plexiform layer, and large puncta also outlined scattered large profiles. In the deep layers, some mitral and granule cell bodies were outlined by puncta, and dense patchy punctate labeling occurred along the internal plexiform layer borders and within the granule cell layer. The salamander olfactory bulb sections exhibited a similar distribution of punctate labeling, although the labeling was sparser.

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49. Olfactory bulb granule cells *in vitro*: anaxonic, GABAergic and spinous

D. Gabeau and C.A. Greer

Department of Neurosurgery & Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06520-8039, USA

Olfactory bulb granule cells *in vivo* exhibit distinctive morphologic and synaptologic features. Included among their characteristics is the lack of axons (anaxonic) and the presence of dendritic spines (gemmules), which both receive afferent synaptic input viaglutamate receptors and make efferent synaptic outputs via gamma-amino-butyric acid (GABA). To explore whether these are intrinsic features of granule cells that persist *in vitro*, we employed immunofluorescent labelling to characterize cultured olfactory bulb neurons from late embryonic and early postnatal Sprague–Dawley rats. To assess neurite characteristics and test for the anaxonic phenotype we used double-labelling with anti-microtubule-associated protein (MAP-2) to label dendrites and anti-growth-associated protein (GAP-43) to label axons. Triple immunolabeling with anti-MAP-2, anti-GAP-43 and anti-GABA was used to determine if anaxonic neurons were GABAergic. Anti-neuron-specific enolase (NSE), which labels neurons in their entirety, and anti-GAP43 were employed to elucidate dendritic spines on anaxonic neurons. Lastly, anti-NMDAR1 and anti-NSE were used to test for the presence of the glutamatergic NMDA receptor. The data show that a GABAergic anaxonic cell type is present in olfactory bulb cultures and that these cells exhibit pedunculated dendritic spines and NMDA receptors. We conclude that olfactory bulb granule cells *in vitro* and *in vivo* express equivalent phenotypes. The data further suggest that these characteristic features are intrinsic to the olfactory bulb granule cell and that *in vitro* models may be useful for studying the determinants of the morphologic and synaptologic characteristics of granule cells.

50. Mapping the distribution of ionotropic glutamate receptors in the olfactory bulb of zebrafish using a channel permeant probe, agmatine (AGB)

J.G. Edwards and W.C. Michel

Department of Physiology, University of Utah School of Medicine, Salt Lake City, UT 84108, USA

The distribution of ionotropic glutamate receptor/channel subtypes in the zebrafish olfactory bulb was examined using glutamate receptor (GR) agonists and activity dependent labeling techniques. Acutely dissected bulbs were exposed to GR agonists/antagonists and AGB, then processed for immunostaining with anti-AGB, anti-GABA or anti-glutamate antibodies. Large (diameter, 13.2 ± 1.6 μm; area, 97 ± 20 μm^2; n = 10), glutamate-positive and GABA-negative cells were predominately located in the inner glomerular layer. Small (diameter, 7.5 ± 1.5 μm; area, 42 ± 15 μm^2; n = 12), glutamate-intermediate and GABA-positive cells were located throughout the bulb, but predominately in the core. Without exogenous AGB, slight AGB staining was observed within nuclei of all cells. With exogenous AGB (5 mM), the intensity of AGB staining increased in both large and small cells. Exogenous AGB staining was blocked with TTX or with a mixture of GR antagonists (AP5 + NBQX), indicating that endogenous electrical activity and associated glutamate release drives labeling. In the presence of TTX, GR agonists (kainate or NMDA) increased AGB labeling in all large cells and in most small cells. Although most small cells in the core were glutamate sensitive (49/50), 35% of the small cells in the glomerular layer (7/20) were glutamate insensitive. The glutamate-sensitive large and small cells are mitral and granule cells respectively. Presumably, the glutamate-insensitive cells are either another interneuron type or glial cells. Our results reveal at least two glutamate-sensitive cell types, but we found no evidence for the differential distribution of ionotropic GR subtypes. We thank Signature Immunologics for the primary antibodies.

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51. Intracerebro-ventricular LHRH injections increase *c-fos* expression in the medial preoptic areaof male hamsters exposed to female pheromones

J.M. Westberry and M. Meredith

Program in Neuroscience, Florida State University, Tallahassee, FL32306-4340, USA

We have previously shown that intracerebro-ventricular (icv) LHRH increases *c-fos* expression in the medial preoptic area (MPOA) of mating, but not of unstimulated male hamsters, and relieves deficits in mating behavior caused by vomeronasal lesions. We investigated whether icv LHRH would produce similar effects in males exposed only to the female pheromones and not given the opportunity to mate. Animals were given pressure injections of LHRH (50 ng in 2 µl) or saline (2 µl) through indwelling cannulae 30 min before exposure to hamster vaginal fluid (HVF), and behavioral testing. Testing was conducted in a glass chamber (14 × 25 cm) constructed so that HVF (diluted 1:10 with distilled water) could be introduced into a well at the front of the chamber. Animals were placed in the chamber for 45 min and given HVF at intervals. We recorded respiration rate, investigatory and other behaviors. Following the behavior testing, the animals were returned to their cages for 45 min, then overdosed with sodium pentobarbital and perfused with 4% paraformaldehyde. Vibratome brain sections (50 µm) were processed for *c-fos* immunocytochemistry using rabbit anti-fos antibody (Santa Cruz 1:50,000), avidin–biotin–horseradish peroxidase complex (Vector), and DAB. *c-fos*-positive nuclei were counted using computer enhanced image analysis software in tissue sections representative of central targets of AOB efferents. Our findings show a significant increase in fos-immunoreactive neurons in the MPOA of animals given LHRH and exposed to HVF. Our preliminary observations suggest that increased fos expression is independent of the behavioral response to HVF.

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52. Activation of an anatomically distinct subpopulation of neurons in the male mouse accessory olfactory bulb (AOB) following exposure to female mouse urine: effect of endocrine status

C.A. Dudley, A. Kumar and R.L. Moss

University of Texas Southwestern Medical Center, Dallas, TX 75235, USA

We have previously reported that exposure of male mice to soiled bedding from female mice resulted in preferential activation of cells located in the anterior portion of the AOB as opposed to those located posteriorly. To more precisely define the nature of the stimulus, we have examined the effect of urine collected from female mice in various endocrine states.

After a 2 h exposure to a given female urinary stimulus, the AOBs of the male mice were processed for *c-fos* immunoreactivity. In the mitral and granule layers of the AOB, the total number of *c-fos*-immunopositive cells was higher following exposure to urine pooled from cycling females (irrespective of stage), urine from proestrus/estrus females or urine from diestrus females (D1 and D2) than after exposure to urine from ovariectomized females or clean bedding. Castrated males exhibited a smaller increase. Analysis of the position of the positively stained cells along the anterior/posterior (A/P) axis revealed that all stimuli produced anA/P ratio of >1. Exposure to urine from females in any of the tested endocrine states resulted in A/P ratios of at least 5.43 in the mitral layer and at least 3.21 in the granule layer while clean bedding exposure yielded ratios no higher than 2.57. The results indicate that female urine of any type preferentially activates cellsin the anterior AOB. The efficacy of female mouse urine in inducing cellular activation in the male mouse AOB is dependent on ovarian hormones, while the ability of the male to respond is gonadally dependent.

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53. Functional mapping of urine-activated glomeruli in the main olfactory bulbs of mice

M.L. Schaefer and D. Restrepo

Program in Neuroscience and Department of Cellular and Structural Biology, University of Colorado Health Science Center, Denver, CO 80262, USA

Individual body odors are of particular importance and provide information about gender, age, individual identity, reproductive status, maternal status and health. Odors that distinguish one individual from another member of the species are called odortypes and are determined by polymorphic genes. Previous work has shown that BALB/c-H-2^d female mice can discriminate between inbred male mice of disparate MHC-type (H-2) and their urine (Yamazaki *et al.*, 1983). Our hypothesis is that genetically determined odortypes expressed in urine elicit unique maps of neuronal activity in the main olfactory bulb (MOB) by recruiting different subsets of functional units (i.e. a glomerulus and surrounding circuitry). Also, that these distributed pattern of odor-induced neuronal activity contribute to the encoding of odortype information. We measured increases in *c-fos* mRNA expression in serial MOB sections of several mice. Expression of *c-fos*, can be induced by neural activity within the olfactory system allowing identification of discrete urine-activated glomeruli. Live BALB/c-H-2^d female mice were exposed to either B6.AKR-H-2^k, C57BL/6J-H-2^b or C57BL/6β2m KO (MHC class I deficient) male mouse urine odor via an olfactometer. The congenic and MHC class I deficient mouse urine odors elicited moderate increases in *c-fos* mRNA in many glomeruli throughout the MOBs of female mice. In addition, the congenic urines exhibited differences in their distributed patterns of odor-induced neuronal activity. We report for the first time anatomical evidence for odortype discrimination: MHC-determined odortypes expressed in novel congenic urines elicit different spatial maps of neuronal activity within the MOBs of female mice.

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54. Functional mapping of the developing olfactory bulb

K.M. Guthrie and C.M. Gall

Department of Anatomy and Neurobiology, University of California, Irvine, CA 92697, USA

Mapping studies employing 2-deoxyglucose or markers of *c-fos* expression have provided evidence for the spatial organization of

odorant responses in the mature olfactory bulb. These techniques have not clearly demonstrated functional topography in the neonatal olfactory bulb, although behavioral experiments indicate that young rats are capable of identifying odor cues and performing discrimination tasks. Moreover, it has been shown that odorant receptors and signal transduction molecules are expressed by olfactory receptor neurons during embryonic development. Using *in situ* hybridization of *c-fos* cRNA, we have re-examined functional responses to odors in the bulbs of young rats beginning at day of birth [postnatal day (PN) 0] to determine if odor stimulation produces distinct spatial patterns of neuronal activity.

At all ages examined (PN0, 2, 4, 6, 8, 11, 14 and 21) exposure to propionic acid odor resulted in elevated *c-fos* mRNA expression within focal populations of neurons distributed in the dorsomedial and lateral glomerular layer. Peppermint odor produced a different pattern of cRNA labeling, with most activated neurons concentrated in the mid-lateral glomerular layer. Laminae beneath these activated glomerular regions contained densely labeled mitral and granule cells. Mitral cell labeling was particularly prominent at the earliest postnatal ages. Control littermates maintained in clean air exhibited less cRNA labeling throughout the olfactory bulbs. During the first postnatal week, the odor-evoked pattern of neuronal activation became more distinct, possibly reflecting the maturation of interneuron populations. Nevertheless, odor-specific patterns of activity are detected in the olfactory bulb beginning at PN0. This result is consistent with odorant receptor mapping and behavioral studies and indicates that functional spatial maps of odor quality are present from birth.

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55. Con A selectively influences neuronal processing of odor stimuli in the rat olfactory bulb

A. Kirner and R. Apfelbach

University of Tübingen, Department of Animal Physiology, D-72076 Tübingen, Germany

According to biochemical and histological studies lectins (carbohydrate binding proteins) are capable to bind to olfactory sensory cells. The lectin concanavalin A (Con A) affects electro-olfactogram responses of some but not all odors when superfused over the olfactory mucosa of a rat preparation *in vitro*. These findings suggest that there are olfactory sensory cells/receptor molecules that respond to particular classes of odors and that the function of these structures can be selectively inhibited by lectins.

In the present study we investigated whether Con A applied to the olfactory epithelium affects odor induced cell activation in the olfactory bulb. Active cells were visualized by Fos immunoreactivity. Without lectin treatment the odors ethyl acetate (EA) and D-carvone produced similar distribution patterns of Fos-labeled periglomerular cells. There were two foci with heavy staining, one in the dorsolateral and the second in the dorso- and mediomedial bulb. However, for D-carvone the foci covered a smaller part of the bulb's surface and were located more anteriorly. After Con A treatment the pattern for EA changed: the focus in the dorso- and mediomedial area disappeared while the staining in the dorso-lateral area was unaffected. Treatment with Con A had no effect on the staining pattern evoked by D-carvone. The selective effect of Con A on Fos staining patterns suggests that some of the receptor molecules involved in EA recognition are affected by Con A while others are not. Con A does not interfere with receptor molecules responsible for D-carvone recognition.

56. Slice blotting: a simple method for visualizing secretion patterns in living brain tissue

G. Lowe

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104-3308, USA

The intricate circuitry of the CNS forms spatially organized structures containing a multitude of transmitters, modulators, enzymes, regulatory and growth factors that are expressed and released in specific patterns from subpopulations of neurons and glial cells. Immunohistochemistry enables anatomists to disclose in great detail the distribution of these substances in fixed tissue. Anatomical studies with immunohistochemical and molecular biological techniques have mapped in fine detail the distribution of these substances in fixed tissue. However, the release of neuroactive substances is often under precise spatial control and is regulated by numerous physiological factors. Understanding such complex intercellular signaling systems will require the development of new spatially resolved methods for detecting secretion in living systems.

This paper describes a simple but powerful general method for visualizing and quantifying the time-averaged spatial pattern of release of specific neurotransmitters and neuromodulators from *in vitro* brain slices. It may have widespread application in neuroscience, especially developmental biology. The method combines standard physiological preparations with immunoblotting protocols from molecular biology, and complements other, more sophisticated approaches to brain slice physiology. It can be applied to study patterns of release of any substance in a spatially organized tissue or process detectable by immunoassay, subject to a variety of stimuli and conditions, such as growth factors, chemoattractants and chemorepellents, morphogens, enzymes, hormones and other paracrine signals.

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57. Dependence of olfactory bulb activation on the duration of odor exposure revealed by fMRI

X. Yang[1], F. Xu[1,2], R. Renken[1], C.A. Greer[2,3], G.M. Shepherd[2] and R.G. Shulman[1]

[1]Department of Molecular Biology and Biophysics, [2]Section of Neurobiology, Medical School and [3]Department of Neurosurgery, Medical School, Yale University, New Haven, CT 06520, USA

In previous studies (Proc. Natl Acad. Sci. USA, 95: 7155), we demonstrated that iso-amyl acetate induced activation in the olfactory bulb (OB) could be mapped by functional magnetic resonance imaging (fMRI) in naturally sniffing rats during prolonged exposures, based on blood oxygenation level-dependent (BOLD) contrast. We showed that under these conditions, activation was strongest in the lateral and central areas, and persisted after the termination of odor stimulation. To optimize the fMRI for functional mapping in the olfactory system, we have now implemented externally controlled odor exposures (32 s). In

the current studies, we observed highly reproducible activation within animals and across animals. However, the 32 s exposures showed significantly different spatial and temporal patterns from the previous long exposures (5–20 min). Spatially, the 32 s exposure activation was predominantly dorsal, and no significant activity was observed in the ventral and lateral area. Temporally, upon the termination of odor stimulation, the activation returned to baseline within 8 s. During long exposures in the same animal, we observed dominant dorsal activation in the first minute, but the ventral activation quickly increased and was invariably the dominant activation in the bulb following 2 min of exposure. These results indicate that the activation in short exposures has sufficient reproducibility for reliable fMRI mapping of odor responses, and that the exposure duration should be an important consideration in interpreting functional studies in OB.

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58. Modulation of neuronal activities in olfactory bulb layers studied by functional magnetic resonance imaging

F. Xu[1,2], X. Yang[2], F. Hyder[2], C.A. Greer[1,3], G.M. Shepherd[1] and R.G. Shulman[2]

[1]Section of Neurobiology, Medical School, [2]Department of Molecular Biology and Biophysics and [3]Department of Neurosurgery, Medical School, Yale University, New Haven, CT 06520, USA

The main olfactory bulb (MOB), specialized in processing and encoding the molecular information of odorants, has the most regular laminar structures in the brain. The quality and quantity information of odorants is believed to be represented by the neuronal activity patterns across the MOB. High-resolution fMRI has been successfully used to map the activity pattern elicited by an odorant and showed that the activated foci are located in the outer bulbar layers (Proc. Natl Acad. Sci. USA, 95: 7155; Yang *et al.*, this conference). Now we have used fMRI to reveal the time-dependent regulation of the neuronal activities in these layers. When 3% amyl acetate vapor was presented to an anesthetized rat for 30 s with 4 min intervals, adaptation to the later exposures was observed. The activity patterns of the outer layer pixels (ONL + GL) and the inner layer pixels (GL + EPL) were similar. Prolonged odor exposure, however, produced different patterns. The activities of different layers showed different temporal activity patterns. The activity of the most peripheral activated pixels did not change significantly with time, whereas the activities of the inner and innermost activated pixels decreased to baseline within 2–3 and 1–2 min respectively, indicating that intrinsic and extrinsic mechanisms are involved in the regulation of the neuronal activity in bulbar layers. The results demonstrated that fMRI can be used to study neuronal modulation in processing and encoding of olfactory information at the level of different laminae.

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61. Disruption of the gene encoding a Dyrk2 kinase homologue causes olfactory impairment in *Drosophila melanogaster*

G. Fedorowicz, N. Kulkarni[1], J. Roote[2], M. Ashburner[2], T. Mackay and R. Anholt[1]

Department of Genetics, North Carolina State University, Raleigh, NC 27965, [1]Department of Genetics, North Carolina State University, Raleigh, NC 27965, [2]Department of Genetics, University of Cambridge, Cambridge, CB2 3EH, UK

Previously, introduction of a *P*-element (*P[lArB]*) in an isogenic strain of *Drosophila melanogaster* gave rise to 14 *smell-impaired* (*smi*) lines with aberrant avoidance behavior to the odorant, benzaldehyde. One *P*-element insertion causing a small (12%) reduction in olfactory avoidance behavior in homozygous flies is localized to chromosomal band 35A, 30 kb downstream from a gene encoding a Dyrk2 kinase ('dual-specificity tyrosine-phosphorylation regulated kinase') homologue and 15 kb upstream from the *wingblister* locus. Crossing *smi35A* homozygotes to lines with deletions in the area of interest and testing the offspring for olfactory behavior identified the Dyrk2 kinase gene rather than the *wingblister* locus as the gene affected by the *P[lArB]* insertion. This result was confirmed by testing flies containing a *PlacW* insertion in the 5′ exon of the Dyrk2 kinase gene (k11509) for olfactory avoidance behavior. The k11509 flies were completely anosmic as heterozygotes, indicating a dominant effect of the mutation on odor-guided behavior, and the wild-type olfactory behavioral phenotype could be restored following mobilization and precise excision of the *P*-element. Immunohistochemical visualization of the β-galactosidase reporter gene in k11509 flies indicated that the Dyrk2 kinase gene is expressed throughout the third antennal segment. Our observations show that phosphorylation events mediated by this enzyme are essential for the expression of normal olfactory behavior.

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62. Specialization of receptor neurons to naturally produced plant odours

H. Mustaparta, T. Røstelien and M. Stranden

Department of Zoology, Norwegian University of Science and Technology, N-7034 Trondheim, Norway

With the purpose to identifiy plant volatiles that are detected by receptor neurons, we have used gas chromatography linked to electrophysiological recordings from single receptor neurons in insects, termed GC-SCR (single cell recordings). Plant volatiles have been collected by drawing air around intact plants or cut plant materials through an adsorbant (head-space technique), followed by washing out the volatiles with a solvent. Single receptor neurons have been tested with these solutions by stimulation via the gas chromatograph (GC). The GC was installed with columns having a split at the end for leading half of the effluent over the insect antenna. Thus, the gas chromatogram of a mixture of volatiles and the effect of each compound could be recorded simultanously. Results obtained from species of beetles and moths have shown that plant odour receptor neurons respond

specifically to one compound or a few structurally similar compounds of which one usually has a marked best effect. Whereas 30 types of receptor neurons have been identified in the pine weevil (a specialist species on conifer trees), only 12–15 types of plant odour receptor neurons have been found in heliothine moths that are characterized as polyphagous species with a broad range of host plants. Furthermore, a large number of one particular receptor neuron type in the moth species suggests the significance of the compound that specifically activates this neuron type. Monoterpenes seems to be most important in the pine weevil and sesquiterpenes in the heliothine moths.

63. Orientation in complex odor landscapes: spatial arrangement of odor sources influences crayfish food-finding efficiency in streams

T.A. Keller, A.M. Tomba and P.A. Moore

Laboratory for Sensory Ecology, Bowling Green State University, Bowling Green, OH 43403, USA

Previous research has demonstrated that chemical stimuli play a fundamental role in the survival, growth and reproduction of organisms. While fluid hydraulics have been shown to alter orientation behavior of aquatic organisms to distant odor sources, it is unclear from these studies whether chemical signal structure or flow dynamics was responsible for differences in animal behavior. This study examined how alterations in chemical signal structure alone (through changes in source arrangement) affect crayfish search behavior to food resources in artificial streams. This study is unique in that we are altered signal structure while keeping hydrodynamics constant. Results demonstrate that crayfish (*Orconectes virilis*) found the source faster when the sources were separated than when they were together. However, the number of successful crayfish did not differ across treatments. Measurements of tracer molecules indicated that source arrangement affects the downstream fine-scale structure of odor plumes. We conclude that odor source arrangement strongly alters search efficiency for *Orconectes virilis,* and that future studies on the mechanisms of chemical orientation need to consider the role of chemical signal structure independent of hydrodynamics effects on chemosensory mediated orientation.

64. Is crayfish dominance communicated through recognition of individuals or dominance status?

R.A. Zulandt, R. Huber and P.A. Moore

Laboratory for Sensory Ecology, Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403, USA

Agonistic interactions are the primary determinants of dominance relationships in many other social systems. Recognition of dominance is essential for the success in future agonistic encounters and may involve recognition of either individuals or of dominance status. In crayfish, sensory mechanisms involved in such processes are most likely chemical or visual. Although preliminary studies had suggested an importance for chemical signals in general, they were not designed to distinguish between status or individual recognition. The present study now aimed to explore this question specifically in the crayfish. The behavioral characteristics of fighting were compared between winner–loser pairs which had fought with each other previously (WL+) and winner–loser pairs derived from different pairs (WL–). We quantified average duration and intensity for ensuing encounters, and estimated the likelihood to initiate, escalate or retreat from fighting. Dominance is recognized via status-specific signals and not via individual recognition as familiar and naive winner–loser pairs exhibited similar behavior. It would be advantageous for dominance status to be recognized by naive individuals, which would lead to dominance being transferred through some sort of visual or chemical signal and not individual recognition.

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65. Olfactory and trigeminal event-related brain potentials to attended and ignored stimuli

M.W. Geisler[1,2], C.B. Middleton[2], A. Dalve-Endres[2] and C.Murphy[1,2]

[1]University of California Medical Center, San Diego, CA and [2]San Diego State University, Department of Psychology, San Diego, CA, USA

Event-related brain potentials (ERPs) were recorded from 26 young adults with equal numbers of male and female subjects using attended and ignored olfactory and trigeminal stimuli. The amplitudes and latencies from the N1, P2 and P3 components were obtained using a single-stimulus paradigm, with an interstimulus interval of 60 s using amyl acetate as the olfactory stimulus and ammonia as the trigeminal stimulus. Subjects estimated the intensity of the stimulus in the attend condition or continued with a visual tracking task in the ignore condition. Odor thresholds were assessed using amyl acetate in a two-alternative forced-choice ascending method of limits procedure, odor sensitivity was measured with the Alcohol Sniff Test. The results indicate that olfactory information is processed 30–70 ms faster than trigeminal information for the N1 and P2 potential and 100 ms faster for the P3 ERP component. N1/P2 interpeak amplitude was greater for the trigeminal than the olfactory stimuli, and greater inthe attended than in the ignored condition. P3 amplitude wasgreater in the attend than ignore condition for olfactory information processing and equivalent for trigeminal information processing. The N1/P2 interpeak amplitude and P3 amplitude for both stimuli were maximally distributed over the Cz and Pz electrode sites, and were lowest at the Fz recording site. These findings suggest that neuronal resource allocation is greatest for attended stimuli and that a painful stimulus demands attentional resources even when ignored.

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66. Odorants increase the variance but not the amplitude of fMRI activation in the ventral temporal lobe of the human

N. Sobel[1], V. Prabhakaran[1], Z. Zhao[2], J.E. Desmond[2], G.H. Glover[3], E.V. Sullivan[1,2] and J.D.E. Gabrieli[1,2]

Departments of [1]Neuroscience, [2]Psychology, [3]Radiology, [4]Psychiatry and Behavioral Science, Stanford University, Stanford, CA 94305 USA

BOLD (blood oxygen level-dependent) fMRI has failed to show extensive increases in odorant-induced activation in primary olfactory (ventral temporal) regions of humans. This failure may be due to technical difficulties of measuring such an increase. Alternatively, the encoding scheme of odors in the ventral temporal region may be such that no such overall increase in blood flow occurs when odorants are presented. Here we test the possibility that odorants do not change the overall amplitude of

ventral-temporal fMRI activation, but do change the variance of this activation.

Eight subjects were scanned using methods previously described (Sobel *et al.*, 1998, J. Neurosci., 18: 8990–9001). Each subject was scanned once with each of four odorants: vanillin, decanoic acid, propionic acid and valeric acid. A functional region of interest (ROI) in the ventral-temporal region was constructed by choosing pixels that were significantly activated by sniffing. The amplitude and variance of activation induced by odorant presence in this ROI was then analyzed.

Odorants did not induce a significant change in amplitude of fMRI activation, but did induce a small but highly significant increase in the variance of activation [4% mean increase, $F(1) = 21.4$, $P = 0.002$].

It is tempting to relate this significant increase in variance ofactivation to a possible odorant-induced reduction in synchronization of neural activity in the ventral temporal regions. It remains to be explained, however, how synchronization and desynchronization of neural activity would map onto the fMRI signal.

67. Olfactory activity in the human cingulate cortex identified by FMRI

B. Kettenmann, S.T. Francis[1], R.W. Bowtell[1], F. McGlone[2], B.Renner, G. Ahne, E. Rolls[3] and G. Kobal

Department of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, Erlangen, Germany, [1]Magnetic Resonance Centre, School of Physics and Astronomy, University of Nottingham, [2]Unilever Research, Port Sunlight Laboratory, Wirral and [3]Department of Experimental Psychology, University of Oxford, Oxford, UK

From fMRI and PET studies it is well known that the cingulate cortex is involved in the processing of emotional aspects of pain.Since emotions are also crucial for odor perception, we hypothesized that the cingulate cortex is activated by olfactory stimuli, too. In order to investigate this hypothesis fMRI based on the BOLD-technique was applied. Imaging was performed using a 3.0 Tesla Echo Planar Imaging scanner. T_2*-weighted coronal images were obtained with a matrix size of 128 × 64 pixels. Anatomical localization was achieved using a multi-slice inversion recovery set of isotropic 3 mm resolution with grey matter nulled. Stimulus delivery was provided by a specialized olfactometer which allowed delivery of odorants with a defined delivery rate, temperature and humidity. Two odorants (Vanillin and H_2S) were delivered to both nostrils in five 501 ms bursts within an 'ON' period of 5 s. This was followed by a 15 s 'OFF' period when non-odorous air was delivered. In addition to the activation of 'primary olfactory', orbitofrontal and temporal areas, we identified significant signals arising from the cingulate cortex in all seven subjects for both odorants. Activated zones were not homogeneous across subjects. Anterior as well as posterior parts of the cingulum were involved. Due to the small number of subjects, systematic differences between odorants were not yet detectable. For the first time we could demonstrate that the cingulate cortex is active during olfactory perception. Future studies are necessary in order to investigate correlations between qualitative hedonic differences and functional topography of this part of the brain.

68. Functional mapping of different olfactory functions in humans

I. Savic[1,2], B. Gulyas[1], M. Larsson[3] and P. Roland[1]

[1]Division of Human Brain Research, Department Neuroscience, [2]Department of Neurology and [3]Department of Clinical Neuroscience and Family Medicine, Division of Geriatric Medicine, Karolinska Institute, Stockholm, Sweden

Introduction: How the brain processes different olfactory functions is currently unknown. To map the underlying networks we employed PET measurements during monorhinal, passive smelling of odorless air (AS), of single odors (OS), during discrimination of odor intensity (OD-i), odor quality (OD-q) and during tests of odor recognition memory (OM).

Methods: Eighteen healthy females participated. The same odor was presented in different concentrations during OD-i, and different odors during OD-q. OD-i and OD-q were tested using paired stimuli in a stronger–weaker and same–different paradigm respectively. In the OM task 10 target and 10 foil odors were presented 60 min after the encoding. The decision was whether thetest odor had been presented during the encoding or not. Significant changes in rCBF during OS were calculated in relation to AS, and during OD-i, OD-q and OM in relation to the collapsed data from AS and OS ($P < 0.05$ corrected, SPM96).

Results: The four olfactory functions shared activation of the right pyriform and orbitofrontal cortex and/orthe left insular cortex, independent on the side of presentation. OD-i also activated the right cerebellum. In addition to these areas, OD-q activatedthe right subiculum, caudate, frontal operculum and midbrain. OM also involved the right thalamus, right middle temporal gyrus, right prefrontal cortex and precuneus, but not the caudate and presubiculum.

Conclusion: The tested olfactory functions are subserved by four levels of hierachically organized brain regions, with pyriform, orbitofrontal and/or insular cortex as always activated core regions. Adding stepwise more regions, which are all directly or indirectly connected to the core regions, subserves intensity discrimination, odor distinction and recognition memory.

69. Olfactory epithelial morphology in children with and without Rett syndrome

D.A. Leopold, X. Cai, S.K. Naidu, M.B. Yablanski, T. Loehrl and G.V.Ronnett

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

Previous studies have shown that olfactory neurons from children with Rett syndrome have few mature characteristics. Normal olfactory histology has not yet been determined for children. To determine whether olfactory neurons can give more information on Rett syndrome, and whether these changes are different from normal children, an expanded biopsy study has been performed. Olfactory biopsies were done on 30 children (ages 3–17 years) and 25 controls (ages 1–15 years). The tissue was processed for light microscopy with several different stains. The normal controls had olfactory histology similar to that already described in adults. Because of the small size of the biopsies, the density of the olfactory area in children cannot be determined. The samples from the children with Rett syndrome have fewer OMP-positive neurons. They also have more NST-positive neurons. The olfactoryneurons in Rett syndrome are clearly abnormal, and may

not only serve as a marker for the disease but also allow further delineation of the disease process.

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70. Neurophysiological differences between embryonic rat trigeminal and geniculate ganglion cells in culture

S. Al-Hadlaq, R.M. Bradley[1], D.K. MacCallum[2] and C.M. Mistretta[1]

Oral Health Sciences Ph.D. Program, Dentistry, [1]Biologic and Materials Sciences, School of Dentistry and [2]Anatomy and Cell Biology, Medical School, University of Michigan, Ann Arbor, MI 48109, USA

During embryonic development, nerves grow from trigeminal and geniculate ganglia to target sensory organs, the fungiform papillae, in the anterior tongue. We are studying functional differentiation of these ganglia during the period of neurite extension to targets. To understand basic electrophysiological properties of the embryonic ganglion cells, whole cell recordings were made from trigeminal and geniculate ganglia from rat embryos at 16 days of gestation; at this stage neurites from trigeminal and geniculate ganglion cells provide a dense innervation of fungiform papillae, although taste buds have not yet developed. Embryonic ganglia were dissected and cultured for 4–8 days, in medium supplemented with the appropriate neurotrophin for ganglion support: nerve growth factor for the trigeminal ganglion and brain derived neurotrophic factor for the geniculate. Whole cell recordings were made from 72 trigeminal and 68 geniculate neurons. Trigeminal neurons had a significantly larger membrane time constant, action potential amplitude, threshold to spike and falling slope on the action potential than geniculate cells. In addition, a greater proportion of trigeminal neurons exhibited multiple action potentials compared with geniculate. Differences in inflections on the falling slope of the action potential also distinguished between cells from these ganglia. The recordings indicate that at an early stage of functional development, when papillae are innervated but before taste buds have formed, trigeminal and geniculate neurons already differ in passive membrane and action potential properties.

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71. Assessing trigeminal-based repellents *in vitro*: comparative studies

A. Savchenko, B. Bryant, J.R. Mason[1] and L. Clark[2]

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, [1]USDA/ADC, Utah State University, Logan, UT 84322 and [2]USDA National Wildlife Research Center, Fort Collins, CO 80521-2154, USA

Study of the chemical sensitivity of the trigeminal system has largely focused on a few domesticated mammals. In order to design, synthesize and evaluate novel chemical repellents against other mammalian and avian species, deeper understanding of the comparative aspects of trigeminal chemical sensitivity would be extremely helpful. Specifically, are there similarities and differences in the underlying mechanisms and their cellular distribution that would allow the design of taxon-specific repellents? We measured the changes in intracellular calcium in cultured trigeminal neurons from rat, chicken, white-tailed deer (*Odocoileus virginiana*) and coyote (*Canis latrans*), induced by prototypical pain-inducing andirritating compounds. Consistent with behavioral data, neurons from mammals responded robustly to capsaicin (1 μM), bradykinin (1 μM) and acetylcholine (10 μM), and neurons from chicken responded robustly to methyl anthranilate (1 μM) and not strongly to capsaicin. However, different from what behavioral and physiological data would suggest, mammalian and chicken neurons were not completely insensitive to irritants that are differentially active in behavioral assays; some mammalian neurons responded to methyl anthranilate and some avian neurons responded to capsaicin. This discrepancy may be explained by differential barrier properties of epithelia and therefore, access of stimuli to nerve endings.

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72. Expression of an acid sensitive ion channel (ASIC) in cultured rat trigeminal neurons

T. Huque, B.P. Bryant and S.A. Mackler[1,2]

Monell Chemical Senses Center, Philadelphia, PA 19104, [1]University of Pennsylvania, Philadelphia, PA 19104 and [2]Veterans Affairs Medical Center, Philadelphia, PA 19104, USA

A family of proton-gated ion channels that function as acid sensors has been cloned recently. To determine whether the mRNA encoding the isoform ASICI is expressed in rat trigeminal neurons, total RNA was extracted from cultured cells and reverse transcribed into the cDNA. RT-PCR amplification was performed using a primer pair derived from published sequences. A single band of the expected size (657 bp) was obtained, gel-isolated and subcloned into pGEM-T-Easy. The sequence of this cDNA was the same as that for both brain and dorsal root ganglion ASICI is expressed with respect to capsaicin sensitivity, the neurotoxicity ofcapsaicin was used to eliminate a specific subpopulation of trigeminal neurons. Samples were then treated with either capsaicin (5 μM) or EtOH (solvent control). After 16 h the cells were lysed, total RNA was extracted and RT-PCR performed. ASICI mRNA was present in both treated and control samples. These data indicate that ASICI is expressed in cultured rat trigeminal neurons and can still be detected after exposure to a concentration of capsaicin that would be expected to cause specific neuronal death.

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73. The effect of specific *n*-acetylcholine receptor blockers on nasal trigeminal nerve responses to R- and S-nicotine in rats

B. Renner, F. Meindorfner, M. Kaegler[1], N. Thuerauf[2], A. Barocka[2] and G. Kobal

Institute of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, D-91054 Erlangen, [1]INBIFO Institut fürbiologische Forschung GmbH, D-51149 Cologne and [2]Department ofPsychiatry, University of Erlangen-Nuremberg, D-91054 Erlangen, Germany

In a previous study we showed that the nasal trigeminal system can distinguish between nicotine enantiomers. We also demonstrated that both stereoisomers could activate the same neuron and that responses to S-nicotine were suppressed by hexamethonium.

The aim of the current study was to identify the receptor types involved in the chemoreception of nicotine. Extracellular single cell recordings were taken from the Gasserian ganglion in rats after nasal stimulation with R- and S- nicotine (stimulus duration 1350

ms; R- and S-nicotine vapor 90 μg/l). Gaseous CO_2 was used as the control stimulus for trigeminal receptors (concentration: 80% v/v). The nicotinic acetylcholine (nACh) blockers hexamethonium and mecamylamine were topically administered in increasing concentrations to the nasal mucosa before stimulation. The local anesthetic oxybuprocain-hydrochloride was tested in the same way with the three different stimulants.

Trigeminal responses to R- and S-nicotine were blocked with concentrations of hexamethonium and mecamylamine that did not affect the responses to CO_2. The effective concentration of mecamylamine was lower than that of hexamethonium for both enantiomers. No response to the three stimulants was observed following nasal administration of the local anesthetic drug. In ongoing experiments with dihydro-β-erythroidine hydrobromide (DHBE) we will further investigate subtypes of the nACh receptors involved in the chemoreception of R- and S-nicotine.

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74. Role of neuronal nicotinic receptors in the activation of neurons in trigeminal subnucleus caudalis by nicotine delivered to the oral mucosa

E. Carstens[1], C.T. Simons[1,2], J.-M. Dessirier[1,2], M. Iodi-Carstens[1] and S.L. Jinks[1]

[1]*Section of Neurobiology, Physiology & Behavior and* [2]*Department of Food Science and Technology, University of California, Davis, CA 95616, USA*

Noxious stimuli delivered to the oral cavity excite trigeminal nociceptors, which send axons into the brainstem to activate neurons in the trigeminal nuclear complex. Using immunohistochemical methods we showed that application of a variety of irritant chemicals to the tongue resulted in increased expression of *c-fos* in neurons in superficial laminae of the dorsomedial aspect of trigeminal caudalis, as well as in other areas. To determine if individual neurons are excited by different irritant chemicals, we recorded from single units in dorsomedial caudalis that responded to mechanical and noxious thermal stimulation of the tongue in anesthetized rats. A large proportion of such neurons responded in a concentration-dependent manner to topical application of a wide variety of irritant chemicals to the tongue, including capsaicin, nicotine, histamine, NaCl, acid, ethanol and others. Neurons responded on average to 75% of the 10 irritants tested. Responses to certain chemicals (capsaicin, nicotine, piperine) exhibited desensitization. Histamine- and nicotine-evoked responses were selectively reduced by respective pharmacological antagonists. Similar findings were made for a separate population of neurons in ventrolateral caudalis that responded to irritant chemicals delivered to the corneal surface. These results indicate that a subpopulation of trigeminal caudalis neurons responds indiscriminately to a variety of irritant chemicals, at least partly via separate molecular transduction mechanisms. Such neurons might mediate a 'common chemical sense' or, alternatively, signal different qualities of chemical irritation based on an across-fiber population code similar to taste.

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75. *C-fos* expression in trigeminal nucleus caudalis neurons evoked by application of carbonated water to the tongue is reduced by blockers of carbonic anhydrase

S.L. Jinks[1], C.T. Simons[1,2], J.-M. Dessirier[1,2], M. Iodi Carstens[1] and E. Carstens[1]

[1]*Section of Neurobiology, Physiology & Behavior and* [2]*Department of Food Science and Technology, University of California, Davis, CA 95616, USA*

We hypothesize that the tingling sensation of carbonated drinks is due to excitation of intraoral nociceptors by carbonic acid, which is formed from CO_2 in a reaction catalyzed by carbonic anhydrase. The nociceptors in turn excite neurons in the brainstem trigeminal complex that signal oral irritation. We used *c-fos* immunohistochemistry to determine if the activation of brainstem neurons by carbonated water is reduced by carbonic anhydrase blockers. In anesthetized rats, carbonated water (50 ml/10 min) was flowed over the tongue which had been pretreated with topical application of (1) isotonic saline, (2) acetazolamide (1%) or (3) dorzolamide (1%). Saline-treated or untreated rats not receiving carbonated water served as controls. Two hours later animals were perfused with 3% paraformaldehyde. Brainstem sections were immunohistochemically processed for fos-like immunoreactivity (FLI). Animals receiving carbonated water + saline showed a significant increase in FLI above saline controls in superficial laminae of the dorsomedial aspect trigeminal nucleus caudalis. Carbonated water also evoked significantly higher FLI compared with unstimulated controls in the nucleus of the solitary tract, ventrolateral caudalis and ventrolateral medulla. Pretreatment with either acetazolamide or dorzolamide significantly reduced FLI in dorsomedial caudalis evoked by carbonated water, compared with animals receiving carbonated water + saline. These results support the hypothesis that carbonated water activates lingual nociceptors via conversion of CO_2 to carbonic acid; the nociceptors in turn excite trigeminal neurons involved in signaling oral irritation.

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76. Responses of neurons in trigeminal nucleus caudalis to intraoral application of carbonated water are reduced by dorzolamide, a blocker of carbonic anhydrase

C.T. Simons[1,2], J.-M. Dessirier[1,2] and E. Carstens[1]

[1]*Section of Neurobiology, Physiology & Behavior and* [2]*Department of Food Science & Technology, University of California, Davis, CA 95616, USA*

We have further tested the hypothesis that the tingling sensation of carbonated drinks is due to excitation of intraoral nociceptors by carbonic acid formed from CO_2 in a reaction catalyzed by carbonic anhydrase. These nociceptors in turn excite neurons in the trigeminal nuclear complex that signal oral irritation. We used electrophysiological methods to determine if single neurons in superficial laminae of the dorsomedial aspect of trigeminal caudalis of anesthetized rats respond to application of carbonated water on the tongue in a manner that is reduced by the carbonic anhydrase blocker, dorzolamide.

Ten wide dynamic range-type neurons recorded in superficial dorsomedial caudalis responded to noxious heat (54°C) and

mechanical stimulation of the tongue. All additionally responded to carbonated water flowed over the tongue (6 ml/45 s; pH = 4.7), and some of the neurons tested also responded to HCl (pH = 1). Following topical application of dorzolamide (1%), the mean response of the 10 neurons to carbonated water was significantly reduced, with recovery over the ensuing 45 min. Dorzolamide did not affect neuronal responses to noxious heat or HCl when tested. These data provide further support for the hypothesis that the sensation elicited by carbonated water is of chemogenic origin, and underscore the importance of neurons in trigeminal nucleus caudalis as a relay in central pathways mediating oral irritation.

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77. Oral irritation by carbonated water is reduced by the carbonic anhydrase inhibitors, acetazolamide and dorzolamide

J.-M. Dessirier[1,2], C.T. Simons[1,2], M. O'Mahony[2] and E. Carstens[1]

[1]Section of Neurobiology, Physiology & Behavior and [2]Department of Food Science & Technology, University of California, Davis, CA 95616, USA

The sensation produced by carbonated drinks is hypothesized to involve activation of intraoral nociceptive trigeminal nerve endings by CO_2, which is converted to carbonic acid in a reaction catalyzed by carbonic anhydrase. We investigated if pretreatment with carbonic anhydrase inhibitors (acetazolamide, dorzolamide) affected irritation by carbonated water using a two-alternative forced-choice discrimination test coupled with magnitude ratings. When acetazolamide (1%) was swabbed onto one side of the tongue, followed by immersion of the entire tongue into fresh carbonated water (6.6 g CO_2/l), significantly more subjects chose the non-treated side as yielding stronger irritation and assigned significantly higher intensity ratings of irritation to that side. Acetazolamide had no effect on irritation produced by citric acid (125 mM) or on tactile sensitivity. After pretreatment with dorzolamide (2%), a significant majority of subjects also chose the non-treated side to yield stronger irritation and assigned significantly higher intensity ratings to that side, when carbonated water was delivered to the tongue for 5 s by pressurized flow. However, dorzolamide did not significantly reduce irritation when carbonated water was delivered for 15 s, possibly due to greater diffusion of CO_2. Dorzolamide had no effect on irritation from pentanoic acid (200 mM) or on tactile sensitivity. These results support the hypothesis that the irritation of carbonated water results partly from the conversion of CO_2 to carbonic acid to excite chemonociceptive lingual afferent fibers.

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78. Oral irritation by carbonated water is reduced by capsaicin desensitization

M. O'Mahony[1], J.-M. Dessirier[1,2,3], C.T. Simons[1,2] and E. Carstens[2]

[1]Department of Food Science & Technology, [2]Section of Neurobiology, Physiology & Behavior, University of California, Davis, CA 95616, USA and [3]Department Sciences Alimentaires, ENSIA, Massy, France

The tingling sensation produced by carbonated drinks is thought to be of chemogenic origin, whereby CO_2 stimulates chemosensitive nociceptive endings in the oral epithelium. Capsaicin is known to desensitize nociceptors, prompting us to investigate if pretreatment of the tongue with capsaicin cross-desensitizes the irritant sensation produced by carbonated water. We used a two-alternative forced-choice discrimination test coupled with intensity ratings. In the first experiment, capsaicin (5 ppm) was applied to one side of the tongue five times at 1 min intervals, followed by a 10 min rest period. Subjects then immersed the entire anterior tongue into carbonated water (6.6 g CO_2/l). A significant majority chose the untreated side of the tongue as yielding stronger irritation, and intensity ratings were significantly higher on that side. In the second experiment, capsaicin (33 ppm) was applied once to one side of the tongue, after which carbonated water was delivered to the tongue bilaterally by pressurized flow for 5 or 15 s. Subjects again reported the untreated side to yield a stronger sensation and assigned significantly higher intensity ratings to that side, although the difference between the two sides was less for the 15 versus 5 s stimulus. CO_2, a small lipophilic molecule, might have diffused further during the 15 s stimulus to activate undesensitized fibers. These results indicate that capsaicin cross-desensitizes irritation from carbonation water, suggesting that a fraction of the sensory fibers involved are capsaicin-sensitive.

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79. The effect of capsaicin desensitization on differential sensitivity for sour taste

J.R. Zuniga and N. Chen[1]

Department of Oral and Maxillofacial Surgery, University of North Carolina, Chapel Hill, NC 27599-7450, USA and [1]Department of Oral and Maxillofacial Surgery, Nanjing Medical University, Nanjing, P. R. China 210029

Two experiments were conducted to measure the ability of 25 human subjects to resolve just noticeable differences (JND) in sour taste using suprathreshold concentrations of citric acid. In the first experiment, the sip-and-spit method with a 2AFC, modified staircase procedure was used to measure whole mouth JND. Five suprathreshold concentrations of citric acid separated by 0.5 log units (0.0003–0.03 M) served as standard taste stimuli. The JND in sour taste intensity, ΔI, was the percent difference between standard and a comparison stimuli that was 0.04 M log units in the direction of the standard stimuli. The first experiment revealed that subjects' ΔI varied with the molar concentration of citric acid in whole mouth testing. A difference in concentration of 38% was measured for concentrations of 0.003, 0.001 and 0.03 M. A difference of 57% was measured for concentrations of 0.001 and 0.0003 M. In the second experiment, desensitization of the oral mucosa was accomplished using capsaicin (10ppm) prior to measuring subjects' ΔI with standard stimuli of 0.003 and 0.03 M. On average, subjects' resolved a 24% reduction in the ΔI of 0.03 M citric acid that was statistically different from pre-capsaicin ΔI. There was no statistical difference in pre- and post-capsaicin ΔI of 0.003 M. The different effects of capsaicin desensitization on 0.003 and 0.03 M citric acid sour taste intensity may be due to the differential effect of capsaicin desensitization of the irritation of oral mucosa by stronger concentrations.

80. Time course of capsaicin burn to a double-step input

D.H. McBurney, C.D. Balaban[1], M. Affeltranger, A. Deithorn and A.Puskar

Departments of Psychology and [1] Otolaryngology and Neurobiology, University of Pittsburgh, Pittsburgh, PA 15261, USA

Previous experiments in this series showed that psychophysical responses to capsaicin can be modeled as the sum of three processes: a phasic (or 'change' detection) mechanism, a tonic (or 'level' detection) mechanism and a rising function that may be characteristic of painful stimulation. Here we present the results of two experiments using a double step in concentration. In the first, subjects rated the burn of capsaicin for 40 min. Thirty ppm capsaicin was presented for the first 15 min, followed immediately and without explanation by 300 ppm for 25 min. Computer simulation using time constants from our previous studies shows that, with the addition of a parameter representing the effect of concentration, the model provides a satisfactory fit to the data (r^2= 0.99). The second experiment presented 300 ppm for 24 min followed immediately and without explanation by 10 ppm for an additional 22 min. The reduction in concentration during the second period caused an undershoot in the response, reflecting the phasic process.

81. Effects of long-term exposure to trigeminal irritants

P. Dalton, T. Hummel[1] and D.D. Dilks

Monell Chemical Senses Center, Philadelphia, PA 19104, USA and [1]Department of Otorhinolaryngology, University of Dresden, Dresden, Germany

Chronic or repetitive exposure to an olfactory stimulus typically results in olfactory adaptation, a stimulus-specific decrease in the detectability and perceived intensity of the olfactory stimulus. In contrast, some reports have suggested that similar exposure to a chemosensory irritant leads to increases in sensitivity, although other evidence suggests that adaptation does occur for irritants. This study examined whether repetitive exposure to an irritant stimulus modulates either the psychological or the physiological response to that chemical. Using a long-term adaptation protocol, we exposed eight men and four women to acetic acid vapor in their home environment. Before, during and after 3 weeks of exposure to acetic acid, we obtained chemosensory event-related potentials (CSERP) as a measure of central processing, nasal mucosal potentials (NMP) as a measure of peripheral response to sensory irritation, intensity ratings and lateralization thresholds to three concentrations of acetic acid (exposure odorant) and three concentrations of acetone (control odorant). Reaction times were also obtained as measures of stimulus detectability. The CSERP and NMP showed good agreement with the psychophysical measures. Both the amplitude of the physiological measures (CSERP and NMP) and the ratings of intensity increased with stimulus intensity, and, on all measures, responses to acetic acid decreased during and following exposure, indicating a long-termeffect from exposure to acetic acid both peripherally and centrally. In contrast, responses to acetone showed similar effects of stimulus intensity but little change over the course of long-term exposure to acetic acid.

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82. Structure–activity relationship of analogs of plant unsaturated alkylamides

I. Mezine and B. Bryant

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA

Recently we reported the isolation and electrophysiological characterization of several plant-derived sensory irritants belonging to unsaturated alkylamides. Those compounds, when applied to the human tongue, give rise to a sensation described as tingling or buzzing. The prior studies have identified the type of trigeminal neurons that are activated by these compounds. In order to investigate the molecular features of the compounds thatare required for specific activity, we have synthesized and confirmed the structure of a number of analogs. The structure ofthe natural irritants can be divided in three regions: the alkylregion (A), amide region (B) and hydrophobic chain (C). Accordingly, the analogs were different in type of alkyl group (A region), O-alkyl analogs and corresponding free acids (region B). Analogs in region C were different in chain length, as well as in number, position and configuration of double bonds. The activity of the analogs was quantified by determination of intracellular Ca^{2+} uptake by cultured trigeminal neurons. Due to the high hydrophobicity of the analogs, we developed a method for the reliable delivery of the analogs in organic solvent—free aqueous media using a particular cyclodextrin derivative (O-methylated β-cyclodextrin). It was found that O-alkyl analogs, the corresponding acids and analogs in region C lacking 2,3-double bond were inactive, and therefore the conjugated alkylamide moiety is necessary for specific activity. Activity of the analog with a shorter hydrophobic chain was decreased. Activity of the compounds wasvery sensitive to varying the level of unsaturation of the hydrophobic chain and conformation of the double bonds.

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83. Interactions of tannins and human salivary proteins assessed by turbidity measurements

H.T. Lawless, C. Hartono, J. Horne and K.J. Siebert[1]

Cornell University Department of Food Science, Ithaca, NY 14853 and [1]Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA

One proposed mechanism generating astringency sensations in humans is the binding of salivary proline-rich proteins by tannins, reducing the mucus coating on oral surfaces and creating sensations of oral dryness. In beverages such as beer and fruit juices, the generation of chill haze by the interaction of polyphenols andproline-rich proteins has been studied using turbidimetry (K.Siebert and P. Lynn, 1997, J. Food Sci., 62: 79). This study examined the turbidity of mixtures of filtered human saliva withtannic acid. Turbidity was measured using a Hach Model 2100AN ratio turbidimeter and increased with increasing tannin concentration over a range of 0–1.58 g/l, from a baseline near zero to 42 Nephelos turbidity units (NTUs) depending upon the individual saliva, time of mixing (30 s or 45 min, with increasing haze development over incubation time) and the ratio of saliva to tannin solution volume. Saliva filtered through Whatman #1 filter

paper had a lower baseline level of turbidity than saliva filtered through cheesecloth and a ratio of 4 ml saliva to 4 ml tannin solution provided the highest turbidity levels. Turbidity measured over a psychophysically meaningful concentration range paralleled astringency response and thus may provide an *in vitro* objective correlate of psychophysically measured astringency.

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84. The influence of gender, allergic rhinitis, and test system on perceptual acuity to nasal irritants

D. Shusterman, R. Loya and J. Balmes

University of California, San Francisco, CA 94143, USA

Nasal irritant sensitivity is an important construct in environmental health science; functional measures, however, lack standardization. We performed duplicate measures of nasal irritant perceptual acuity on 12 subjects (evenly divided by gender, five with and seven without seasonal allergic rhinitis) using two different test systems: (1) carbon dioxide (detection) and (2) *n*-propanol (localization). The *a priori* hypotheses included: (1) rhinitics will display lower thresholds than non-rhinitics; (2) females will display lower thresholds than males; and (3) estimates of perceptual acuity using the two test systems will be positively correlated. CO_2 detection thresholds were obtained using an ascending series, method of limits, presenting 3 s pulses of CO_2 (15–45% v/v), paired with air in random order (12 s ISI), dirhinally by nasal cannula. The detection threshold was defined as the lowest level at which a subject could distinguish the CO_2 ('irritating') stimulus from air on four consecutive trials 45 s apart. Localization thresholds were obtained by simultaneously presenting stimuli (*n*-propanol vapor at 4063–23 700 ppm in air) and blanks (saturated water vapor in air) to opposite nostrils, with laterality randomized. The localization threshold was defined as the lowest concentration at which the subject could correctly identify laterality on six trials 60 s apart. Although individual mean CO_2 and propanol thresholds were positively correlated ($P<0.05$), only propanol localization thresholds were predicted by gender and allergic rhinitis status in univariate and multivariate models (females and rhinitics showing lower thresholds than their counterparts). The questions of susceptibility markers for nasal irritant sensitivity and the generalizability of nasal irritant perceptual acuity across test compounds deserve further attention.

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85. Objective correlates of nasal irritation caused by exposure to ethanol vapor

R.A. de Wijk, A. Jalowayski, G. Pilla-Caminha and W.S. Cain

Departments of Surgery and Pediatrics, University of California, San Diego, CA, USA

The research explored the question of whether subchronic exposure to a subjectively just-irritating level of a volatile organic compound (VOC) causes objective changes in nasal mucosal functioning. If so, then these might supplement subjective responses and even offer some indication of why a vapor irritates in the first place. Potential objective effects include neurophysiological responses [negative mucosal potential NMP)], secretions [weight of secretion, albumin (protein) content from extravasation into the mucosa], leukocytic cells [number of polymorphonuclear leukocytes (PMNs)], mucociliary functioning (clearance time) and patency (nasal expiratory flow patterns and nasal airway resistance). A 45 min exposure to ethanol vapor (37°C, 80% RH) at 15% v/v, flowed continuously into the nose but not inhaled, caused various changes, some specific to the presence or absence of signs of existing inflammation in the 19 subjects studied. (Clean air served as control.) Amplitude of the NMP, for example, was enhanced by the exposure to ethanol. Nasal airway resistance increased, as did weight of secretions, but just in subjects free of existing inflammation. On the other hand, expiratory flow increased in persons with evidence of existing inflammation. Hence, mucosal inflammatory status plays a role in responsiveness to exposure. Screening subjects for inflammatory status should play a role in studies of how exposure to VOCs reflects itself in mucosal effects.

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86. Predicting everyday responses from psychophysical data: problems encountered and a solution proposed

J.C. Walker[1,2], V.V. Polyakov[2], V.L. Connell[1], A.D. Barreto[3], M.Kendal-Reed[2], M.A. Howell[1] and C.J. Smith[1,3]

[1]R & D, R. J. Reynolds Tobacco Co., Winston-Salem, NC 27102, [2]University of North Carolina School of Dentistry, Chapel Hill, NC 27599 and [3]Wake Forest University School of Medicine, Winston-Salem, NC 27103, USA

Everyday exposures to ambient mixtures of odorants or irritants typically last from several min to a few hours, and cause stimulation of both ocular (trigeminal) and nasal (olfactory and trigeminal) chemoreceptors. However, laboratory studies with human participants typically record only perceptual judgements following brief (a few seconds) presentations of a single compound to only the nose. In many cases, the concentrations actually presented may not be known. Quantitative models to predict the impact of everyday exposures to environmental chemicals are needed. We have developed a protocol for providing input data for such models. Normal and anosmic individuals (both genders, aged 21 to 70) are exposed (nasal only, ocular plus nasal) during 90 min test sessions, conducted in a 46.1 m^3 controlled environment room, to precisely controlled concentrations (0.01, 0.1, 1, 10, 15 ppm) of propionic acid. Breathing parameters and propionic acid concentration are recorded continuously, with the following measured intermittently: eye blink rate, sensory/symptom ratings of room air, psychological state, information processing speed, blood pressure and heart rate.

87. Ocular trigeminal chemoreception: comparison with nasal trigeminal chemoreception and development of a quantitative structure–activity relationship (QSAR)

J.E. Cometto-Muñiz, W.S. Cain, M.H. Abraham[1] and R. Kumarsingh[1]

Chemosensory Perception Laboratory, Department of Surgery (Otolaryngology), University of California, San Diego, La Jolla, CA92093-0957, USA and [1]Department of Chemistry, University College London, 20 Gordon Street, London WC1 0AJ, UK

Using members of homologous chemical series we have measured

eye irritation thresholds and detectability (i.e. psychometric) functions for eye irritation. Stimuli were delivered from 'squeeze bottles' and from recently developed glass vessels adapted for ocular exposures. Gas chromatography served to measure vapor-phase concentrations in all containers. The sensory method consisted of a two-alternative forced-choice procedure with presentation of ascending concentrations. Subjects tested included normosmics and anosmics. The results showed that eye irritation thresholds fell close to nasal pungency thresholds, and declined with carbon chain length in all series tested, as seen before with odor and nasal pungency thresholds. The detectability function for eye irritation fell into register with that for nasal pungency for the stimuli 1-butanol, butyl acetate, and toluene, and was slightly shifted towards lower concentrations for 2-heptanone. Ocular trigeminal sensitivity, whether measured as detection thresholds or as detectability functions, was similar in normosmics and anosmics for all substances.

A single QSAR, based on a solvation equation model, successfully described human eye irritation thresholds and Draize eye scores in rabbits. The approach had served well in the past to describe nasal pungency thresholds. The applicability of the model to the two trigeminal responses suggests that the rate of transfer of the stimulus from one (bio)phase to another or its distribution between (bio)phases forms a key step for both chemoreception processes.

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88. Measurement of eye redness from exposure to vapor

A.A. Jalowayski, R.A. de Wijk and W.S. Cain

Chemosensory Perception Laboratory, UCSD, La Jolla, CA 92093-0957, USA

Exposure of the eyes to vapors can induce irritation, which may reveal itself objectively as redness. In development of noninvasive techniques to assess magnitude of redness, we have devised ways to create images of the eyes and present these for evaluation in pair-comparison by judges. We will illustrate the technique with data for 10 subjects (18–44 years old) exposed to 15% v/v ethanol vapor (37°C, 80% RH) uniocularly (left eye) at 1.5 l/min for 45 min via a modified swimmer's goggle. Exposure to clean air served as a control. The level of ethanol equaled twice the psychophysical threshold for irritation in momentary (5 s) exposures. The longer exposures caused little or no sustained feelings of irritation. A slit-lamp table, modified with precision-arms to hold a camera, reflecting umbrella and flash, and a small fill-in flash, provided the scaffolding to photograph exposed and unexposed eyes with an Olympus OM-1 camera with Ektachrome Elite 100 ASA slide film and 100 mm macro lens. A slide scanner digitized the images, which were cropped to uniform size and displayed contiguously via high-intensity projection. A panel of 10 judges evaluated 480 pairs for the image with the greater redness in each instance. The index d' showed that exposure to clean air caused no change in redness, whereas exposure to ethanol caused a significant increase. The results will be compared with other measurements, such as tear-film break-up and weight and composition of ocular secretions, made on the same subjects.

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89. Culture-specific chemosensory drivers of food preference in Japan, Indonesia, Singapore and Australia

G.A. Bell and H.J. Song

Centre for ChemoSensory Research, University of New South Wales, Australian Technology Park, Sydney, Australia, 1430

A person's food preferences are largely determined by experience, which in turn is generated by their culture. The highly variable importance of chilli in the diets of four cultures studied here (low in Japan; low to medium in Australia; medium in Singapore; and high in Indonesia) suggests that chemosensory experience may create a bias toward one or more of the chemosensory modalities in driving food preferences.

Sensory evaluations were performed by consumers in four countries on a number of different food products but with generally the same perceptual assessments required of all subjects: Japan, 44 products and 285 people; Singapore, 10 products and 100 people; Indonesia, 56 products and 105 people; Australia, 22 products and 75 people. Data were obtained from unstructured scales of perceived sensory intensity for taste (sweet, sour, salty, bitter), overall olfactory strength, trigeminal (oral burn), as well as overall liking of the product.

In Japan, perceived taste of sweetness, saltiness and umami were common drivers of food preference. As predicted, Japanese consumers considered attenuated trigeminal components of flavour (low level of acidity and low chilli burn) as very important. Australian consumers revealed by contrast an optimal, medium level of spiciness that determines preference. Singaporean consumers revealed that perceived sourness and chilli strength positively determined preference. Drivers of overall liking of products for Indonesian consumers included chilli, pepper and odour.

While previous research has generally shown very little difference between members of cultures in chemosensory perceptual judgements, this overview of recent studies in the four countries in which a common psychometric procedure was used in all four, suggests that culturally driven experience builds up in different chemosensory modalities such that preference becomes driven in different ways in each culture.

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90. Genetic taste status associates with fat food acceptance and body mass index in adults

V.B. Duffy[1,2], K. Fast[2], Z. Cohen[2], E. Chodos[2] and L.M. Bartoshuk[2]

[1]*School of Allied Health, University of Connecticut, Storrs, CT 06269-2101 and* [2]*Department of Surgery, Yale School of Medicine, New Haven, CT 06520, USA*

Humans show genetic variation in taste and oral somatosensation. One marker for this variation is 6-*n*-propylthiouracil (PROP) bitterness; concentrated PROP is intensely bitter to supertasters and weak or tasteless to nontasters. Supertasters also give highest intensity ratings to oral somatosensory stimuli (e.g. dairy fat, oils, and irritants). This genetic variation has associated with fat liking/disliking and body mass index (BMI) in small samples of adults. We examined the association between PROP bitterness

(delivered on impregnated filter paper) and liking/disliking of nine high-fat foods and BMI. Subjects (360 females and 249 males, aged18–92 years) rated PROP bitterness and degree of liking/disliking on the Labeled Magnitude Scale (Green *et al.*, 1993). The foods formed a statistically reliable group. Females rated highest PROP bitterness, though the sex difference was not significant inolder women (>50 years). Older women also had fewer supertasters than did younger women. PROP bitterness showed a negative relationship with BMI, the effect being strongest in normal weight individuals; however, a positive relationship was observed in overweight/obese young adults. PROP bitterness wasnegatively correlated with liking of high-fat foods in females; this association was independent of the effects of BMI and age. Inmen, only age contributed significantly to fat liking. These datasupport previous findings of a relationship between higher PROP tasting and lower liking of high-fat foods in females. The relationship between BMI and PROP may depend on age, sex and degree of obesity.

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91. Genetic sensitivity to 6-*n*-propylthiouracil (PROP) influences food preferences in preschool children. 22. Human taste and psychophysics

K.L. Keller, L. Steinmann, R.J. Nurse and B.J. Tepper

Department of Food Science, Rutgers University, New Brunswick, NJ 08901, USA

Individuals who can taste the bitter compound 6-*n*-propyltiouracil (PROP) are more sensitive to bitter and fat tastes in foods (Tepper, 1998, Am J. Hum. Genet. 63: 1271–1276). While adults have been the most studied population, sensitivity to PROP might play a rolein the development of food preferences during childhood. Inthis study, we tested the hypothesis that 4- to5-year-old childrenwho are PROP tasters will dislike foods which are higher in bitter and fat intensities. Fifty-two preschool children were classified as tasters ($n = 33$) or nontasters ($n = 19$) of PROP using a suprathreshold screening solution. Children used a five-point facial hedonic scale to rate liking for common foods which differ in bitter (raw and cooked broccoli, orange juice and grapefruit-orange juice, semisweet and milk chocolate, American and sharpAmerican cheese) or fat (whole and skim milk, turkey andbeef hotdogs) components. Foods were randomly presented, one at a time. Tasters of both genders liked American cheese less than nontasters ($P < 0.05$). Taster girls liked raw broccoli and whole milk less than nontaster girls ($P < 0.05$). This effect was not seen in boys. These data suggest that PROP taste ability plays a role in preference for several bitter or fat-containing foods in young children and that girls may be more sensitive to these effects than boys. Because girls generally mature more rapidly than boys, these observed gender differences suggest that developmental factors might be involved in the acquisition of childhood food patterns.

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92. Flavor imprinting in infants

J.A. Mennella, S.H. Khan, P.L. Garcia and G.K. Beauchamp

Monell Chemical Senses Center, Philadelphia, PA 19104-3308, USA

The study of the acceptance of formulas containing protein hydrolysates provides a model system for evaluating the role of early human sensory experiences on later flavor and food preferences. To most adults the flavors of these formulas are judged highly unpalatable, having unpleasant taste and olfactory characteristics. When first introduced to these formulas, infants younger than four months of age will readily accept and consume them, whereas older infants will strongly reject them. However, if an infant is exposed to these formulas during the early period of acceptability, the formula remains acceptable for a considerable period of time thereafter. The present study aimed to determine whether the acceptance pattern that develops is specific to the hydrolysate flavor profile experienced. To this end, we studied the infants' acceptance of two types of hydrolysate formulas which differed in their characteristic flavor profiles, as determined by adult sensory evaluation. The three groups of subjects were infants who were 6 months of age and older and currently being fed one or the other of these two protein hydrolysate formulas and similarly aged infants who had only experienced a milk-base formula prior to the testing sessions. We found that infants who were currently feeding hydrolysate formulas consumed more of the formula with which they were familiar. In other words, the acceptance pattern that develops may be specific to the flavor profile experienced.

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93. Strategies to enhance food acceptance in infants

C.J. Gerrish and J.A. Mennella

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA

The transition from an exclusive milk diet to a mixed diet consisting of milk and solid food can be facilitated by repeated exposure to the food or by providing the infant with bridges of familiarity such that the infant experiences a commonality of flavors in the two feeding situations. However, the type of exposure required to enhance acceptance may not have to be with the actual food. Rather, animal model research suggests that experience with a variety of flavors influences the acceptance of novel foods during weaning. The present study, which builds upon these previous findings, determined the infants' acceptance of carrots before and after a 9 day exposure period to either carrots, potatoes or a variety of vegetables (i.e. peas, potatoes, squash). As expected, the infants who were repeatedly exposed to carrots ate significantly more carrots after, relative to before, the exposure period. In contrast, infants given repeated exposure to potatoes showed no such increase. However, infants who experienced a variety of vegetables increased their intake of carrots after the exposure period. These and other preliminary results confirm that repeated exposure to an initially novel vegetable enhances the human infants' acceptance of that vegetable and suggest that exposure to a variety of foods facilitates the acceptance of novel foods and flavors.

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94. Cephalic phase hormonal responses to high and low fat foods in women

S.R. Crystal and K.L. Teff

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA

The macronutrient composition of the diet is thought to influence satiety and subsequent food intake. High-fat, palatable foods are often over consumed although the physiological mechanisms driving the increased intake are poorly understood. The sensory qualities of food elicit neurally mediated hormones which may participate in stimulating food intake. To evaluate whether foods differing in fat content elicit differential cephalic phase hormonal responses, normal weight women (n = 13) underwent three experiment conditions administered in a counter balanced order during the first 10 days of their menstrual cycle. On each test day, following an overnight fast, arterialized venous blood was drawn. Four baseline blood samples were drawn every 10 min prior to subjects either remaining fasted (control) or tasting, but not swallowing high-fat cake or nonfat cake for 3 min. Blood samples were taken every 2 min for 14 min, followed by every 5 min for 15min. Area under the curve for cephalic phase pancreatic polypeptide release was significantly greater when subjects tasted the high-fat cake (306.99 ± 163.09 pg/ml) but not the nonfat cake (194.01 ± 104.20 pg/ml), compared with the control (–15.96 ± 45.83 pg/ml) [$F(2,16) = 3.48$, $P = 0.05$]. In contrast, no significant changes in plasma insulin were observed. Findings indicate differential cephalic phase hormone release for foods differing in fat content. Sensory evaluation of the cakes indicated that subjects were not aware of the fat content. Therefore, the differential hormonal release occurs in response to oral sensory stimulation. Differential effects between restrained and unrestrained eaters were also investigated.

95. Characterization of the chorda tympani nerve terminal field in the rat nucleus of the solitary tract with anterograde DiI transport

D.W. Pittman, K.N. Rathmann and R.J. Contreras

Department of Psychology, The Florida State University, Tallahassee, FL32306-1270, USA

Perinatal NaCl intake has been shown to produce both behavioral and electrophysiological changes in adult offspring. Elevated maternal dietary NaCl produces an increase in NaCl preference inadult offspring and an increase in the amiloride-sensitive response to NaCl stimulation of the chorda tympani nerve (CT). Restriction of maternal dietary NaCl produces a decrease in NaCl preference in adult offspring and a corresponding decrease in the amiloride-sensitive response to NaCl stimulation CT. DiI labeling of CT afferents in the nucleus of the solitary tract (NST) was used to study the effect of perinatal NaCl intake in adult offspring. Rats were raised on either basal (0.1% NaCl), intermediate (1% NaCl) or high (6% NaCl) chow from conception until adulthood. DiI was applied to the central end of the transected CT for anterograde transport in 90 day old rats. The brainstems were sectioned horizontally at 50 μm. Analysis by fluorescence microscopy revealed consistent DiI labeling of both efferent cells and the afferent CT terminal field in the NST. The average volume of labeled CT terminal field for the highs consisted of a larger percent of the total NST than the basals or intermediates, which did not differ. It appears that 0.1% NaCl is above the threshold needed to produce changes in the CT terminal field, but a 6% NaCl may produce an enlargement of the CT terminal field in the NST similar to the effects of 0.03% NaCl deprivation (King and Hill, 1991, J. Comp. Neurol., 303: 159–169) using HRP histochemistry.

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96. Effects of brief pulses of tastants on neuronal sensibilities in the nucleus of the solitary tract

C.H. Lemon, P.M. Di Lorenzo and C.G. Reich

Department of Psychology, Binghamton University, Binghamton, NY13902-6000, USA

The effects of brief taste stimulus presentation on the response tosubsequently presented taste stimuli were recorded as a way of studying the role of inhibition in the nucleus of the solitary tract (NTS). Single NTS neurons were isolated in urethane-anesthetized rats. Stimuli consisted of sucrose (0.5 M), NaCl (0.1 M), HCl (0.01M) and quinine-HCl (0.01 M). Initially, each stimulus was presented individually. Experimental trials consisted of a 100 ms 'prepulse' stimulus immediately followed by a 1 s water rinse, a 3 s 'test' stimulus and a water rinse. These prepulse-test trials continued until every possible stimulus combination was exhausted. Cluster analysis was used to suggest neuronal groups based on similarities among initial response profiles. Three groups emerged: 'HQN-tuned' responded well to HCl, quinine and NaCl; 'S-tuned' were narrowly tuned to sucrose; 'N-tuned' responded well only to NaCl. The prepulse procedure differentially affected the response to the best stimulus across neuronal groups. In HQN-tuned neurons, HCl and quinine test responses were generally suppressed by any prepulse, even when prepulse and test stimuli were identical. Sucrose test responses were suppressed in S-tuned neurons following prepulses of sucrose or quinine. However, NaCl test responses in N-tuned neurons were resistant to change following any prepulse stimulus. Results imply that the best stimulus of an NTS neuron is a good predictor of the effects of a prepulse on its responses to taste stimuli. These effects may be be the result of different patterns of inhibitory input.

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97. Salt taste discrimination by rats depends upon differential responses across gustatory neuron types

S.J. St John and D.V. Smith

Department of Anatomy & Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Rats discriminate NaCl from KCl on the basis of taste. Recently it was demonstrated that performance in a discrimination task is reduced in proportion to the concentration of amiloride (0–100 μM) mixed with NaCl and KCl (A.C.Spector *et al.*, 1996, J. Neurosci., 16: 8115). At 30 μM and higher, rats responded to amiloride-adulterated NaCl solutions as if they were KCl. We attempted to define the neurophysiological correlates of this behavior by examining across-neuron patterns in the rat solitary nucleus. The anterior tongue was stimulated with NaCl and KCl at the concentrations (0.05, 0.1 and 0.2 M) and amiloride doses (0, 3and 30 μM) used in the Spector *et al.* study. We recorded the responses of 29 single neurons to all 18 salt + amiloride combinations as well as the four prototypical stimuli: 0.5 M sucrose, 0.1 M

NaCl, 0.01 M HCl and 0.02 M quinine hydrochloride. On the basis of the best 5 s net response to the prototypical stimuli, these neurons were classified by hierarchical cluster analysis as S-best (n = 6), N-best (n = 15) or H-best (n = 8). Across-neuron patterns evoked by isomolar concentrations of salts were clearly different. Consistent with the behavioral results, however, amiloride dose-dependently reduced the difference in the across-neuron patterns evoked by NaCl and KCl, primarily by eliminating the differential response of N-best neurons. The behavioral discrimination between NaCl and KCl, therefore, depends upon the differential activity evoked by these salts across neuron types.

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98. Gustatory quality and intensity affect Fos expression in the rNST

S.P. Travers, E.J. Hauswirth and J. Hanin

College of Dentistry, Ohio State University, Columbus, OH 43210, USA

Both chemical species and concentration profoundly affect neurophysiological gustatory responses. Central consequences of gustatory quality also are evident using Fos immunohistochemistry. Results from our laboratory (Harrer and Travers, 1996, Brain Res., 711; Hu and Travers, 1996, Chem. Senses, 21; Travers and Hu, 1997, Chem. Senses, 22) indicate that sucrose and quinine stimulation elicit distinctive distributions of Fos-like immunoreactivity (FLI) within the rostral NST (rNST). The present study extends these observations to the intensive domain for quinine (0.0003, 0.003 and 0.03 M) and to additional chemicals (0.3 M NaCl and 0.1 M citric acid). Adult rats (n = 18) were implanted with intraoral cannulae, then adapted to experimental conditions. For testing, 7 ml of a tastant were delivered over 30 min. Controls included water and no stimulation. Following stimulation, rats were anesthetized, perfused and standard immunohistochemical techniques used to detect FLI. Fos positive neurons were plotted at five levels of rNST, each divided into six segments to characterize Fos topography (King *et al.*, 1997, Chem. Senses, 22). Increasing the quinine concentration monotonically increased the number of FLI neurons, but their spatial distribution, characterized by a marked medial clustering, remained constant (rs $\geq$ +0.96). Citric acid produced numbers of FLI neurons comparable to 0.03 M quinine, but with a different, more diffuse pattern (rs between acid and quinine $\leq$+0.2). NaCl evoked minimal Fos expression. These results demonstrate that quinine activates a population of NST cells with a circumscribed distribution, implying a specialized function. Further, the weak effects of NaCl imply that gustatory neurons may not be homogeneous in their competence for Fos expression.

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99. Lithium chloride-induced taste aversion and *c-fos* expression in area postrema-lesioned rats

C.M. Spencer, L.A. Eckel[1], R. Nardos and T.A. Houpt

Department of Biological Science, Florida State University, Tallahassee, FL32306 and [1]Bourne Laboratory, Department of Psychiatry, Weill Medical College of Cornell University, White Plains, NY 10605, USA

During the acquisition of a lithium chloride (LiCl)-induced conditioned taste aversion (CTA) in rats, administration of systemic LiCl is correlated with an induction of *c-fos*-like immunoreactivity (c-FLI) in many brain regions. Previous studies have shown that lesions of the area postrema (AP) interfere with the acquisition of LiCl-induced CTAs. We hypothesized that AP lesions will also attenuate the induction of c-FLI during CTA acquisition.

Twelve male Sprague–Dawley rats received AP lesions (APX) using a cautery device. An additional seven rats received sham lesions (APS) in which the AP was exposed but not cauterized. Following a 2–3 week recovery period, rats were tested for the acquisition of a LiCl-induced CTA. After 3 days on a 18 h water-deprivation schedule, all rats were exposed to a novel taste (5% sucrose) for 30 min, followed by LiCl (i.p., 0.15 M, 12 ml/kg). There was no significant difference between the lesioned and sham groups in sucrose intake (APX 11.7 $\pm$ 1.8 g versus APS 8.7 $\pm$ 0.8 g; P = 0.24, t-test). After 48 h, all rats were again exposed to sucrose for 30 min. The APX rats failed to exhibit a significant taste aversion to sucrose (APX 14.5 $\pm$ 2.8 g versus APS 4.0 $\pm$ 0.5 g; P = 0.01, t-test). The brains of these rats will be processed for c-FLI following an acute injection of LiCl or vehicle. We predict that the APX rats will exhibit significantly less c-FLI after LiCl than the APS rats in brain regions activated during the acquisition of LiCl-mediated taste aversions.

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100. The influence of a modified salt diet on dendritic remodeling in the rostral nucleus of the solitary tract (rNST) of the rat

Y.-Z. Liu, L. Schweitzer[1] and W.E. Renehan

Division of Gastroenterology, Henry Ford Health System, Case Western Reserve University, Detroit, MI and [1]Department of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY, USA

Recent studies conducted in our laboratory have demonstrated that gustatory neurons that respond to NaCl, KCl and ammonium chloride exhibit considerable dendritic remodeling during postnatal life. This remodeling is evidenced by changes in a number of dendritic features as well as the size and orientation of the dendritic field. We have postulated that the changes that we observed in dendritic morphology may be linked to changes in the salt content of the animals' diet (the NaCl content of rat milk is ~38 mM while the concentration of NaCl in normal rat chow is ~170 mM). If true, such an association would lend credence to the argument that the morphology of higher order neurons in the rNST is influenced by the information that is conveyed to this nucleus by salt-sensitive primary afferents. We tested this hypothesis by maintaining weaned rats on a diet that had a NaCl concentration of 39.3 mM. A comparison of the dendritic morphology of salt-sensitive neurons from normal adults with the dendrites of neurons from animals maintained on the modified salt diet demonstrated that the cells from animals in the experimental group were more extensive in the rostrocaudal plane (t = –2.27, df = 24, P = 0.033), less extensive in the dorsoventral axis (t = –3.5, df = 24, P = 0.002) and exhibited a trend toward a lesser total dendritic length (t = –1.65, df = 24, P = 0.127). These data support our hypothesis that the dendritic morphology of certain gustatory neurons in the rNST is sensitive to the concentration of NaCl in the diet of weaned animals.

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101. Postnatal development of hyperpolarizing inhibitory post-synaptic potentials in the rat gustatory nucleus of the solitary tract studied *in vitro*

G. Grabauskas and R.M. Bradley

Department of Biologic and Materials Science, School of Dentistry, University Michigan, Ann Arbor, MI 48109-1078, USA

An increasing body of evidence indicates that inhibitory neurotransmission has an important role in processing gustatory information in the adult nucleus of the solitary tract (rNST). Studies in other brain areas have revealed that during development the characteristics of inhibitory neurotransmission change. To determine developmental changes in inhibitory synaptic activity in the rNST, the characteristics of stimulus evoked inhibitory post-synaptic potentials (IPSP) in rat brainstem slices at 0–7 days and >55 days (adult) have been studied. The IPSPs of 0–7 day animals have an 11.5 ± 1 ms rise time constant and 236 ± 23 ms decay time constant compared with 7.0 ± 2 and 64 ± 9 ms in adult animals. In addition, tetanic stimulation-evoked IPSPs in 0–7 day animals have a sustained phase of hyperpolarization even after tetanic stimulation is terminated, whereas in adult animals tetanic stimulation-evoked IPSPs exponentially decay immediately after termination of the tetanic stimulation. This indicates a developmental difference in neurotransmitter-receptor binding-unbinding characteristics. Also the dose–response curve of the IPSPs to the $GABA_A$ receptor antagonist bicuculline (BMI) shifts to the left during development. The EC_{50} to BMI was 8 μM for 0–7 day animals and 0.75 μM for adult animals, a >10-fold change in sensitivity during maturation. These developmental changes in characteristics of the IPSPs indicate that newborn animals have GABA receptors with different pharmacological and kinetic properties which may play an important role in shaping synaptic activity in early development of the rat gustatory system.

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102. Ultrastructural localization of GABA during postnatal development in the rat rNST

M.E. Brown, W.E. Renehan[1], E. Langevin and L. Schweitzer

Department of Anatomical Science and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292 and [1]Henry Ford Hospital, Detroit, MI 48202, USA

Previous light microscopic studies using post-embedment immunohistochemistry demonstrate that GABA is present in the neonatal rat rNST at higher levels than any later age. In the current study, electron microscopy and post-embedment immunogold labelling were used to study the ultrastructural distribution of GABA in the developing rNST. Our results showed that at postnatal day 1 (PND1), while almost half of the neurons as well as many neuritic profiles were immunoreactive for GABA, few GABA-ergic synapses were found. At this age, colloidal gold particle counts show that GABA is abundant in axons. By PND10, the percentage of GABA-ergic synapses more than doubles to equal the proportion found in the adult. Synapses onto cell somas also increase postnatally. Few synapses of any kind contacted the neonatal cell somas, but by PND20 more terminals were observed synapsing onto a greater proportion of the somatic perimeter, and the majority of these were GABAergic, a finding similar to results previously demonstrated in the adult. At PND20, when weaning begins, the concentration of GABA in synapses has doubled, and the proportion of GABAergic synapses is mature. However, a subpopulation of high vesicular density GABAergic terminals previously observed in the adult, has yet to emerge. These results support three phases of GABA development in the rNST: a phase of early synaptogenesis (PND1–10) a phase of synaptic re-arrangement with respect to postsynaptic targets (PND10–20) anda late developmental phase after weaning, when a second population of GABAergic terminals matures.

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103. Immunohistochemical localization of GABA receptors in the developing rNST of the rat

W.L. Heck, W.E. Renehan[1] and L. Schweitzer

Department of Anatomical Science and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292 and [1]Henry Ford Hospital, Detroit, MI 48202, USA

The early phase [post-natal day (PND)1–PND10] of the developing rNST of the rat is characterized by synaptogenesis. Current studies in our laboratory demonstrate that during this phase, GABA is in a high proportion of somata, neurites and puncta; but of the few synapses present, <20% are GABAergic. This suggests that GABA has a non-synaptic role early in development. In order for GABA to function, it must bind to its receptors. The goal of this study was to determine the expression of GABA receptors in the developing rNST of the rat. Standard immunohistochemical techniques for light microscopy were employed on brainstem sections from PND1, 5, 10, 15, 20 and adult (>60). In the adult, there was diffuse neuropil staining for $GABA_A$ receptors in the rNST as well as more discrete staining in somata and processes. During development, there was an increase in the neuropil and somatic staining between PND1 and PND10. This was followed by a decrease in immunoreactivity such that by PND15, the adult pattern of staining was established. $GABA_B$ receptor immunoreactivity in the adult rNST was also found in both cell somata and processes. There was an interesting transformation in the pattern of immunoreactivity that changed from diffuse to punctate during development. These results suggest that both types of receptors are present in the neonate, that there is an apparent increase in $GABA_A$ receptor immunoreactivity in parallel with the increase ofGABAergic synapses and that the distribution of $GABA_B$ receptors also changes.

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104. Naltrexone blocks enkephalin-induced inhibition of gustatory responses in the nucleus of the solitary tract

C.-S. Li and D.V. Smith

Department of Anatomy & Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21021, USA

Previous immunohistochemical studies have shown that some cells in the gustatory zone of the nucleus of the solitary tract (NST) contain the opioid neuropeptide met-enkephalin (ENK). Cells within the gustatory zone also express δ- and μ-opioid receptors.

Our previous electrophysiological studies in the hamster have demonstrated that microinjection of ENK into the vicinity of taste-responsive NST cells significantly decreased the responses to both chemical and anodal stimulation of the tongue in 24% of the cells tested. These data indicated that gustatory cells of the NST are subject to opioid-mediated inhibition. In the present study, we examined the influence of naltrexone (NLTX), a broad-spectrum opioid receptor antagonist, on gustatory responses in the hamster NST. Taste-responsive neurons were characterized by recording action potentials extracellularly and stimulating the tongue with 0.032 M sucrose, 0.032 M NaCl, 0.0032 M citric acid, 0.032 M QHCl and anodal current (25 μA, 0.5 s, 0.33 Hz). ENK and NLTX were microinjected near the recorded cell using a multibarrel pipette assembly. ENK inhibited the taste-evoked responses of a subset of NST neurons in a dose-dependent fashion (0.5, 5 and 50 mM). Administration of 50 mM NLTX alone had no effect, showing that these cells are not maintained under a tonic opioid inhibition. However, microinjection of NLTX diminished the inhibitory effect of 50 mM ENK on the responses of NST cells (n =14). These data further characterize the role of opioid peptides in modulating brainstem taste activity.

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105. Individual rostral NST neurons project to both parabrabrachial nuclei in rat

C.D. Collins, C.F. Gill, W.D. Moore and M.S. King

Biology Department, Stetson University, DeLand, FL 32720, USA

A previous investigation in our laboratory demonstrated that the projection from the rostral nucleus of the solitary tract (rNST) to the parabrachial nucleus (PBN) in rat is bilateral, with an ~25% contralateral component (Brain Res., 1996, 737: 231–237). The current study was designed to determine if individual rNST neurons project bilaterally to the PBN. This was addressed in seven male Wistar rats by stereotaxically injecting rhodamine labeled (red) latex microspheres into the right PBN and fluorescein-labeled (green) microspheres into the left PBN (0.4 μl, Molecular Probes). Following a 10–14 day transport period, the rats were perfused with 4% paraformaldehyde and 40 μm thick coronal sections were examined for retrogradely labeled neurons. In a mean of 16 sections per rat, the number and location of rNST neurons containing red or green microspheres or both was determined. Of the 5855 rNST neurons that contained red microspheres, 986 (16.8%) were located in the left rNST, contralateral to the pontine injection site. Even though only 1158 rNST neurons contained green microspheres, a similar proportion (18.7%) was found in the contralateral rNST. 56 rNST neurons contained both retrograde tracers. These double-labeled neurons represent 4.8% of the rNST cells labeled with green microspheres and 1.0% of the projection neurons containing red microspheres. These results support previous findings that the projection from the rNST to the PBN is bilateral and indicate that some rNST neurons project to both PBN.

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106. Up-regulation of Fos in orexin-A-immunoreactive neurons of the hypothalamus after taste nerve stimulation

T.A. Harrison

James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA

Our previous work demonstrated Fos up-regulation in localized populations of neurons in the rostral nucleus of the solitary tract (rNST), and in the gustatory regions of the parabrachial nucleus (PBN) which receive rNST projections, after electrical stimulation of the chorda tympani and lingual-tonsillar (LT) nerves. We now have examined the hypothalamus, a target of gustatory efferents from the PBN, for Fos up-regulation after taste nerve stimulation. To address the potential functional consequences of increased hypothalamic activation, we examined Fos-irradiated (-ir) neuron distribution in relation to two hypothalamic factors implicated in regulation of feeding: melanin concentrating hormone (MCH) and orexin-A (hypocretin 1). The LT nerve was stimulated with trains of pulses for 1 h in anesthetized rats. Brains were perfusion fixed 1 h post-stimulation. Fos was visualized immunocytochemically with avidin-biotin amplification and an HRP/DAB procedure, or with Texas Red for fluorescence double-labeling of MCH or orexin-A with FITC (antibodies courtesy of J.-K. Chang, Phoenix Pharmaceuticals, Inc.). Results indicate a 35–50% increase in the number of Fos-ir profiles in the tuberal hypothalamus of nerve-stimulated rats compared with sham-operated controls. All Fos-ir neurons in this area were positive for orexin A-ir in both sham and stimulated rats. Fos-ir was not observed in MCH-ir neurons in this area. Thus, LT stimulation selectively increased the number of orexin-A neurons expressing Fos in the tuberal hypothalamus. It remains to be determined if taste input from the PBN directly up-regulates Fos in these neurons.

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107. Neuronal correlates of satiety

D. Small[1,2], R.J. Zatoree[1,2], A. Dagher[1] and M. Jones-Gotman[1,2]

[1]McConnell Brain Imaging Center and [2]Neuropsychology Unit, Montreal Neurological Institute, McGill University, Quebec, Canada

Rolls and colleagues have reported a dissociation in monkeys such that the cells in the primary gustatory area (PGA) respond to food stimuli regardless of hunger, whereas taste and smell cells in the secondary and tertiary orbitofrontal (OFC) areas respond only if the animal is hungry (E.T. Rolls, Crit. Rev. Neurobiol., 11: 2). The point along the neuroaxis at which chemosensory-responsive cells become modulated by satiety is unknown in humans. Furthermore, satiety encompasses both hedonic (i.e. the pleasantness of the food) and motivational (i.e. desire to eat) aspects, which may themselves be dissociated. To investigate which cortical taste and smell areas are modulated by hunger we performed successive positron emission tomography scans on eight Ss as they became sated with chocolate. Ss rated how pleasant they found the chocolate and how much they would like another piece. Our data suggest that PGA and OFC are both modulated by satiety. Regional cerebral blood flow (CBF) decreased with satiety in the PGA and increased with satiety in the caudolateral OFC. In all cases, differences were observed bilaterally, but greater in the right

hemisphere. Additionally, differential activation was observed in many limbic areas including the ventral tegmental area, thalamus, hypothalamus, anteromedial temporal lobe, inferior temporal lobes, area 14/32 of the frontal lobe and cingulatge cortex. Interestingly, CBF in area 31 of the posterior cingulate cortex was negatively correlated with motivational ratings but not pleasantness ratings or scan order. We speculate that this may be related to performing an act (eating) despite a clear motivation not to do so.

108. Generation of an immortal olfactory receptor neuron cell culture

R.D. Barber[1,2], D.E. Jaworsky[1], K.-W. Yau[1,2] and G.V. Ronnett[1,3]

[1]Department of Neuroscience, [2]Howard Hughes Medical Institute and [3]Department of Neurology, Johns Hopkins University School of Medicine, 725 N. Wolfe St, Baltimore, MD 21205, USA

The H-2Kb-tsA58 transgenic mouse (Jat *et al.*, 1991, Proc. Natl Acad. Sci. USA, 88: 5096–5100) harbors coding sequences for a temperature-sensitive mutant of the SV40 TAg under the control of an interferon-inducible promoter. This permits direct derivation of conditionally immortal cell lines from primary cultures. We have set out to generate a conditionally immortal olfactory receptor neuron (ORN) cell culture from H-2Kb-tsA58 transgenic mice.

Olfactory epithelia were cultured in selective media at 'permissive' conditions, defined as culture at 33°C in the presence of murine γ-interferon. This results in TAg-induced immortalization, whereas, in 'non-permissive' conditions (at 37°C in the absence of interferon), TAg expression is inhibited, cell proliferation is reduced and cells express 'mature' or 'differentiated' characteristics. Cells have been maintained through multiple passages and culture conditions have been optimized with additional reagents.

Immunocytochemistry, Western blot analysis and PCR amplification of first-strand cDNA have been used to characterize the ORN cell culture. The data demonstrate (i) TAg expression is limited to cells cultured in permissive conditions; (ii) the neuronal markers NST and NCAM can be detected in both permissive and non-permissive culture conditions; (iii) the olfactory-specific markers OCNC1, OCNC2, adenylyl cyclase type III, G_{olf} and OE1 can be detected in both conditions; and (iv) OMP expression appears to be selective for cells cultured at non-permissive culture conditions.

These data indicate that, in permissive conditions, this culture system contains a conditionally immortal population of immature ORNs and that expression patterns consistent with those observed in mature ORNs can be induced in cells at non-permissive conditions.

109. Primary olfactory neuroepithelial cultures are GDNF responsive

A.M. Cunningham and K.L. Doyle

Sensory Neurobiology Group, Garvan Institute of Medical Research, 384 Victoria St, Darlinghurst, NSW 2010, Australia

Glial cell line derived neurotrophic factor (GDNF) is a potent mammalian neuronal growth factor and although classically target derived it has been demonstrated to have additional modes of trophic action. Recently, we demonstrated GDNF expression by mature olfactory receptor neurons (ORNs) in the rat olfactory neuroepithelium (ON), but not the surgically target deprived ON (Buckland and Cunningham, 1999, Neuroscience, 90: 337–347), indicating GDNF expression in ORNs is dependent upon synaptic contact with the olfactory bulb. To determine whether ORNs were responsive to GDNF, we grew primary mixed cultures from neonatal rat turbinates under serum free conditions. Apoptosis was compared in control versus GDNF treated cultures at 3, 6 and 9 days. GDNF treatment resulted in fewer TUNEL-positive cells at day 3, indicating that GDNF protected against apoptosis at this time. GDNF treatment resulted in both increased neuronal numbers and differentiation of ORNs, as determined morphologically and immunocytochemically. S100 immunoreactive glial cells also increased in number and appeared more differentiated. Combined immunocytochemistry and TUNEL showed that GDNF protected cells which were growing in clusters closely associated with neurons from apoptosis, and some of these protected cells were characterized as precursor cells. Our data suggest that GDNF may play an important role in the normal differentiation of the ON acting both on precursors and later in the differentiation pathway.

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110. Cloning and characterizing NIP: a PDZ domain-containing protein interacts with the cytoplasmic domain of neuropilin-1

R.R. Reed[1,2,3] and H. Cai[1,2]

[1]Howard Hughes Medical Institutes, [2]Department of Neuroscience and [3]Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

Neuropilin-1 (Npn-1), a receptor for semaphorin III exerts its guidance effects on extending neurites including olfactory nerves. The molecular mechanism of Npn-1 signaling remains unclear. We have isolated a Npn-1-interacting protein (NIP) from olfactory tissue, which interacts with the cytoplasmic domain of Npn-1 in a yeast two-hybrid system. NIP contains a central PDZ domain and a C-terminal acyl carrier protein (ACP) domain. The physiological interaction is supported by coimmunoprecipitation of Npn-1 and NIP in a mammalian expression system and from olfactory bulb extract. The C-terminal three amino acids of Npn-1 (-Ser-Glu-Ala-COOH), which is conserved from *Xenopus* to human, and the PDZ domain-containing C-terminal two-thirds of NIP are responsible for this interaction. NIP as well as Npn-1 is broadly expressed in mice as assayed by Northern and Western analysis. Immunohistochemistry and *in situ* hybridization experiments revealed that NIP expression overlaps with that of Npn-1. Recently, a protein called GIPC was identified that is identical to NIP by virtue of its interaction with the C terminus of RGS-GAIP. GIPC is suggested to be involved in clathrin-coated vesicular trafficking. We hypothesize that NIP/GIPC participates in regulation of Npn-1-mediated signaling as a molecular adapter for coupling Npn-1 to membrane trafficking machinery in the dynamical axon growth cone. This work is supported by HHMI.

111. Convergent ideas on olfactory organ development in the zebrafish *Danio rerio*

K.E. Whitlock

Section of Genetics and Development, Biotechnology Building, Cornell University, Ithaca, NY 14853, USA

The olfactory organ develops from the olfactory placode, a structure for which the mechanisms controlling its development are not clearly understood. I have shown that, surprisingly, there is a large field of cells lying along the edge of the neural plate which will give rise to the olfactory organ. Within this field cells are segregated with respect to their future fate and function. The field of cells lying medially to the olfactory placode field will give rise tothe olfactory bulb and this field must converge anteriorly to form the future telencephalon. Finally, the olfactory placode arises in the absence of cell division leading me to propose that the olfactory placode forms from the convergence of the cellular fields along the edge of the neural plate.

Once these fields have condensed to form the olfactory placodes, the placodes must establish contact with the central nervous system (CNS). The first axons exiting the placode are an early classof pioneer neurons that pre-figure the primary olfactory pathway. These pioneer axons are followed by the axons of the olfactory sensory neurons. The pioneer neurons are a transient population whose function is to target the axons of the sensory neurons to the developing olfactory bulb. During development, thepioneer neurons arise from the field of cells giving rise to theolfactory placode. But, they arise from a region distinct fromthat giving rise to the first sensory neurons and, clones containing both sensory neurons and pioneer neurons were never observed.

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112. Perturbation of gastrulation does not block taste bud genesis

L.A. Barlow

Department of Biological Sciences, University of Denver, Denver, CO80208 and the Rocky Mountain Taste and Smell Center, UCHSC, Denver, CO 80262, USA

Until recently taste bud induction was thought to rely upon nerve contact. However, new results from amphibians and mammals indicate that much of taste bud development is nerve-independent. In fact, in amphibians, taste bud genesis is intrinsic to the local epithelium; this feature is acquired by a restricted region of the embryo, i.e. presumptive oropharyngeal endoderm, by the time gastrulation is completed. Given that tissue-level signaling during gastrulation is critical for induction of the nervous system, we hypothesized that the oropharyngeal endoderm (which later will make taste buds) is also induced during gastrulation. To test this idea, gastrulation was disrupted in salamander embryos by exposing them to hypertonic saline throughout gastrulation. Normally, the presumptive oropharyngeal endoderm is first to involute at Spemann's Organizer as gastrulation begins. However, when embryos are exposed to double normal salt concentrations, these cells move away, or exogastrulate, from the Organizer and ultimately the oropharyngeal epithelium is located distally in the exogastrulated tissue. The resultant, severely disrupted embryos were examined for taste buds. Surprisingly, taste buds were found in exogastrulae, and most importantly were restricted to the distal exogastrulated region. This result indicates that the future oropharyngeal endoderm is induced and develops taste buds without undergoing normal gastrulation. I am currently examining whether persistent contact with Spemann's Organizer during exogastrulation provides signals necessary for the development of the oropharyngeal endoderm and therefore of taste buds, or whether this taste bud-bearing region is specified by mechanisms independent of gastrulation.

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113. Neuropilin-1 dependent repulsion guidesgeniculate axons destined for lingual tastebuds

M.W. Rochlin, R. O'Connor[1], R.J. Giger[2], J. Verhaagen[3], M.Tessier-Lavigne[1] and A.I. Farbman

Neurobiology & Physiology, Northwestern University, Evanston, IL 60208, [1]Department of Anatomy, University of California—San Francisco & Howard Hughes Medical Institute, San Francisco, CA94143-0452, [2]Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA and [3]Netherlands Institutefor Brain Research, Amsterdam, Netherlands

Geniculate ganglion axons pioneer lingual innervation. We investigated the role of diffusible factors in their guidance. Previously, we showed that trigeminal axons are first repelled, then attracted by their presumptive targets, including pre-tongue explants. We now report that geniculate axons are repelled by pre-tongue explants and hyoid arch, through which they grow before entering the mandibular arch (Ib). Blocking neuropilin-1, asemaphorin III receptor, neutralizes the repellant influence. Geniculate axons *in vivo* avoid regions that are repellant *in vitro*. Specifically, these axons enter arch Ib on E13, avoiding its repellant ventral surface. Next, they curve medially, avoiding therepellant anterior tongue. On E14, they turn away from the midline, advancing into the lateral anterior tongue. By then, thelateral tongue has downregulated its repellant influence, but the midline remains repellant. In semaphorin III knockout mice,trajectories of trigeminal and geniculate axons are only partially disrupted, indicating that the repellant is involved in, butnot crucial for pathfinding to the target region. We find no evidence of diffusible attractants for geniculate axons, suggestingthat short range cues guide these axons at early stages. Curiously, semaphorin III mRNA persists in dorsal tongue epithelium through E17, when geniculate axons penetrate the epithelium. E17 geniculate axons are less repellant-sensitive thanE13 axons, and tongue explants evidence no long range repulsion. Thus, at early stages, long-range chemorepulsion influences pioneer lingual axon navigation, but later, modificationsof repellant activity and sensitivity may underlie a transitionto short-range roles: regulating arborization or synaptic differentiation.

114. Trigeminal collaterals in the olfactory epithelium and bulb: a route for direct modulation of olfactory information by trigeminal stimuli?

T.E. Finger[1], M. Schaefer[1,2], B. Böttger[1] and W.L. Silver[3]

[1]Rocky Mountain Taste & Smell Center and Department of Cellular & Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262, [2]Neuroscience Program, UCHSC, Denver, CO 80262 and [3]Department of Biology, Wake Forest University, Winston-Salem, NC27109, USA

The olfactory epithelium is richly invested with peptidergic (substance P and CGRP) trigeminal polymodal nociceptors that respond to numerous odorants as well as irritants. Peptidergic trigeminal fibers also enter the olfactory bulb to terminate within the glomerular layer. In order to test whether the trigeminal fibers in the olfactory bulb are collaterals of the epithelial trigeminal fibers, we utilized dual retrograde labeling techniques to identify the trigeminal ganglion cells innervating each of these territories. Nuclear Yellow was injected into the dorsal olfactory epithelium while True Blue was injected into the olfactory bulb of the same side. Following a survival period of 3–7 days, the trigeminal ganglion was examined for the presence of double-labeled cells. A small population of double-labeled, small, elongate cells was present within the ethmoid nerve region of the ganglion. In order to test whether these cells also had an axon extending into the spinal trigeminal complex, a third tracer, rhodamine dextran, was injected into the rostral end of the spinal trigeminal tract. A few triple-labeled cells then could be found. These results indicate that some trigeminal ganglion cells with sensory endings in the olfactory epithelium also have branches reaching directly into both the olfactory bulb and the spinal trigeminal complex. Such an arrangement provides an avenue by which trigeminal stimuli applied to the mucosa to stimulate the polymodal nociceptors could not only transmit this information to the brainstem trigeminal complex, but, by axon reflex, also directly modulate olfactory processing in the bulb.

115. Activation of trigeminal neurons by acid, capsaicin, and nicotine

S.A. Simon and L. Liu

Departments of Neurobiology and Anesthesiology, Duke University Medical Center, Durham, NC 27710, USA

A variety of chemical stimuli interact with sensory neurons to produce pain and irritation. Three commonly used stimuli are capsaicin, acid and nicotine. We have investigated how these chemicals interact with neurons from rat trigeminal ganglia (TG) using RT-PCR, whole-cell voltage and current clamp, and changes in $[Ca^{2+}]_i$. In rat TG, RT-PCR revealed the presence of subunits for: the acid-sensing ion channels ASIC-α, ASIC-β and DRASIC; the vanilloid receptor, VR1; and neuronal types of nicotinic receptors α2–α7, α9 and β2–β4. In TG neurons all three agonists can induce increases in $[Ca^{2+}]_i$ and evoke action potentials. Acid (pH 5.9) activated a variety of inward currents that differed in their kinetics [transient (T) or sustained (S)], reversal potentials (30mV transient and 0 mV sustained), p$K_{50\%}$s, and ability to beinhibited by amiloride (T) and capsazepine (S). Desensitizing the neuron with the vanilloid zingerone desensitized the S- but notthe T-type current. Capsaicin also activated a variety of capsazepine-sensitive T- and S-type currents that differed in reversal potentials (20 mV, T; 0 mV, S), suggesting the presence of subtypes of vanilloid receptors. Lowering the pH to 5.9 shifted the capsaicin dose response from 0.7 to 0.6 μM. The S-type acid current has many characteristics in common with the VR1 receptor, but the T-type has similarities with those of the ENaC family. Nicotine also evoked a variety of kinetically distinct currents that are found in capsaicin and acid sensitive neurons.

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116. Trigeminal mechanisms of oral irritation

E. Carstens

Section of Neurobiology, Physiology and Behavior, University of California, Davis, CA 95616, USA

To characterize the role of neuronal nicotinic acetylcholine receptors (nAChRs) in oral irritation, we mapped the locations and numbers of brainstem neurons that express the immediate–early gene, c-*fos*, after application of nicotine to the tongue, eitheralone or after pretreatment with cholinergic antagonists. Anesthetized rats received one of the following stimuli delivered to the dorsal anterior tongue: (1) 0.9% NaCl followed by nicotine 1% = 61 mM; (2) the nAChR antagonist, mecamylamine 0.1% = 4.9 mM + nicotine; (3) the muscarinic antagonist atropine 0.1% = 1.46 mM + nicotine; (4) atropine 1% = 14.6 mM + nicotine; (5) 0.9% NaCl control; or (6) no stimulus control. Two hours later animals were perfused with 3% paraformaldehyde. Brainstem sections were immunohistochemically processed for fos-like immunoreactivity (FLI). Nicotine evoked significant increases in FLI compared with controls in dorsomedial and ventrolateral trigeminal caudalis, nucleus of the solitary tract (NTS), ventrolateral medulla and area postrema. Mecamylamine significantly reduced nicotine-evoked FLI in dorsomedial and ventrolateral caudalis, ventrolateral medulla and area postrema. Atropine 1% significantly reduced FLI in each of the same areas and also NTS, while 0.1% atropine significantly increased FLI in NTS with no significant effect in the other regions. These results are consistent with the idea that nicotine activates nAChRs residing on nociceptive fibers innervating the tongue which, in turn, excite neurons in trigeminal caudalis and other brainstem regions.

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117. Responses to irritation of the nasal mucosa using short- and long-lasting painful stimuli

T. Hummel

Department of Otorhinolaryngology, University of Dresden, Fetscherstraße 74, D-01307 Dresden, Germany

In humans intranasal irritation can be produced using short- and long-lasting painful stimuli. Short-lasting pain can be induced bygaseous CO_2, while long-lasting pain may be induced by a stream of dry air. The two types of stimuli have been explored regarding their major determinants, e.g. effects of stimulus duration, stimulus intensity or repeated stimulation. Short-lasting, non-inflammatory pain stimuli seem to provide specific indicators of A_{delta}-fiber function, while responses to long-lasting, inflammatory pain appear to be indicative of C-fiber function. Intranasal irritation can be assessed by means of psychophysical techniques

including threshold measurements, ratings of suprathreshold stimuli or lateralization measurements when stimuli are presented to either the left or the right nostril. Electrophysiological measures include the recording of peripheral responses from the respiratory epithelium and the recording of cortical responses. In addition, functional magnetic resonance imaging may be used to look intothe cerebral processing of intranasally induced irritation. Responses to both types of painful stimuli are specifically modulated by antinociceptive drugs. As these well-investigated models allow the detailed and precise analysis of modulatory effects on intranasal nociception, they also appear to be suited for the investigation of subtle changes of intranasal irritation, e.g. induced by environmental agents.

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118. Current understanding of the oral sensory effects of capsaicin in humans

B.G. Green

The John B. Pierce Laboratory and Department of Surgery (Otolaryngology), Yale School of Medicine, 290 Congress Ave, New Haven, CT 06519, USA

Capsaicin is by far the most heavily studied of all sensory irritants, yet many questions remain concerning the nature of its perceptual effects and their relationship to physiological and cellular mechanisms. This talk will provide an overview of capsaicin's psychophysical characteristics in the oral-pharyngeal region in humans, with emphasis placed on desensitization, sensitization and the more recently identified phenomenon of 'stimulus-induced recovery'. Hypotheses about how these phenomena may be related to one another, and why their expression can vary so profoundly across individuals, will be presented and discussed.

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119. Comparison of sonication and calcium shock methods of preparing olfactory cilia

K. Washburn[1,2], T. Turner[2] and B.R. Talamo[1,2]

Departments of [1]Neuroscience and [2]Physiology, Tufts University School of Medicine, Boston, MA 02111, USA

Calcium plays an important regulatory role in olfactory signal transduction. Methods were sought to prepare olfactory cilia without exposing the tissue to high calcium, which might alter theassociation of different macromolecules with the membrane preparation. Various physical and sonication protocols were explored to optimize a preparation of olfactory cilia from CF-1 mouse olfactory epithelium. These preparations were evaluated by analysis of basal adenylyl cyclase activity as well as GTP-dependent forskolin- and odorant-activated adenylyl cyclase. Specific activity, yield, and the effects of permeabilization with detergent or addition of phosphodiesterase inhibitors were compared. Western blots were analyzed for the presence of transduction components adenylyl cyclase type III (ACIII) (antibody from Santa Cruz), the 1c2 isoform for phosphodiesterase (PDE1c2) (antibody from J.Beavo), and heterotrimeric G-protein subunits Galpha-olf and Galpha-i2 (antibodies from Santa Cruz). By these criteria, the cilia prepared by sonication appear to be very similar to those isolated by calcium shock. Further studies will examine the association of various regulatory enzymes with each preparation.

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120. Calcium-activated neutral protease in olfactory tissues of the catfish and rat

D.L. Kalinoski, S. Hilmi and S. Herman

Monell Chemical Sense Center, 3500 Market St, Philadelphia, PA19104-3308, USA

Calpain, an intracellular protease activated by calcium, is inferred to be a regulator of many calcium-dependent cell functions. Studies in several tissues have demonstrated that calcium-induced degradation of the inositol 1,4,5-trisphosphate receptor/channel (IP3R) is, at least in part, due to activation of calpain, and that thismechanism may play a role in regulating IP3R activity by down-regulation of receptor in response to increased intracellular calcium concentrations. We have begun to examine whether this mechanism may play a role in olfactory function by determining the ability of a number of calpain inhibitors to alter molecular masses of IP3R as determined by SDS–PAGE and Western blotting with IP3 receptor type-specific antibodies. Western blots of olfactory cilia proteins prepared by calcium shock technique in the presence or absence of calpain inhibitors could identify no high-molecular-weight IP3Rs, but instead identified two major degradative fragments of ~120 kDa. However, when cilia were prepared by sonication with selected calpain inhibitors immunoreactive bands of ~260 kDa were labeled. These results suggest that the high calcium concentrations employed to isolate olfactory cilia may irreversibly activate certain calcium-dependent processes. Immunoblots with anti-calpain antibodies identified bands of ~80kDa in all subcellular membrane fractions isolated from olfactory epithelium by differential centrifugation suggesting that this soluble protease closely associates with cell membranes. Immunohistochemical and immunoblotting studies to further identify the types and location of calpains present within the olfactory epithelium of the rat and catfish are being undertaken.

121. Localization of IP3 receptor type 1 and type 3 olfactory epithelium of rat and catfish

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

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

Odorant molecules interact with specific receptors on cilia of olfactory receptor neurons leading to rapid and transient elevations in the intracellular second messengers cyclic AMP (cAMP) and inositol 1,4,5-trisphosphate (IP3). These elevations in second messenger lead to activation of specifically gated calcium channels, and an increase in calcium influx in olfactory cilia resulting in membrane depolarization. To further our understanding of how IP3 responses are processed in the olfactory neuron, subsequent to the elevation of IP3, we have utilized RT-PCR to clone cDNA fragments from the olfactory tissue corresponding to of IP3 receptors type 1 and type 3 (IP3R1 and IP3R3). *In situ* hybridization was performed to determine the expression patterns of these IP3R types within the olfactory epithelium. The results from *in situ* hybridization using non-radioactively labeled receptor probes in olfactory epithelium of both rat and catfish demonstrate that both IP3R 1 and IP3R 3 are found in this tissue. In the rat, a strong labelling corresponding to the IP3R 1 and IP3R 3 messengers is

restricted to some turbinates, and distributed as a 'cluster' of some mature olfactory neuron within the olfactory epithelium. Diffuse but significant labelling is localized in the cytoplasm of sustentacular cells. In the catfish olfactory epithelium, the labelling is generalized to all basal cells and olfactory immature neuron. Strong staining is localized in individual and punctate mature olfactory neuron. Immunohistochemical studies are being performed to further characterize the distribution of these proteins within the olfactory epithelium of the rat and catfish.

122. Regulation of the generation of cGMP in the olfactory system of *Manduca sexta*

A.J. Nighorn and P.J. Simpson

ARL Division of Neurobiology, The University of Arizona, Tucson, AZ85721, USA

The intracellular messenger cGMP is thought to play a role in both peripheral and central processing of olfactory information in the insect, *Manduca sexta*. In the antennae, pheromone stimulation has been shown to result in a slow rise in cGMP levels. More centrally, in the antennal lobe, its role has been assumed from the high amount of NADPH diaphorase staining found there. To investigate these phenomena more closely, we have cloned five different isoforms of guanylyl cyclase (GC) as well as nitric oxide synthase (NOS), the enzyme that generates nitric oxide (NO). GCs are usually separated into two different classes, soluble GCs that are stimulated by NO and receptor GCs that are stimulated by ligand binding or interactions with guanylyl cyclase activating proteins. We have cloned two sGC isoforms, one rGC isoform and two novel GCs that are soluble yet insensitive to NO. We find that it is one of these NO-insensitive isoforms (MGCI) and perhaps a classic rGC (MGCII) that are likely to mediate the rise of cGMP in the antennae in response to pheromonal stimulation. We also find that both NO-sensitive and -insensitive GCs are expressed in the antennal lobe and may play a role in the processing of odorant information.

123. Kinases and phosphorylation in lobster olfactory system

T.D. Stoss, F. Xu and T.S. McClintock

Department of Physiology, University of Kentucky, Lexington, KY 40536, USA

Phosphorylation is a common mechanism signalling pathways useto transmit information and is particularly important in feedback regulation. The ability of phosphorylation to regulate both homologous and heterologous pathways is especially relevant to lobster olfactory receptor neurons, which contain dual interacting olfactory transduction pathways. In preliminary experiments, we found that a complex odorant (Tetramarin extract) or GTP-γ-S caused increased phosphorylation of proteins of the lobster outer dendritic segment (ODS). Subsequent experiments determined that the odorant increased the phosphorylation of proteins at 209, 72, 65, 59, 46, 34 and 32 kDa. Many of these proteins were also phosphorylated in response to cAMP. Because odorants stimulate phospholipase C-β and adenylyl cyclase, PKC and PKA may be partly responsible for odor stimulated phosphorylation. Other kinases that may also be involved are lobGRK1, a G-protein-coupled receptor kinase that is expressed in the ODS, and calcium-dependent kinases, which would be activated by calcium permeating through the inositol 1,4,5-trisphosphate receptor.

Lobster brain homogenates are being used to identify conditions permitting specific activation of various kinases. Phosphorylation in brain homogenates was stimulated by GTP-γ-S and by a mixture of neurotransmitters that are expressed in the olfactory lobe. Subsequent experiments revealed that cAMP increased the phosphorylation of proteins of 209, 178, 151, 104, 80, 71, 66, 58, 53, 48, 44 and 40 kDa. Experiments to investigate the activation of other kinases in both brain and the olfactory organ are in progress.

124. Functional characterization of *Drosophila* organs involved in olfaction and pheromone response

S.-K. Park, Q. Wang, S. Shanbhag[1], A. Steinbrecht[1] and C.W.Pikielny

[1]*Department of Neuroscience and Cell Biology, Robert Wood Johnson Medical School/UMDNJ, 675 Hoes Lane, Piscataway, NJ 08854, USA and* [1]*Max-Planck-Institut für Verhaltensphysiologie, D-82319 Seewiesen, Germany. e-mail: pikielcl@umdnj.edu; http://www2.umdnj.edu/flylbweb/*

We are using molecular genetic methods to investigate two different chemical senses in *Drosophila*.

First, we have previously shown that members of a family ofodorant-binding proteins (OBPs) are expressed in different patterns on the surface of the antennae, suggesting a topographical segregation of chemosensory hairs or sensilla involved in general olfaction. To test this hypothesis we are using OBP promoters to direct expression of cell death genes and inactivate specific subsets of sensilla in otherwise normal adults. Behavioral analysis of several types of ablated flies suggests that different sensilla are necessary for the detection of sometimes closely relatedodors. Surprisingly, some ablations have differential effects on attraction to an odorant and repulsion by higher, toxic concentrations of the same odorant, suggesting the existence of two sets of sensilla responding to the same odorant but differing in their ability to mediate different behavioral responses.

Second, other workers have suggested that *Drosophila* males recognize female pheromones using sensilla on their front legs. We have cloned two genes that are only expressed in a few cells of male front legs, suggesting a role in pheromone perception. While leg sensilla are known to be involved in taste, double-immunolabeling suggests that pheromone response and gustation are mediated bytwo non-overlapping sets of hairs. Directed cell ablation shouldallow us to test the involvement of these cells in pheromone response.

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125. Annexin in the rat olfactory epithelium

A.I. Farbman, J.A. Buchholz and E. Weiler

Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208-3520, USA

Previous studies from our laboratory showed that after ablation of the olfactory bulb cell dynamics in olfactory epithelium change inthree significant ways: (1) the rate of cell proliferation in the neuronal lineage is up-regulated; (2) the rate of cell death in the neuronal lineage is up-regulated; and (3) more supporting cells are

recruited to phagocytose dying neurons. We studied olfactory mucosa from normal and unilaterally bulbectomized rats 60 days after surgery. Tissues from operated (n = 25) and unoperated animals (n = 25) were pooled, and aliquots were subjected to SDS–PAGE to determine whether the two groups had different protein profiles. In silver-stained gels a 38 kDa band was more prominent in the bulbectomized olfactory mucosa. Microsequencing of that band revealed several components, the most prominent of which was annexin-I, a member of a protein family associated with cell membrane activities. Immunohistochemical analysis of olfactory epithelium revealed annexin-I-like reactivity in a population of non-neuronal cells different from ordinary supporting cells, previously identified by our laboratory (Carr *et al.*, 1991, Neuroscience, 45: 433) and in cells lining the ducts ofBowman's glands. This is the first report of annexin-I immunoreactivity in olfactory epithelium. The number of annexin-I immunoreactive cells in bulbectomized animals does not appear tochange significantly in accordance with the 38 kDa band in ouroriginal observation but does follow the same pattern as the non-neuronal cells observed in our earlier study.

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126. Olfactory marker protein may function as a mitogen in rat olfactory epithelium

P.I. Ezeh and A.I. Farbman

Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208-3520 USA

Previous studies have shown that addition of picomolar quantities of olfactory marker protein (OMP) to the media of organotypic cultures of olfactory mucosa increased the number of basal cells incorporating BrdU, a marker for DNA synthesis (Carr *et al.*, 1998, J. Neurobiol., 34: 377). In the present study we asked whether OMP could act as a mitogen *in vivo*. Adult rats (n = 6) were injected i.p. with tritiated thymidine ([^{3}H]TdR) and then injected i.a. (in one of the carotid arteries) with 50 µg of OMP. Control rats (n = 6) were injected with [^{3}H]TdR and i.a. with TBS solution. After 1 h animals were killed and the olfactory mucosa (both sides) and a portion of liver were removed and homogenized on ice. The homogenates were filtered and the filters counted in a liquid scintillation counter. The counts in olfactory tissue of OMP-injected animals were 23% higher than those in TBS injected animals (ANOVA, P = 0.009), and the counts in the liver were 17% higher (ANOVA, P = 0.01). In some OMP-injected animals the olfactory mucosae of the two sides were counted separately, and the counts on the side ipsilateral to the injected carotid artery were higher than on the other side. The results substantiate those of the *in vitro* studies and support the notion that OMP acts as a mitogen in the olfactory epithelium.

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127. Exposure-induced odor sensitivity: evidence for peripheral involvement

K.K. Yee and C.J. Wysocki

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA19104-3308, USA

Exposure-induced odor sensitivity may involve peripheral or central components of the olfactory system. Ability to disconnect the epithelium from the bulbs provides a unique opportunity to examine how odorant exposure affects each component. In one study, sensitivity of CBA/J mice was determined for androstenone (AND) and amyl acetate (AA). Animals were then exposed to AND (n = 3) or AA (n = 3) for 10 days (16 h/day) after bilateral olfactory nerve transection (BTX) to determine the effects of exposure on peripheral components. After 45–50 days of recovery, sensitivity to the exposed odorant increased by 8-fold whereas sensitivity to the non-exposed odorant remained unchanged. Similar changes were observed in surgical sham animals for both exposures. In another study, mice were exposed before BTX to examine the effects of exposure on central components. Sensitivity for AND (n = 4) or AA (n = 4) was determined before the animals were exposed to the same odorant for 10 days (16 h/day). After exposure, animals were re-tested and sensitivity for each odorant increased by 6-fold. All the animals then received a BTX procedure. After 45–50 days of recovery, sensitivity for each odorant remained at pre-surgical exposure levels. Results suggest that the effects of odor exposure are specific. Although further work is needed to determine the precise peripheral mechanism, these studies provide additional evidence for peripheral involvement in changes in sensitivity to odorants and demonstrate the remarkable capacity of the olfactory system to maintain odor sensitivity after injury.

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128. A distinct ubiquitin-positive ultrastructural array in the supranuclear region of olfactory epithelial supporting cells following extended odor exposure of rats

V.McM. Carr, A.I. Farbman and B.Ph.M. Menco

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

We have previously described the appearance of immunoreactivity (IR) for the stress proteins ubiquitin and HSP70 in the supranuclear region of olfactory epithelial (OE) supporting cells following exposure of rats to a variety of odorants (Neurosci. Abstr., 1997, 23: 738) and ketamine (Neuroreport, 1993, 5: 197). Similar IR patterns also occurred following heat stress. To gain more insight into the nature of this response, we examined sections of the septal OE of two male rats at the ultrastructural level following a 6 h exposure to lavender essential oil extract, an odorant known to induce this response. Observations were compared with those of comparable sections from two unexposed rats. Sections of the paraformaldehyde-fixed cryoprotected, freeze-substituted, Lowicryl-embedded OE were subjected to post-embedding immunocytochemistry for ubiquitin using gold-conjugated secondary antibodies (Microsc. Res. Tech., 1995, 32: 337). Results showed that many of the supporting cells in the exposed animals exhibited a supranuclear electron-opaque granular array from which membranous organelles were largely excluded. This array was co-extensive with the IRs of a panel of antibodies to ubiquitin, a protein that targets proteins for proteolysis, suggesting that the array is at least partly comprised of proteins being degraded. In contrast, the electron-opaque array was not observed in supporting cells of unexposed animals or in ubiquitin(–) supporting cells of odor-exposed rats. Instead, the supranuclear regions of these cells contained numerous mitochondria, smooth endoplasmic reticulum and Golgi apparatus.

This suggests that organelle-associated proteins may be the targets for ubiquitinylation under physiologically stressing conditions.

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129. Increased HSP70(+) olfactory receptor neuron (ORN) density and expansion of bulbar projections following methyl bromide (MeBr) lesion of rat olfactory epithelium (OE)

V.McM. Carr, G. Ring[1], S.L. Youngentob[2], J.E. Schwob[1] and A.I.Farbman

Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208, [1]Department of Anatomy & Cell Biology, SUNY Health Science Center, Syracuse, NY 13214 and [2]Department of Physiology, SUNY Health Science Center, Syracuse, NY 13214, USA

A rat ORN subpopulation showing intense cytoplasmic immunoreactivity (IR) for Mab 2A4 to human HSP70 has been described previously (J. Comp. Neurosci., 1994, 348: 150). 2A4(+)ORNs occur throughout OE regions II–IV (see Buck, Annu. Rev. Neurosci., 1996, 19: 517) and project to just 2–3 glomeruli in each olfactory bulb (OB), located lateroventrally at 15–20% of the ant.-post. OB axis and medioventrally at 35–40%. The OE locations and OB projections are consistent throughout 2A4(+)ORN neurogenesis (J. Comp. Neurosci., 1999, 404: in press). We have now examined the 2A4(+)ORN OE distribution and OB projections 6–8 weeks after MeBr lesioning of the OE (J.Comp. Neurosci., 1995, 359: 15), near the end of subsequent OE reconstitution and OB reinnervation. Non-lesioned rats showed completely normal projection patterns. Following bilateral MeBr lesion, rats showed increased 2A4(+)ORN OE densities. However, 2A4(+)ORN distribution patterns remained unchanged. Corresponding large increases in the number of glomeruli receiving 2A4(+)axons also occurred. Some of the additional glomeruli received just one or a few small 2A4(+)axonal bundles;but in other glomeruli 2A4(+)axons clearly occupied a substantial portion of the glomerular volume. Significantly, additional 2A4(+)glomeruli were confined to the ventral one-thirdand anterior half of each experimental OB, i.e. the region of 2A4(+)axonal projection pathways in normal rats. Thus, while 2A4(+)ORNs in reconstituted OE project to the appropriate OB region during reinnervation, the MeBr lesioning of the OE has compromised the more specific targeting abilities of these axons within this region.

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130. Feline olfactory anatomy

O. Crenshaw, M. Haskins[1], P. Ulrich and N.E. Rawson

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104 and [1]Department of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Few studies have been reported describing the anatomy and biology of the feline olfactory system. As an obligate carnivore species, it is of interest to examine olfactory neurobiology in the feline to fill-in a gap in our understanding of the evolution of the olfactory system. Thus, in order to obtain a basic understanding of the feline olfactory system, we have studied the anatomy of the domestic shorthaired cat at the macro- and microscopic level. Standard histological techniques were used to obtain 25–40 μm sections which were stained for morphological studies using hematoxylin & eosin, or for immunohistochemistry. Anatomical examination indicates an extensive and intricate turbinate system extending nearly to the end of the nose. The olfactory bulbs extend into the olfactory epithelium, which projects both below and above the anterior extension of the bulbs. Immunohistochemical staining with the neuronal marker NCAM indicates extensive coverage of the septum and turbinates with olfactory epithelium. The cellular structure of the epithelium is similar to that of other species, with a layered appearance and multiple cell types, including round, basal cells, bipolar receptor neurons and supporting cells. These studies have been funded in part by NIH grant DK25759.

131. Bowman's glands and nasal mucous contain gonadotropin-releasing hormone

C.R. Wirsig-Wiechmann and H. Matsumoto[1]

Departments of Cell Biology and [1]Biochemistry and Molecular Biology, University of Oklahoma, Oklahoma City, OK 73104, USA

Gonadotropin-releasing hormone (GnRH) appears to modulate olfactory neuron sensitivity to odors. GnRH is present in terminal nerve (TN) fibers in the lamina propria which underlies the olfactory epithelium. To test our hypothesis that GnRH accesses the olfactory receptor neuron dendrites via mucous secretions from the nasal glands, we conducted an immunocytochemical analysis of olfactory mucosa for the presence of GnRH. In sections of tiger salamander (*Ambystoma tigrinum*) nasal cavity, the Bowman's glands and mucous overlying the olfactory epithelium were immunoreactive for GnRH. Multiple controls including antibody absorption with GnRH, and elimination of theprimary antibody failed to label Bowman's glands and mucous. Antibodies to FMRFamide, another peptide found in the amphibian TN, labeled TN fibers but did not label glands or mucous. Preliminary mass spectrometric analysis of olfactory mucous for the presence of the GnRH peptide has indicated that GnRH levels in mucous are <1 pmol/μl. These findings suggest that GnRH, secreted from the terminal nerve or produced by the glands, may access the surface of the olfactory epithelium via nasal gland secretions.

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132. Expression of estrogen receptor α(ERα) mRNA and protein in the olfactory mucosa

K.J. Fong, A.M. Robinson, R.C. Kern and D.Z. Pitovski

Department of Otolaryngology Head and Neck Surgery, Northwestern University Medical School, Chicago, IL 60611, USA

The mammalian olfactory mucosa consists of a specialized neuroepithelium and secretory lamina propria within the nasal cavity that is exposed to external stress. Neurogenesis, however, continues in this tissue throughout life, thus endowing the animal with the ability to replace neurons that have been lost as a result of physical, chemical or infectious insults. Systemic regulation of this process is poorly understood; however, psychophysical studies indicate that females tend to have a superior sense of smell throughout life. Furthermore, females on postmenopausal estrogen tend to be relatively protected against age-related diminution in olfactory sensation. Given the established neuroprotective role of estrogens elsewhere in the nervous system, the

current study was designed to determine the presence and distribution of estrogen receptor α (ERα) within the olfactory mucosa. The reverse transcriptase-polymerase chain reaction (RT-PCR) technique was implemented to examine the expression of Erα mRNA in the olfactory mucosa. To assure the presence of olfactory mucosa in the nasal tissue samples, RT-PCR was utilized to determine the presence of olfactory marker protein.

In parallel, using the technique of immunocytochemistry we demonstrated the localization of ERα in the olfactory receptor neurons of female Sprague–Dawley rats. Rat uterus was used as anERα-positive control tissue. When the ER21 antibody was preincubated with the peptide against which the ER21 antibody was developed, only a minimal level of nonspecific staining was detected. The results support the hypothesis that estrogen may serve as a systemic regulator of olfactory development, neuronal turnover and plasticity.

133. Does estrogen protect olfactory neurons from apoptosis through up regulation of the Bcl2 proto oncogene?

A.M. Robinson, K.J. Fong, R.C. Kern and D.Z. Pitovski

Department of Otolaryngology Head and Neck Surgery, Northwestern University School of Medicine, Chicago, IL 60611, USA

Multiple lines of evidence have suggested that estrogen may exert a neuroprotective effect, preventing neurons from natural occurring cell death and the effect of variety of stressors. The mechanism of action for these effects is not completely clear, but may involve estrogen mediated regulation of the *Bcl2* proto-oncogene. The family of Bcl2-related proteins is highly conserved across the phylogenetic tree and is involved in the regulation of death of many cell types, including neurons. Recent studies suggest that *Bcl2* proto-oncogene may play a key role by suppressing programmed cell death (F. Jourdan *et al.*, 1998, Neuroreport, 9: 921). Estradiol, the major physiologic estrogen, has been shown tohave a profound effect on the regulation of the *Bcl2* gene expression in neuronal and non-neuronal cells, suggesting a critical role for this steroid in mediating Bcl2-dependent regulation of natural and induced apoptosis. The current study is designed to explore the hypothesis that estrogen up-regulates *Bcl2* expression in the olfactory neuroepithelium. Since apoptosis is a well-described process in the olfactory neuroepithelium, up-regulation of *Bcl2* should decrease overall neuronal cell death. To this end we have correlated the expression pattern of Bcl2 in the rat olfactory neuroepithelium with altered serum estradiol levels. In brief, ovariectomized rats were treated with variable doses of estradiol replacement. Quantitative RT PCR analysis of the mRNA was performed using rat-specific PCR primers for the *Bcl2* gene.

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134. Apoptotic death of olfactory neurons during different estrogen conditions

D.Z. Pitovski, K.J. Fong, A.M. Robinson and R.C. Kern

Department of Otolaryngology Head and Neck Surgery, Northwestern University School of Medicine, Chicago, IL 60611, USA

Programmed cell death (or apoptosis) is the most common form of physiological death and is characterized by cytoplasmic blebbing, nuclear shrinkage, chromatin condensation and internucleosomal DNA fragmentation. Apoptosis can be triggered by multiple mechanisms, including growth factor deprivation, cytokine treatment, hormone dependent or sensitive cells, and antigen receptor engagement. The anti apoptotic action of estrogen is a well recognized phenomenon that, among other things, frequently confounds establishment of up-regulation of *Bcl2* gene expression in many cell types. A key question within these studies is whether estrogen withdrawal/or augmentation (naturally or experimentally) results in changes in olfactory neuronal apoptosis. Although a wealth of information is available as to the anti-apoptotic effects of estrogen in many cell types, including brain neurons, nothing isknown about whether olfactory neuronal apoptosis (and conversely cell viability) is modulated. In these studies we have examined the pattern of olfactory neuronal apoptosis in normal female rats and in rats subjected to different serum levels of estrogen. The process of apoptosis was examined by the TUNEL technique. In each case, a quantitative analysis was made on the olfactory neurons that exhibit characteristics of apoptosis. In addition, we have determined whether estrogen is sufficient to promote protection for the olfactory neurons from apoptotic death. Preliminary data indicates that olfactory neurons undergo cell death, and this death exhibits definitive characteristics of apoptosis. Interestingly, the data also suggests that estrogen may be involved in regulating olfactory neuronal apoptosis.

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135. The olfactory epithelium of the zebrafish and the giant danio: morphological and proliferative differences

K.R. Poling and P.C. Brunjes

102 Gilmer Hall, University of Virginia, Charlottesville, VA 22903, USA

The zebrafish is a popular and easily manipulated developmental model. We previously demonstrated that olfactory deafferentation has less of an effect on olfactory bulb size in zebrafish than in the giant danio, a closely related species. The present study compares epithelial morphology and proliferation between species. Adults were fixed by immersion in paraformaldehyde (morphology) or methacarn (proliferation). For morphology, 2 μm plastic sections were stained with toluidine blue O. For proliferation, 20 μm cryosections were processed for PCNA immunocytochemistry. Using image analysis, rosette surface area and the area of sensory and non-sensory epithelia were measured. Both total rosette and sensory epithelial surface areas were 25% larger in the giant daniothan in the zebrafish, even when scaled to body size. Most proliferation in zebrafish was limited to the sensory portion of the epithelium (about half of the lamellar surface area). In the giant danio, the sensory epithelium extends almost entirely over the lamellae and proliferation is more widespread. The increased sensory epithelial area of the giant danio indicates it has a larger number of sensory afferents, perhaps explaining why rosette removal has a greater impact on the olfactory bulb in this species. With its extensive sensory epithelium and pronounced proliferation, the giant danio appears to have an enhanced olfactory system compared with the zebrafish. Further studies using these two species will determine if their different sensory structures are solely responsible for differing susceptibility to injury, and whether they exhibit different behavioral characteristics.

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136. Activity-dependent labeling of the olfactory organ of blue crabs suggests that pheromone-sensitive and food odor-sensitive receptor neurons are packaged together in aesthetasc sensilla

H.S. Cate, R.A. Gleeson[1] and C.D. Derby

Department of Biology, Georgia State University, Atlanta, GA 30303 and [1]Whitney Laboratory, University of Florida, St Augustine, FL 32084, USA

Decapod crustaceans are known to use sex pheromones as intraspecific communication signals, despite there being no obvious sexual differences in the anatomy of their peripheral and central olfactory systems. We have begun to explore processing of sex pheromones in crustaceans using the blue crab *Callinectes sapidus*. Previous behavioral studies (Gleeson, 1991) suggest that sex pheromones present in the urine of pubertal females are detected by olfactory receptor neurons (ORNs) innervating aesthetasc sensilla on the lateral filament of the male's antennules. In the present study of male crabs, we ask the question, are the receptors for pheromones and food odors colocalized in the same aesthetascs? We used the agmatine (AGB) activity-dependent labeling technique (Steullet *et al.*, this meeting) on male antennules. Urine samples from pubertal females and males were used as sex pheromones (since the pheromone molecules in urine have not been purified or identified), Tetramarin (a commercial fish food) was used as food odor, and seawater was the control. Results show that AGB labeling was greater for male antennules stimulated with either food odor or urine (2–4%) than the control (<1%). Both food odor and urine labeled ORNs in most aesthetascs of the lateral filament, thus suggesting that there is not a subset of aesthetascs exclusively responsive to pheromones. We are currently pursuing several lines, including purifying and identifying the pheromone molecules in urine, and exploring the peripheral and central organization of the pheromone-processing pathway.

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137. Abnormal growth in the olfactory epithelium induced by a parasitic nematode

E. Weiler

Northwestern University, 2153 North Campus Drive, Evanston, IL 60208, USA. e-mail: emw884@nwu.edu

Abnormal growth of olfactory and vomeronasal epithelia was discovered in hamsters (*Phodopus campbelli*) stroked by a parasitic nematode. The olfactory epithelium, showed protrusions extending from turbinates into the nasal cavity and fusions with the epithelium of neighboring turbinates. This abnormal epithelial growth appeared partially as a layered structure and partially as disorganized clumps of tissue which contained differentiated (OMP-positive) olfactory neurons. In some regions the abnormal protrusions formed a layer of olfactory tissue on top of respiratory epithelium. The depth of fusion of turbinates to the septum extended to the lamina propria of the septal olfactory mucosa and showed also signs of destruction of bony tissue. In the most extreme case, the epithelia on the two sides of the septal wall had become connected and formed common olfactory nerve bundles. Sensory epithelium of the vomeronasal organ also developed protrusions. These fused with other protrusions of both sensory and non-sensory epithelium within the VNO. The hitherto undescribed nematode might excrete a potent growth factor inducing uncontrolled proliferation in the olfactory epithelium or alters expression patterns within the olfactory tissues and might provide a useful tool for understanding olfactory neurogenesis, growth and control of structural organization.

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138. A portable artificial nose based on multiple olfactory principles

J.S. Kauer, D.R. Walt[1] and J. White

Department of Neuroscience, Tufts University School of Medicine, Boston, MA 02111 and [1]Department of Chemistry, Tufts University, Medford, MA, USA

The processing of odor information by biological olfactory systems depends heavily on neuronal signals that are encoded both in space across many cells at different levels of the pathway, and in time during and after the stimulus pulse. A number of artificial systems have been developed which also use patterned activity across various kinds of sensor arrays to recognize odors e.g. Persaud and Dodd, 1982, Nature). Improved discrimination performance in these systems can also be achieved by including response time course in the analysis. We have previously shown that robust odor recognition can be obtained from a device that exploits both spatial and temporal information (White *et al.*, 1996, Anal. Chem.) in a system using optical sensors in a research grade, bench-top microscope, a CCD video camera and positive-pressure odor delivery system. In the present paper we describe a more portable version of this device that also incorporates negative pressure odor delivery by sniffing, an array of wide-dynamic range photodiode detectors and an embedded microcomputer for controlling stimulus delivery, data acquisition/storage and pattern recognition. After training, the device is capable of discriminating and identifying a number of odorant substances within seconds.

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139. Techniques for quantifying information in olfactory sensor arrays

T.K. Alkasab, J.S. Kauer and J. White

Department of Neuroscience, Tufts University School of Medicine, Boston, MA 02111, USA

Information theoretic analyses quantitatively estimate the extent to which responses of a sensor or sensors depend on input parameters and therefore could be useful in the study of both artificial and biological olfactory systems. We have used this approach to estimate the mutual information between a panel of odorants and temporal responses of an artificial olfactory system. Repeated responses of a sensor to each odorant are used to develop noise models and an estimated conditional probability matrix. From these, mutual information, indicating the contribution the sensor could make toward discrimination between the applied odorants, can be estimated. In addition, similar estimates

can be made for sensor pairs or groups, which can be used to assess the degree of information overlap between sensors. We have usedthese techniques to analyze the responses of an array of fluorescent chemical detectors to a panel of ten odorants. We found that the sensors with the highest mutual information had previously been shown to be the most empirically useful for discrimination. We expect that this approach can be applied to the rational design of artificial olfactory devices for specific tasks by allowing optimization of both total information and information overlap within a sensor array. Similar methods can be used to analyze data from biological olfactory systems, both to assess the dependence of neuronal responses on stimulus parameters and to measure the degree of similarity of overall response profiles between different elements.

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140. Functional expression of AMPA and kainate receptors in the olfactory bulb

M.S. Horning and P.Q. Trombley

Department of Biological Science, Florida State University, Tallahassee, FL32306, USA

Glutamate dominates excitatory synaptic transmission in the olfactory bulb (OB) and activates both NMDA and non-NMDA receptors on mitral/tufted (M/T) cells and interneurons. However, the receptor identity underlying non-NMDA receptor components has not been determined and it is unknown if they are mediated by AMPA receptors, kainate receptors or both.

AMPA evoked AMPA receptor-mediated currents in the majority of OB neurons. Smaller kainate receptor-mediated currents were also observed in most neurons by co-applying the selective AMPA receptor antagonist, GYKI 52466, with kainate.

Alternative splicing of AMPA receptor subunits GluR1–4 generates 'flip' and 'flop' splice variants, which influence the kinetics of the current. Kinetic properties of AMPA-evoked currents we obseved in OB neurons suggest that both flip and flop variants are expressed. Furthermore, *I–V* relationships were linear and relatively insensitive to changes in external concentrations of calcium, indicating the expression of the GluR2 subunit in its edited form, which acts as a calcium filter.

Co-application of copper effectively blocked currents mediated by AMPA or kainate receptors. However, co-application of zinc had variable effects on AMPA or kainate receptor-mediated currents, consisting of potentiation, inhibition, or no effect. The effects of zinc on AMPA receptors appear to correlate with flip and flop variants.

Results from immunocytochemical staining for kainate subunits GluR5, -6 and -7 and AMPA subunit GluR2 suggest that both receptors may be presynaptically and postsynaptically localized on OB dendrites. Presynaptically, these receptors may be involved infacilitating or inhibiting neurotransmitter release, and post-synaptically, they may contribute to fast excitatory transmission in the OB.

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141. Presynaptic inhibition of primary olfactory afferents mediated by different mechanisms in the lobster and turtle

M. Wachowiak and L.B. Cohen

Department of Molecular and Cellular Physiology, Yale University, New Haven, CT 06520 and Marine Biological Laboratory, Woods Hole, MA02546, USA

We recently demonstrated, using optical imaging with voltage-sensitive dyes, that presynaptic inhibition of olfactory receptor neurons can regulate olfactory input to the CNS in the spiny lobster, *Panulirus argus* (J. Neurophysiol., 80: 1011–1015). Here, we test whether GABA or histamine, inhibitory transmitters in olfactory interneurons, can mediate presynaptic inhibition, and also whether presynaptic inhibition exists in vertebrate olfactory afferents. In the lobster, application of GABA or histamine in calcium-free saline suppressed shock-evoked action potential propagation into receptor cell axon terminals as measured with voltage-sensitive dyes. The suppression was blocked by picrotoxin and cimetidine, antagonists to GABA and histamine receptors respectively. Thus, GABA- and histaminergic interneurons appear to converge on receptor cell axon terminals to dually regulate olfactory input. We used this protocol to test for presynaptic inhibition in the. In contrast, in the turtle (*Terrapene carolina*) olfactory bulb, GABA and dopamine had no effect on action potentials in receptor cell axon terminals. However, we also measured calcium in the terminals by labeling receptor cells with dextran-conjugated calcium indicators (Neuron, 18: 737–752). In normal saline, stimulus-evoked calcium influx showed substantial paired-pulse suppression (50–60%). This suppression was reduced by the $GABA_B$ antagonist saclofen. Calcium influx was reduced by the $GABA_B$ agonist baclofen and the D_2 agonist quinpirole. These results indicate that GABA and dopamine mediate presynaptic inhibition by reducing calcium influx without affecting action potentials, while in the lobster, GABA and histamine directly affect action potential invasion into the terminal. Overall, these findings suggest that, while mediated bydifferent cellular mechanisms, presynaptic regulation of olfactory input to the CNS is a feature common to vertebrates and invertebrates, and may be important in processing olfactory information.

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142. Spatio-temporal properties of odor elicited responses in the turtle olfactory bulb measured with the voltage-sensitive styryl dye, RH414

Y.-W. Lam, L.B. Cohen, M. Wachowiak and M. Zochowski

Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520, USA

Odorant elicited oscillations in local field potential recordings from vertebrate olfactory bulbs were first recorded 50 years ago in mammals (Adrian, 1950) and 25 years ago in turtles (Beuerman, 1975). We made optical measurements of the response to several odorants in an *in vivo* turtle (*Terrapine carolina* or *T. ornata*) preparation. We measured the optical signals with a 464 element diode array using a 4.5× objective; the area of the object plane imaged onto each detector was $166 \times 166\ \mu m^2$.

Four different population signals were detected: a DC response and three oscillations. The three distinguishable oscillations (rostral, medial and caudal) differ from each other in latency, frequency, spatial position, propagation direction and concentration dependence. They appear in specific temporal patterns but do not seems to be strongly causally related because any of the three types can appear without the other two. The spatial origin of the caudal oscillations sometimes changes noticeably from cycle to cycle, suggesting that different neurons are responsible for the consecutive bursts of activity.

We measured oscillations in response to amyl acetate, cineole, butyric acid and pyridine. Preliminary analyses indicate that the propagation direction, the frequency of the oscillation and the area of the bulb involved in the oscillations were similar for all four odors. However, the rise time of the DC component and the latency for the medial oscillation differed for amyl acetate and cineole (the two most frequently studied odors). Thus, some parameters of these population signals differentiate among odorants.

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143. Odor plume structure and dynamics: electrophysiological measurement and central processing

N.J. Vickers[1,2], T.A. Christensen[2], T.C. Baker[3] and J.G. Hildebrand[2]

[1]Department of Biology, University of Utah, Salt Lake City, UT 84112, [2]ARL, Division of Neurobiology, University of Arizona, Tucson, AZ 85721 and [3]Department of Entomology, Iowa State University, Ames, IA 50011, USA

Electroantennograms (EAGs) have been widely used in studies of insect olfaction for both the identification of biologically active odors or their components and as an indication of the presence or absence of odors. Because odor is distributed heterogeneously owing to physical forces of turbulence and shearing in the moving fluid, EAGs have proved particularly useful in characterizing the spatio-temporal structure of odor plumes in air.

We report on several experiments that have utilized EAGs as a means to understand the spatio-temporal structure of odor input that a moth encounters during upwind flight. A stationary EAG was utilized to determine the structure of a pheromone plume under changing physical conditions (wind speed, distance). In another experiment, an EAG was mounted on a moth and then transported upwind by the flying male, allowing resolution of the temporal structure of a pheromone plume coincident with the behavioral responses of the male. More recently, we have succeeded in stimulating a preparation with a plume created within a miniature wind tunnel while recording simultaneously from an intact EAG and central olfactory projection neurons in the ipsilateral antennal lobe of male moths. Analysis of these various data sets reveals the temporal information present in an odor plume and the features extracted and represented at the primary processing level of the olfactory system.

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144. Contextual influences on the central processing of chemical signals by ensembles of olfactory interneurons in the moth antennal lobe

T.A. Christensen and J.G. Hildebrand

Arizona Research Labs Division of Neurobiology, University of Arizona, POBox 210077, Tucson, AZ 85721-0077, USA

We are using multiunit recording methods to characterize patterns of pheromone-evoked activity in ensembles of neurons associated with the glomeruli of the macroglomerular complex (MGC) in the antennal lobe of male *Manduca sexta*. In order to understand how spike patterns are translated into the appropriate signals to elicit goal-oriented upwind flight, it is important to consider that the animal must perform this discrimination task under a wide range of stimulus conditions. We are therefore investigating how changes in the olfactory environment affect activity patterns in MGC networks. While the overall pattern of the response reflects the time course of the stimulus, there is considerable variability in a number of different spike-train parameters, including spike latency, and the number and temporal pattern of evoked spikes. With longer duration stimuli, moreover, new units may be recruited into the ensemble. Since functional interactions among neurons may enhance the representation of sensory stimuli, we also examined the responses of pairs of MGC neurons in the ensemble for evidence of odor-induced synchronization. For each cell pair, spike-train cross-correlations show that coincident events tend to occur only early in the response to a single pulse, regardless of stimulus duration (50–1000 ms). We furthermore found that a pulsatile stimulus evokes a greater degree of coactivity in the ensemble than does a continuous stimulus over the same time period. These data show that ensemble codes in the MGC are not odor-specific, but are greatly affected by stimulus context.

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145. Number and timing of spikes as measures of mitral/tufted cell response strength

K.M. Dorries and J.S. Kauer

Department of Neuroscience, Tufts University School of Medicine, Boston, MA 02111, USA

A widely held assumption in sensory coding is that the number of action potentials reflects the strength of neuronal response to a stimulus. Some important recent conclusions on mitral/tufted cell (MT) response properties—e.g. by Mori and colleagues—are based on measuring numbers of spikes. Earlier work characterizing MT responses failed to find direct effects of stimulus strength on numbers of spikes because MTs respond with complex patterns that include both excitation and suppression. Results of this study indicate that, using a stimulus paradigm that simulates sniffing, the number of spikes does relate to stimulus strength, but not always with a direct correlation. Single unit responses from ~100 salamander MT layer cells included either suppression or bursts of spikes that followed the temporal pattern of a pulse train stimulus. Two distinct burst patterns were differentiated by the timing of the bursts within each sniff cycle: ~25% of cells fired in the rising phase of sniff cycles (early), ~18% fired in the falling phase (late). Approximately half of the remaining cells responded with simple suppression at all concentrations and the rest fell into none of these response categories. While increasing concentrations did

elicit significantly more spikes in late bursting cells, early bursting cells responded with significantly fewer spikes at higher concentrations. These observations suggest that number of spikes may be an effective measure of MT responses only if the timing of the spike bursts and the stimulus are also known.

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146. Goldfish olfactory bulb relay neurons demonstrate a great variety of response characteristies during epithelial cross-adaptation experiments with various pheromones

L.G. Lüthje and H.P. Zippel

Physiologische Institut der Universität, Humboldtallee 23, D-37073 Göttingen, Germany

Physiological responses from mitral cells and ruffed cells were recorded extracellularly and simultaneously. Eleven pheromones and control stimuli (preovulatory: 17,20β-dihydroxyprogesterone, 17,20α-dihydroxyprogesterone, 4-pregnen-20β-ol-3-one, 17,20β,21-trihydroxyprogesterone, androstenedione; ovulatory: prostaglandin $F_{2\alpha}$, 15-ketoprostaglandin $F_{2\alpha}$; probable alarm pheromone: hypoxanthine-3(N)-oxide, hypoxanthine; and two amino acids: Arg, Gln) were applied for 15 s at similar concentrations (10^{-9} M). During the 15 s stimulus applications various effects were recorded during cross-adaptation and compared with the effects recorded after 180 s application of tap water: (1) no response during stimulus application, i.e. a full cross-adaptation; (2) a significantly reduced response, i.e. a partial cross-adaptation; (3) a response similar to that after the interstimulus water phase,i.e. no cross-adaptation; and (4) an opposing response incomparison to the response recorded after water—excitation instead of inhibition (and vice versa). In the latter case blockade of inhibitory (or excitatory) receptor neurons resulted in the release of antagonistic glomerular inputs. Recordings from olfac- tory bulb relay neurons therefore demonstrate (1) a great variety ofglomerular inputs and (2) significantly different responses in contrast to EOG.

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147. Goldfish olfactory bulb relay neurons demonstrate a great variety of response characteristies during epithelial cross-adaptation experiments with amino acids

H.P. Zippel and H.-G. Willms

Physiologische Institut der Universität, Humboldtalless 23, D-37073 Göttingen, Germany

Physiological responses from mitral cells and ruffed cells were recorded extracellularly and simultaneously using a single tungsten electrode. Eleven L-amino acid stimuli (acetic: Asn, Gln; basic: Arg, Lys neutral: Ala, Ser, Val, Leu, Met; and aromatic: Tyr, Phe) were applied for 15 s at similar concentrations (10^{-7} M). After recordings of stimulus effects interrupted by 180 s interstimulus water phases the same experiments were conducted with the samestimuli, but with one of the stimuli applied instead of water during the 180 s interstimulus phases. During the 15 s stimulus applications various effects recorded in comparison to the effects were recorded after application of tap water: (1) no response during stimulus application, i.e. a full cross-adaptation; (2) a significantly reduced response, i.e. a partial cross-adaptation; (3) aresponse similar to that after the interstimulus water phase, i.e. no cross-adaptation; and (4) an opposing response in comparison tothe response recorded after water—excitation instead of inhibition (and vice versa). In the latter case blockade of inhibitory (or excitatory) receptor neurons resulted in the release of antagonistic glomerular inputs. Recordings from olfactory bulb relay neurons therefore demonstrate (1) a great variety of glomerular inputs and (2) significantly different responses in contrast to EOG.

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148. Electrophysiology of identified mitral and tufted cells in the main olfactory bulb (MOB)

P.M. Heyward, Y. Tian and M.T. Shipley

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

Tufted cells are a heterogeneous population of neurons distributed throughout the external plexiform layer (EPL) of the MOB. Some are output cells and may be similar to mitral cells, the principal output cells of the MOB. Many tufted cells may, however, be intrabulbar neurons. The membrane properties of tufted cells are unknown. We are using whole-cell recording in mouse MOB slices to investigate the electrophysiology of mitral and tufted cells, filled with biocytin for post-recording identification. The data show that tufted cells and mitral cells have different electrophysiological properties. The majority of mitral cells are bistable. They can maintain two levels of membrane potential: a relatively hyperpolarized potential, subthreshold for spike generation, and a relatively depolarized potential, perithreshold for spike generation. Olfactory nerve (ON) input can switch their membrane potential between these two levels of excitability. Bistability was found rarely in tufted cells and in cells morphologically similar to mitral cells, with large soma and extensive basal dendrites. Most tufted cells maintained a single resting potential. These smaller cells had sparse basal dendrites, or basal dendrites branching densely near the soma, and an apical tuft in a single glomerulus. ON input did not induce transition to a perithreshold state, but resulted in depolarization to spike threshold and spike generation, consistent with a greater excitability in tufted cells than in mitral cells. We hypothesize that morphological subpopulations of tufted cells have different electrophysiological properties, which may correspond to different functions in olfactory processing.

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149. Multiple effects of zinc and copper on neuronal excitability

P.Q. Trombley, M.S. Horning and L.J. Blakemore

Department of Biological Science, Florida State University, Tallahassee, FL32306, USA

Whole-cell voltage- and current-clamp recordings were used to examine the effects of zinc and copper on the excitable properties of rat mitral/tufted (M/T) cells in primary culture. Zn (100 μM) or Cu (100 μM) blocked both GABA-evoked currents and inhibitory synaptic transmission mediated by $GABA_A$ receptors. In contrast, although Zn and Cu had similar effects on NMDA-mediated currents, they had qualitatively different effects on excitatory synaptic transmission. Excitatory postsynaptic potentials (EPSPs)

and action potentials (APs) were eliminated by Cu (30 μM), whereas Zn (100 μM) only reduced EPSPs and converted the pattern of APs from asynchronous to synchronous.

Because of the qualitative difference in the effect of Zn and Cuon excitatory activity, we examined the direct effects of Zn (100μM) and Cu (30 μM) on membrane excitability. Zn application reduced the number of APs in response to long step depolarizations by 30–40%. In contrast, Cu application prevented burst firing, and usually only one AP could be evoked. Under voltage-clamp, step-depolarizing M/T cells generated transient and sustained outward K^+ currents. Cu dramatically reduced or eliminated transient currents in most neurons. In contrast, Zn slightly reduced transient currents evoked from –90mV, but potentiated the current evoked from –50 mV. The sustained outward K^+ current was dramatically reduced by Cu and slightly reduced by Zn. These results suggest that Zn and Cu may influence odor information processing through differential effects on neuronal excitability. These effects include modulation of voltage-gated membrane currents in addition to modulation of amino acid receptors.

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150. Excitatory interaction among olfactory bulb mitral cells in the absence of synapses

F.-M. Zhou, M. Ennis, B. Davis, and M. Shipley

Department of Anatomy and Neurobiology, University of Maryland at Baltimore, Baltimore, MD 21201, USA

Actions of glutamate as a neurotransmitter are generally believed to be restricted to synapses. Recent studies, however, suggest that neurotransmitter spillover may occur. Main olfactory bulb (MOB) mitral cell dendrites express glutamate receptors and can release glutamate, but they do not form synapses with each other. To test if dendritically released glutamate may influence neighboring mitral cells through non-synaptic mechanisms, we applied whole-cell patch clamp techniques to mouse MOB slices. We focused on NMDA receptor mediated currents recorded in the presence of CNQX and bicuculline. Under these conditions, electrical stimulation in the olfactory nerve layer, lateral olfactory tract, granule cell layer or mitral cell layer and external plexiform layer induced an inward synaptic-like current with a rise time of ~100 ms, a duration of ~10 s and an amplitude of ~200 pA in mitral cells at –70 mV. This current was suppressed by Mg^{2+} and D-APV. In small juxtaglomerular cells, which receive excitatory synaptic inputs from mitral cell apical dendrites, similar stimulation paradigms induced flurries of typical EPSCs, indicating repeated glutamate release. It is possible that action potentials arrive at release sites at different times. Consequently, glutamate release at these release sites may be asynchronous and apparently long lasting. Released glutamate may spillover and activate non-synaptic NMDA receptors on neighboring mitral cell dendrites. We suggest that the prolonged mitral cell NMDA current was mediated by converging inputs from neighboring mitral cells, thereby providing an example for synaptic-like excitatory interactions among central neurons in the absence of synapses.

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151. NMDA receptor-dependent, recurrent and neighboring excitation of mitral cell dendrites in the rat olfactory bulb

V. Aroniadou-Anderjaska, M. Ennis and M.T. Shipley

Department of Anatomy and Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

The primary function of neuronal dendrites is to receive, integrate and process the signals transmitted to them from the axon terminals of the presynaptic neurons. However, the dendrites of certain neuron types have also presynaptic functions, as they can release neurotransmitters. In the olfactory bulb, most neuronal interactions are mediated by dendrodendritic synapses. Dendritic transmitter release could potentially affect the parent dendrite, as well as local neuronal elements that have receptors specific for and accessible to the released transmitter. Here we report that, under conditions that facilitate NMDA receptor activity (reduced $GABA_A$ inhibition and extracellular Mg^{2+}), a single action potential evoked by brief intracellular current pulses in mitral cells is followed by a prolonged depolarization, which is blocked by an NMDA receptor antagonist. This depolarization is also evoked bya presumed calcium spike in the presence of tetrodotoxin. A similar NMDA receptor-dependent prolonged depolarization is also elicited by stimulation of the lateral olfactory tract at current intensities subthreshold for antidromic activation of the recorded neuron. These observations suggest that glutamate released from the dendrites of mitral cells excites the same and neighboring mitral cell dendrites. Further evidence suggests that both the apical and lateral dendrites of mitral cells participate in this recurrent excitation. These dendrodendritic interactions may play a role in the prolonged, NMDA receptor-dependent depolarization of mitral/tufted cells evoked by olfactory nerve stimulation.

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152. Some periglomerular cells of the rat accessory olfactory bulb may be excited by GABA

G.V. Goldmakher and R.L. Moss

UT Southwestern Medical Center, Dallas, TX 75235, USA

Periglomerular (PG) cells of the accessory olfactory bulb (AOB) are GABAergic and form synapses with each other. It has been suggested that some PG cells in the olfactory system may accumulate intracellular chloride such that GABA may act as an excitatory neurotransmitter. We have examined the voltage-activated (22 cells) and GABA-activated (>50 cells) currents of PG cells from the accessory olfactory bulb of the rat using whole-cell patch and perforated-patch recording on dissociated cells. Voltage studies indicated that PG cells are excitable neurons, possessing voltage-gated sodium and potassium currents similar to those found in other neurons in the CNS and capable of conducting action potentials.

At a holding potential of –60 mV, PG cells responded to GABA(20 cells) with inward currents (ranging from 150 to 800 pA at 50 mM GABA) which could be enhanced by 100 mM chlordiazepoxide (5 cells) and completely blocked by 50 mM bicuculline (15 cells), suggesting that these currents are mediated by $GABA_A$ receptors. Gramicidin-perforated patch recording wasperformed on 30 cells, and two groups of cells could be distinguished based on the reversal potential (E_{rev}) of their

GABA-induced currents. Sixteen of the cells exhibited currents with an E_{rev} = –44.1 ± 8.5 mV, and 14 of the cells exhibited currents with E_{rev} = 1.5 ± 6.5 mV. These data suggest that a subset of PG cells may be excited by GABA.

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153. Calcium influx through NMDA receptors directly triggers neurotransmitter release at olfactory dendrodendritic reciprocal synapses

W.R. Chen and G.M. Shepherd

Yale University Section of Neurobiology, 333 Cedar Street, C303 SHM, New Haven, CT 06510, USA

It has recently been demonstrated that NMDA receptors play a dominant role in olfactory dendrodendritic feedback inhibition while non-NMDA receptors make little direct contribution (Chen and Shepherd, 1998; Isaacson and Strowbridge, 1998; Schoppa *et al.*, 1998). However, the mechanisms underlying these different actions remain to be resolved. We have hypothesized that the action of NMDA receptors is due to a direct role of calcium influx through these receptors in triggering feedback GABA release while non-NMDA receptors mediate a single-synapse EPSP which is too small to activate sodium spikes and high-threshold calcium channels for neurotransmitter release. To test this hypothesis, we have applied caged Ca^{2+} compounds to mitral cell secondary dendrites to trigger glutamate release and activate dendrodendritic feedback circuit. The uncaging-evoked feedback IPSP had the same properties as the spike-evoked feedback IPSP. It was very sensitive to extracellular Mg^{2+} ions and was blocked by APV, an NMDA receptor antagonist. In contrast, non-NMDA receptor blocker CNQX had little effect on the uncaging-evoked IPSP. A mixture of calcium-channel blockers, at doses high enough to block both low- and high-threshold calcium channels, had little effect on the uncaging-evoked feedback IPSP. Taken together, these results indicate that GABA release in the feedback inhibition can be independent of voltage-gated calcium channels and instead it can be triggered directly by calcium influx through NMDA receptors.

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154. Group I metabotropic glutamate receptors modulate transmission from mitral/tufted to granule cells *in vitro*

K.J. Ciombor, V. Aroniadou-Anderjaska, M.T. Shipley and M. Ennis

Department of Anatomy and Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Metabotropic glutamate receptors (mGluRs) are located on several cell types in the olfactory bulb [mitral/tufted (M/T), periglomerular and granule cells], but their physiological actions are not known. Here, we investigated the effects of mGluR activation on field potentials (FPs) recorded in the glomerular layer (GL) and granule cell layer (GCL) during stimulation of the olfactory nerve (ON) and mitral cell layer (MCL) in rat olfactory bulb slices. The ON-evoked GL FP represents ionotropic glutamate receptor-dependent synaptic activation of M/T cell apical dendrites. The GCL FP reflects activation of granule cells by glutamate released from M/T cell lateral dendrites.

The group I/II mGluR agonist ACPD proportionately decreased the GL and GCL FPs evoked by ON shocks in a dose-dependent manner (50–200 μM; 20–50% reduction). The GCL FP evoked by LOT or MCL shocks was simultaneously suppressed. The effects of ACPD were mimicked by the group I agonist DHPG (100 μM), but not by the group II agonist CCG (2μM), and were blocked by the group I/II antagonist MCPG (500μM). When the GL was isolated from the deeper layers, 200μM ACPD did not affect the ON-evoked GL FP, suggesting that ACPD mainly acts below the GL. In agreement with this, ACPD decreased the GCL FP evoked by MCL shocks after removal of the GL. These results indicate that activation of group I mGluRs decreases synaptic activation of granule cells by M/T cells, possibly by presynaptic inhibition of glutamate release from M/T cell lateral dendrites.

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155. Group II metabotropic glutamate receptor reduces synaptic transmission from mitral to granule cells in the rat accessory olfactory bulb

C. Jia, W. Chen, M. Ma and G.M. Shepherd

Section of Neurobiology, Yale Medical School, New Haven, CT 06510, USA

The mitral cells of the rat accessory olfactory bulb (AOB) form dendrodendritic reciprocal synapses with granule cells. Synaptic transmission from mitral to granule cells is glutamatergic and from granule to mitral cells is GABAergic. The group II metabotropic glutamate receptor (mGlu II) has been shown to suppress GABA release from the granule cell and thereby presynaptically reduces granule to mitral cell inhibitory transmission in the AOB. The reduced inhibition of mitral cells is believed to induce a long-term change in synaptic transmission in the AOB that underlies olfactory learning (Kaba *et al.*, 1994, Science).

We analyzed the role of mGlu II in the modulation of mitral to granule cell excitatory transmission. Stimulation of the lateral olfactory tract (LOT) to activate mitral cells evoked an EPSP in granule cells. This EPSP was reduced by bath application of DCG-IV, a mGlu II agonist. The field potential recorded in the granule cell layer in response to LOT stimulation was also reduced by DCG-IV. The effect of DCG-IV was not abolished by the NMDA receptor antagonist APV. Whole cell recording from the mitral cells showed a feedback IPSP following an action potential in the absence of Mg^{2+}; this feedback IPSP as well as the spontaneous IPSP was reduced by DCG-IV. Preliminary data showed that the reduction of synaptic transmission from mitral to granule cells by DCG-IV was presynaptic. These results indicated that mGlu II reduced mitral to granule cell synaptic transmission.

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156. Long-term potentiation in the rat accessory olfactory bulb

C. Jia, M. Ma, W. Chen and G.M. Shepherd

Section of Neurobiology, Yale Medical School, New Haven, CT 06510, USA

The accessory olfactory bulb (AOB) is the first relay station in the vomeronasal system and is believed to play a critical role in learning processes, such as the Bruce effect in mice. The principle neurons of the AOB, the mitral cells, form dendrodendritic reciprocal synapses with the inhibitory interneurons, the granule cells. These synapses have been hypothesized to be the sites of modulation in learning. In the present study, modulation of mitral

to granule cell synaptic transmission was analyzed in slice preparations of young adult rat AOB. A bipolar stimulating electrode was place in the lateral olfactory tract or in the external plexiform layer to activate the mitral cells. The extracellular field EPSP (fEPSP) generated by granule cells was recorded in the granule cell layer and external plexiform layer. Perforated patch recordings were made from mitral cells and granule cells. Three tetanus stimulations of 100 pulse at 50–100 Hz separated by 20 s evoked long-term potentiation (>1 h) of the fEPSP as well as granule cell EPSP. The increase fEPSP was accompanied by an increased IPSP recorded in mitral cells, suggesting that potentiation of mitral to granule cell synaptic transmission may result in a decreased mitral cell excitability. This study not only may help to understand the processing of odor information but also learning in general. We are analyzing the mechanism of the long-term potentiation.

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157. Increase in network excitability following high-frequency stimulation of association fibers in piriform cortex

J.S. Stripling and J.L. Cauthron

Department of Psychology, University of Arkansas, Fayetteville, AR 72701, USA

Repeated high-frequency stimulation (HFS) of association fibers in the piriform cortex (PC) potentiates late components of potentials evoked in the PC and olfactory bulb (OB). For a short period of time following HFS, expression of the potentiated component occurs in an all-or-none fashion at near-threshold stimulation intensities. The present research examined the all-or-none characteristics of this phenomenon. Potentiation was induced in freely moving male Long–Evans rats by daily HFS (10-pulse trains at 100 Hz) of PC association fibers. The expression of potentiation was then characterized by test stimulation before and after HFS. Before HFS the potentiated component was expressed in a graded fashion at high current intensities. Following HFS the potentiated component was expressed at low current intensities in an all-or-none fashion at a variable latency and with a dramatic increase in magnitude. These effects decayed gradually to pre-stimulation levels over a 15 min period. In addition, the potentiated component sometimes occurred spontaneously following HFS. These findings indicate that HFS causes an increase in excitability in the PC due to regenerative activity in a synaptically activated network with a sharp threshold for reverberating positive feedback. Available evidence points to deep cells in the PC and endopiriform nucleus as likely participants in this network. This increase in excitability may play a role in olfactory processing.

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158. Cortical mechanisms of olfactory coding: adaptation and cross-adaptation to odorants presented singly and in mixtures

D.A. Wilson

Department of Zoology, University of Oklahoma, Norman, OK 73019, USA

Behavioral adaptation to odors occurs more rapidly than depression of peripheral EOG responses (Hummel *et al.*, 1996), and there is limited behavioral cross-adaptation between diverse odorants (Berglund and Engen, 1993; Pierce *et al.*, 1996; Stevens and O'Connell, 1996). Similarly in the rat, odor habituation of neural responses in the anterior piriform cortex (1) occurs more rapidly than in mitral/tufted cells; (2) is associated with afferent synaptic depression within the piriform cortex; and (3) appears to be odor-specific (Wilson, 1998a,b). The present study further examined cross-adaptation in piriform cortex, and in addition, examined the effects of habituation to odor mixtures on responses to the mixture components.

Rats were anesthetized with urethane and were naturally respiring. Odorants were delivered with a computer-controlled Picospritzer forcing air through odorant saturated syringe filters into a clean airstream (600 ml/min). Responses of layer II/III anterior piriform cortex single-units to 2 s test odor stimuli were analyzed before and after a 50 s habituating stimulus. Habituating stimuli consisted of (1) a 1:1 mixture of the same test odor and airthrough a clean syringe filter; (2) a 1:1 mixture of the same testodor and a different odor; or (3) a different odor. Response magnitude to the test odor was examined 10–30 s after the termination of the habituating stimulus and 2 min later.

The results suggest minimal cross-adaptation between odorants, and that habituation to an odor mixture has relatively minor effects on subsequent responses to the components. These results correspond with human behavioral findings.

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159. Reduced habituation is achieved with a five minute inter-stimulus interval in the olfactory event-related potential

S. Wetter[1,2], M.W. Geisler[1,2] and C. Murphy[1,2]

[1]SDSU Department of Psychology, 6363 Alvarado Ct, Ste 101, San Diego, CA 92120-4913 and [2]UC San Diego Medical Center, San Diego, CA92103, USA

Recent research on the olfactory event-related potential (OERP) using a 60 s inter-stimulus interval (ISI) has revealed a significant decrease in component amplitude after the first trial, with a leveling off for the remaining trials. In addition, a previous study by the authors demonstrated that a 10 min ISI can completely reduce habituation in the OERP. Studies which manipulate the ISI in olfactory and other modalities demonstrate an association between higher amplitudes and longer ISIs, suggesting that habituation occurs at short time intervals between each stimulus presentation. The present study attempted to further investigate the effects of habituation by using a 5 min ISI. OERPs were recorded monopolarly at the Fz, Cz and Pz electrode sites for three trials. Repeated measures ANOVAs demonstrated no significant reduction in component amplitudes across trials and no significant difference in latencies over trials, indicating no habituation effect at this ISI. Mean differences across trials were similar in pattern to those of the 10 min ISI. The only differential pattern was present at the N1–P2 interpeak interval. Specifically, there was a slight (non-significant) increase in N1–P2 amplitude for the 10 min ISI and a slight decrease for the 5 min ISI across trials. These results indicate that, similar to the 10 min ISI, a reduction in habituation can be achieved with a 5 min ISI.

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160. Sensitization of the human EEG during chemical exposure

M. Fernandez[1,2], I.R. Bell[1,2] and G.E.R. Schwartz[1]

[1]*Department of Psychology, The University of Arizona, Tucson, AZ 85721 and* [2]*Veterans Affairs Medical Center, Tucson, AZ 85723, USA*

This study tested the sensitization model proposed by Bell *et al.* (1992, Biol. Psychiat., 32: 218) to study chemical sensitivity. The sensitization model indicates that an intense stimulus or trauma which elicits a strong response can initiate sensitization in limbic pathways. Less intense stimuli presented subsequently, in the sameor different modality, can elicit an amplified response. Three groups of subjects were tested: (1) women with chemical sensitivity (CS); (2) sexually abused women without chemical sensitivity (SA);and (3) healthy women without chemical sensitivity or sexual abuse history (N). All subjects were exposed to odorant (peppermint, vanilla, propylene glycol) and control stimuli (empty bottle) weekly for 3 weeks. Electroencephalographic (EEG) activity was recorded while subjects sniffed the odorant and control stimuli. EEG findings revealed that the CS and the SA groups showed alpha sensitization (i.e. increases in the amplitude spectrum of the alpha frequency band) across experimental sessions, while the N group showed little change over time. Additionally, EEG findings revealed that the CS group generated greater alpha activity than the other groups. These results suggest that intermittent exposure to odorants elicits sensitization in chemically sensitive women and in sexually abused women without chemical sensitivity. Moreover, these findings suggest that chemically sensitive women are unlike sexually abused women without chemical sensitivity and healthy women without chemical sensitivity or sexual abuse history in the amount of EEG alpha activity they generate.

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161. Response profiles of midbrain and forebrain neurons in the central olfactory pathway of the American lobster, *Homarus americanus*

DeF. Mellon[1,2] and J. Atema[2]

[1]*University of Virginia, Department of Biology, Charlottesville, VA 22903 and* [2]*Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543, USA*

Previous electrical recordings from single cells in the olfactory midbrain and the hemi-ellipsoid body of the freshwater crayfish *Procambarus clarkii* have revealed several type-specific response profiles. For example, type I neurons in the crayfish olfactory midbrain respond to odors applied to the lateral antennular filaments with EPSPS and superimposed impulse trains. Type II midbrain cells respond to the same odors with prolonged hyperpolarizing responses that inhibit ongoing spike activity. Minimal testing ofsimilar cells in the lobster suggests that these cells are broad spectrum and respond to both 0.02% tetramarin solution and 10^{-4} M mixtures of several amino acids.

Recordings from lateral forebrain (hemi-ellipsoid) interneurons of the lobster revealed, as in the crayfish, oscillatory activity consisting of depolarizing compound synaptic potentials at a frequency of ~0.5 Hz. At their peak, these periodic depolarizations generated one or two action potentials; moreover, occasional impulse bursts (3–10 spikes at 100 Hz) punctuated peak depolarizations, as also occur in homologous crayfish neurons, especially following antennular stimulation. These findings strengthen the hypothesis that oscillatory activity in the lateral forebrain is a general feature of the crustacean central olfactory pathway.

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162. The macroglomerular complex in two related species of moths: specified subdivision according to input information

B.G. Berg, T.J. Almaas and H. Mustaparta

Department of Zoology, Norwegian University of Science and Technology, N-7034 Trondheim, Norway

Projections of functionally different receptor neuron types in the macroglomerular complex (MGC) of the antennal lobe have been studied in two allopatric species of heliothine moths: *Heliothis virescens* using *cis*-11-hexadecenal and *Helicoverpa assulta* using *cis*-9-hexadecenal as the major pheromone component. Histological investigations including camera-lucidae drawings and 3-D computer reconstructions have revealed that the MGC consists of four compartments in *H. virescens* and three in *H.assulta*. In both species one large unit (the cumulus) was located at the entrance of the antennal nerve. A second large compartment was located dorso-medially of the cumulus, and one or two smaller units ventrally. Electrophysiological recordings from single sensilla (tip-recordings) combined with cobalt application revealed marked axon terminals in the MGC. In both species the cumulus received input from receptor neurons tuned to the major pheromone component. The dorso-medial compartment received input from neurons tuned to *cis*-9-tetradecenal, which is important as a second pheromone component in *H. virescens* and as a behavioural antagonist in *H. assulta*. Whereas the two ventral compartments in*H. virescens* received input from neurons responding to two behavioural antagonists, the single ventral compartment in *H. assulta* seemed to receive information about the second pheromone component. Thus, a striking similarity is expressed between these and other heliothine species by the function of the cumulus as a relay for transmitting information about the major pheromone component, whereas the function of the other compartments differs between the species.

163. Developmental and activity-dependent cell death in the rat olfactory bulb

B.K. Fiske[1], C.C. Norman[2] and P.C. Brunjes[1,2]

[1]*Neuroscience Graduate Program, University of Virginia, Charlottesville, VA 22903 and* [2]*Department of Psychology, University of Virginia, Charlottesville, VA 22903, USA*

The developing nervous system exhibits periods of regulated cell death that help sculpt the maturing brain. The olfactory bulb is an amenable structure for examining this process due to its strict laminar organization, relatively simple circuitry and the fact that afferent neuronal activity is important for cell survival. Indeed, reducing odorant access to one side of the nasal cavity by surgically closing an external naris on postnatal day 1 (P1) results in an ~25% volume reduction of the ipsilateral bulb by P30. Thedecrease is due in part to a loss of late-arising tufted cells andpostnatally generated interneurons, apparently through an

apoptotic pathway (e.g. J. Najbauer and M. Leon, 1995, Brain Res., 674: 245–251). The present study examined the time course of cell death in both normal rats and rats which had a single naris surgically occluded on P1. Using TUNEL labeling, bulbs were examined at P5, P10, P15, P20 and P30. TUNEL+ nuclei were seen at all ages and in every layer. Naris closure increased TUNEL+ labeling by P30. Preliminary data suggest (a) the increase occurs much earlier and (b) changes in the glomerular layer precede those in the granule cell and subependymal zones, suggesting a superficial-to-deep cell death gradient. Understanding these patterns of cell death will aid in deciphering processes through which bulb wiring is established.

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164. Pheromone-sensitive mitral cells in the goldfish respond to more than one odotope

L.R. Hanson, J. Caprio[1] and P.W. Sorensen

University of Minnesota, St Paul, MN 55108 and [1]Louisiana State University, Baton Rouge, LA 70803, USA

In this study we take a first look at how projection neurons in the vertebrate olfactory bulb discriminate pheromones. Electro-olfactogram (EOG) and extracellular recordings from single olfactory bulb projection neurons were recorded simultaneously in sexually mature male goldfish. Five mixtures of odorants with different functions and molecular structures were tested at biologically relevant concentrations: sex steroids (pre-ovulatory sex pheromones), amino acids (feeding), prostaglandins (post-ovulatory sex pheromones), bile steroids (non-reproductive pheromones) and nucleotides (possible feeding cues). EOG cross-adaptation experiments demonstrated that these mixtures are processed by completely independent sets of olfactory receptors. We have now completed recordings from over a dozen neurons in bulbar regions identified by field potential recordings to respond to sex steroids. Bulbar neuron responses were categorized as being excited, inhibited, or insensitive based on firing rate. Approximately 50% of the neurons were excited by the sex steroid mixture and about half of these were excited by at least one additional mixture. Most of the broadly tuned neurons were excited by both sex steroids and amino acids. This broad tuning of neurons could be the result of input from multiple glomeruli or the presence of multiple receptors on olfactory receptor neurons. In conclusion, our results demonstrate that mitral cells in the goldfish frequently respond to more than one odotope, and suggest that they may be processing information about complex mixtures with possible biological function (see Kihslinger and Sorensen, this symposium).

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165. Heterogeneity of IPSCs in mouse olfactory bulb granule cells

Z. Nusser and I. Mody

Departments of Neurology and Physiology, UCLA School of Medicine, Los Angeles, CA 90095, USA

Granule cells of the olfactory bulb provide GABAergic output to mitral and tufted cells, and receive GABAergic inputs from other granule cells and from short-axon cells. It is unclear whether the different inputs to the granule cells are mediated by the same type of receptor. To investigate the properties of synaptic $GABA_A$ receptors on granule cells, we recorded miniature inhibitory postsynaptic currents (mIPSCs) in the presence of tetrodotoxin (1μM) and kynurenic acid (3–5 mM). mIPSCs occurred relatively infrequently (0.2–2.3 Hz) and the distribution of inter-event intervals was well fitted by a single exponential. In most cells, fast and slowly rising mIPSCs were detected, resulting in a bimodal distribution of the 10–90% rise times, suggesting two distinct populations of events. Fast IPSCs had 10–90% rise times of 410 ± 114 μs (mean ± SD), variable amplitudes (mean = 68 ± 13 pA; range: 13–215 pA) and weighted decay times of 15.8 ± 6.6 ms. In contrast, the slow IPSCs had ~3-fold slower rise times (1.15 ± 0.26ms), 55% smaller amplitudes (29 ± 3.9 pA), but weighted decay times (16.8 ± 6.8 ms) similar to that of the fast events. As the amount of charge transferred by the slow mIPSCs is only 55% of that of fast mIPSCs, we conclude that these two types of IPSCs have different kinetics at their respective sites of generation. Future experiments will be required to identify the origin of presynaptic elements, generating these different types of IPSCs, and their functional significance.

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166. Neural substrates for sex and individual recognition by odors in female golden hamsters

R.E. Johnston and A. Petrulis

Department of Psychology, Cornell University, Ithaca, NY, USA

As part of a research program to understand the neural mechanisms of social recognition and memory (individual, kin, sex, species, etc.), we have examined the areas of the brain of female hamsters necessary for behaviors based on sex recognition by odors and contrasted this with discrimination of individuals by odors. Cuts of the lateral olfactory tract eliminate both abilities. Lesions of the agranular insular (prefrontal) cortex, which have been shown to be involved in learned discriminations of arbitrary odors, did not result in deficits in any of our tasks (preferences for odors of males, preferential scent marking to odors of males or individual discrimination). Lesions of the corticomedial amygdala eliminated female's preferences for male odors, but did not influence individual discrimination or preferential marking toward males. Thus, the corticomedial amygdala is important for sex preferences but is not necessary for all behaviors based on sex discrimination and it is not necessary for individual discrimination. In contrast, lesions of the entorhinal cortex and associated structures eliminated the discrimination of individual odors but did not eliminate sex preferences. Thus, we have shown a dissociation of the central olfactory processes underlying sex discrimination, sex preferences and individual discrimination by odors.

167. Organization of the ophidian amygdala: chemosensory pathways to the hypothalamus

A. Martínez-Marcos, E. Lanuza[1] and M. Halpern

Department of Anatomy and Cell Biology, HSCB, SUNY, Brooklyn, NY11203, USA and [1]Department de Biologia Animal, Universitat de València, 46100 Burjassot, València, Spain

Although recent studies in squamate reptiles have importantly clarified how chemical information is processed in the reptilian

brain, how the amygdala relays chemosensory inputs to the hypothalamus to influence chemically guided behaviors is still poorly documented. To identify these chemosensory pathways, the amygdalo-hypothalamic projections, intra-amygdaloid circuitry and afferents from the lateral cortex (LC) to the amygdala were investigated by injecting conjugated dextran-amines into the hypothalamus, amygdala and LC of garter snakes. The amygdala was divided into olfactory recipient (ventral anterior and external amygdalae), vomeronasal recipient [nucleus sphericus (NS) and medial amygdala (MA)] and non-chemosensory [e.g. posterior dorsal ventricular ridge (PDVR) and dorsolateral amygdaloid nucleus (DLA)] subdivisions. Rostroventral (LCrv) and dorsocaudal subdivisions of the LC were distinguished.

In addition to receiving afferents from the main olfactory bulb, the olfactory amygdala receives afferents from NS and projects to the NS, PDVR and dorsal hypothalamus. The NS has only a minor projection to the lateral hypothalamus, whereas the MA, which receives afferents from the LCrv and NS, has projections to the ventromedial hypothalamic (VMH) and lateral posterior hypothalamic nuclei. Among the non-chemosensory amygdaloid structures, the PDVR receives afferents from the LCrv and the olfactory amygdala and projects to the VMH, whereas DLA receives afferents from the LCrv and NS and projects to the periventricular hypothalamus.

These results substantially clarify the olfactory and vomeronasal tertiary connections and demonstrate that parts of the non-chemosensory amygdala play a major role in relaying chemosensory information to the hypothalamus.

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173. Chronic recordings from the rat chorda tympani nerve

R.M. Bradley and S. Grabauskiene

Department Biological and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA

Neurophysiological recordings from the chorda tympani nerve (CT) are usually performed on acute preparations in which the nerve is cut. While these recordings provide much information about the characteristics of CT response properties, they have limitations. In particular, it is not possible to record CT activity in behaving animals over a prolonged time period. To overcome this limitation a cuff electrode, constructed from a low toxicity silicone compound in which a recording electrode is embedded, has been implanted around the CT. The electrode is an insulated small diameter platinum iridium wire, bonded to a connector in a machined titanium headcap housing. Electrodes are implanted around the uncut rat CT under aseptic conditions and secured in position by further application of the silicone compound. The headcap is attached to the skull using surgical quality titanium screws. After 2 weeks recovery the rats are briefly anesthetized (~30 min), connected to conventional electrophysiological recording equipment and a standard series of taste stimuli flowed over the anterior tongue for 20 s interspersed with 30 s water rinses. In successful recordings from five rats, the signal to noise ratio is excellent and stable responses to taste stimuli are recorded. Recordings are obtained as early as 2 weeks after implantation and can be repeated for up to 16 weeks. Recordings are terminated either because the electrode assembly fails or because the neural signal becomes progressively weaker. This preparation has potential for examining the relationship between feeding behavior and neural activity in chronic experiments.

174. Chemical, thermal, and pharmacological sensitivities of lingual geniculate ganglion neurons in rats

R.F. Lundy Jr[1,2] and R.J. Contreras[1]

[1]Department of Psychology, Florida State University, Tallahassee, FL32304-1072 and [2]Department of Behavioral Science, Penn State College of Medicine, Hershey, PA 17033-4181, USA

To characterize further the unique features of chorda tympani neurons, we recorded extracellular responses from single geniculate ganglion neurons to application of sucrose, NaCl, HCl and QHCl (standards) at different temperatures (35, 25 and 15°C), and to NaCl, KCl and NH_4Cl in the absence and presence of amiloride, amiloride-5(*N*,*N*-dimethyl)-chloride (DMA) and 4-aminopyradine (4-AP). Based on the response characteristics of 73 neurons to the standards, 8 neurons were classified as sucrose-specialist, 18 as NaCl-specialist, 12 as NaCl-generalist, 33 as HCl-generalist and 2 as QHCl-generalist. The sucrose- and NaCl-specialists responded narrowly to sucrose and NaCl respectively. The generalist neurons responded to salt, acid, and alkaloid stimuli; chemical effectiveness was NaCl > HCl = QHCl in NaCl-generalists, Hcl > NaCl > QHCl in HCl-generalists and QHCl = NaCl > HCl in QHCl-generalists. Among the three salts the order was NaCl = KCl = NH_4Cl in NaCl- and QHCl-generalists, and NH_4Cl > NaCl > KCl in HCl-generalists. Of the 39 neurons tested, 23 responded to cooling of the tongue (1°C/s from 35 to 10°C) and chemical stimulation. Twenty of these dually responsive neurons were HCl-generalist. The responses to the standards were reduced progressively at lower temperatures in HCl- and QHCl-generalist neurons, but not in NaCl-specialist neurons. In the presence of amiloride and DMA, antagonists of Na^+ channels and Na^+/H^+ exchangers respectively, the responses to NaCl in NaCl-specialist neurons, but not in HCl- and NaCl-generalist neurons, were inhibited. The K^+ channel antagonist, 4-AP, produced a modest suppression on responses to NH_4Cl only in HCl-generalist neurons.

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175. Salt-evoked lingual surface potentials in humans

A. Mogyorósi[1,3], G.L. Heck[2], V. Lyall[2,3], J.A. DeSimone[2] and G.M.Feldman[1,3]

Departments of [1]Medicine and [2]Physiology, VCU/Medical College of Virginia, Richmond, VA 23298 and [3]Hunter Holmes McGuire VAMC, Richmond, VA 23249, USA

Salt-evoked lingual surface potentials (LSPs) have only been measured in animals. Using a gustometer modified from a device used in animal experiments (Ye *et al.*, 1993, J. Neurophysiol., 70: 167), salt-evoked LSPs were measured in two volunteers. First, increasing concentrations of NaCl (50–500 mM) were applied. A dose–response relationship between the NaCl concentration and the measured LSP was observed. In the first volunteer, at higher salt concentrations (250 and 500 mM), after an initial fast phase response there was a slower increase in voltage. This biphasic

response was similar to salt-evoked LSPs in dogs (DeSimone *et al.*, 1984, J. Gen. Physiol., 83: 633). The slow phase response was absent in the second volunteer, suggesting interindividual differences in LSPs.

When gluconate$^-$, a relatively impermeant anion, was substituted for Cl- in one volunteer, solutions of 250 and 500 mM concentration induced greater voltages than did NaCl. Thus the ability of the anion to cross cell membranes or tight junctions between cells may influence Na^+-evoked LSPs.

To assess the role of the epithelial sodium channel (ENaC) in salt-evoked LSP, amiloride, an inhibitor of ENaC, was used in one volunteer. At 100 μM, amiloride markedly reduced but did not abolish the LSP evoked by 250 mM NaCl. The slow component of the salt-evoked LSP was completely abolished, while the fast component was unchanged.

These pilot data indicate that it is possible to characterize and to quantitate salt-evoked LSPs and to identify the component due to ENaC activity.

176. Rate coding in hamster taste buds?

B.A. Varkevisser[1,3], D.A. Peterson[2], T. Ogura[1,3], C.W. Anderson[2] and S.C. Kinnamon[1,3]

Departments of [1]Anatomy and Neurobiology and [2]Computer Science, Colorado State University, Ft Collins, CO 80523 and [3]Rocky Mountain Taste and Smell Center, Denver, CO 80262, USA

In 1983, taste receptor cells were shown to generate action potentials (Roper *et al.*, 1983, Science, 220: 1311–1312), a phenomenon not seen in other peripheral sensory receptors. However, little is known about the role of action potentials in the encoding of taste qualitites. This study examines taste bud action potential responses measured extracellularly from hamster taste buds using the loose-patch technique (Avenet *et al.*, 1991, J. Membr. Biol., 124: 33–41). Features of each response, such as inter-burst interval and area of burst, were then presented to an artificial neural network for pairwise classification of taste stimuli. Salt responses could be distinguished with up to 80% accuracy from sweet responses, including both NC-01 (an artificial sweetener) and sucrose. In contrast, sucrose and NC-01 responses could not be distinguished. Pruning of features revealed that the inter-burst interval and total number of bursts in time window was sufficient to distinguish between taste stimuli with an accuracy equaling that of tests using all features. This suggests that rate coding among taste buds may participate in the encoding of taste qualitites.

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177. The water response in taste cells: expression of aquaporin-1, -2 and -5 and the characterization of hypoosmotic-induced currents in mammalian taste cells

T.A. Gilbertson, I. Kim, N.L. Siears, H. Zhang and L. Liu

Pennington Biomedical Research Center, LSU, Baton Rouge, LA 70808, USA

Gustatory afferent nerve fibers in a variety of species respond tooral exposure to hypoosmotic stimuli, which has been called thewater response. Currently, however, there is comparatively little known about the effects that osmotic changes have on the activity of taste cells (TRCs) directly. Moreover, the mechanism by which TRCs control the movement of water and, hence, regulate their volume has not been examined. To begin to explore these questions, we have used molecular and immunocytochemical techniques to try and identify candidate molecules that may contribute to volume regulation in mammalian TRCs. Three members of the aquaporin family of water channels found in the kidney have been identified in taste buds. Rat taste cells were labeled basolaterally with antibodies against aquaporin (AQP)-1 and -2, and apically with an anti-AQP5 antibody. Using RT-PCR, we cloned both AQP1 and AQP2 from taste cells in rat and hamster. Partial cDNA sequences show these taste cell AQPs from rat to be >95% identical to those found in kidney. Electrophysiological experiments were performed to investigate the effects of osmotic changes on taste cell activity. Solutions varying in osmolarity (± 30, 60 and 90 mOsm) but not in ion concentrations were perfused onto taste cells during patch-clamp recording and changes in whole cell currents and cell capacitance were recorded. Hypoosmotic solutions cause a 15% increase in membrane area and activate an apparent stretch- sensitive Cl channel. We propose a transduction mechanism for water based upon these preliminary results.

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178. Multiple sensitivity of rat fungiform taste cells: whole cell responses to apical chemical stimulation

T.A. Gilbertson, H. Zhang, J.D. Boughter Jr[1] and D.V. Smith[1]

Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808 and [1]Department of Anatomy & Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Several transduction mechanisms have been demonstrated in mammalian taste cells, but little is known about their distribution within and across receptor cells. We recorded whole-cell responses of 49 rat fungiform taste cells that were maintained within an intact tongue epithelium in a modified Ussing (MU) chamber, which allowed us to flow tastants across the apical membrane while monitoring the activity of the cell with a patch pipette. Stimuli were: 0.1 M sucrose, 0.032 M NaCl, 0.1 M KCl, 0.1 M NH_4Cl, 3.2 mM HCl and 3.2 mM quinine-HCl (QHCl); for some cells (n = 19), 3.2 mM citric acid was used in place of NH_4Cl. Thecells were adapted to distilled H_2O flowing over their apical surfaces. In voltage-clamp configuration, cells showed voltage-activated outward currents, characteristic of taste cells. Application of tastants to the apical membrane resulted in either inward or outward currents; those >5 pA were considered reliable responses. Sucrose and QHCl always elicited outward currents, which were associated with conductance decreases. NaCl, KCl, NH_4Cl and HCl always produced inward currents, which were accompanied by increases in conductance. Over half the cells responded to more than one of these stimuli and some responded to three, four or five of them. Of the 49 cells included in this study, 14 were tested with all six of the stimuli. Although a few of these cells responded to only one of the stimuli, most (64%) responded to two or more.

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179. Effects of external osmolarity on taste receptor cell volume and intracellular pH

V. Lyall[1], G.L. Heck[1], J.A. DeSimone[1] and G.M. Feldman[1,2]

[1]*Department of Physiology, Virginia Commonwealth University, Richmond, VA 23298 and* [2]*Hunter Holmes McGuire Veterans Affairs Medical Center, Richmond, VA 23249, USA*

The chorda tympani response to NaCl and taste receptor cell (TRC) volume are modulated by solution osmolarity, suggesting that TRC volume modulates salt taste (Lyall *et al.*, 1998, Chem. Senses, 23: 618). To investigate the cellular mechanisms involved in TRC volume regulation TRCs were isolated from rat fungiform papillae. We monitored cell volume with calcein and intracellular pH (pH_i) with BCECF. In hypertonic solutions TRC volume decreased. There was no volume recovery in mannitol. In contrast, increasing osmolarity with NaCl caused TRCs to shrink and then recover volume spontaneously (regulatory volume increase; RVI). Increases in osmolarity with these solutes also affected pH_i. Addition of mannitol decreased pH_i (~0.25 pH units), which returned to control values only after mannitol was withdrawn. Increasing the NaCl concentration initially acidified TRCs, which then slowly alkalinized to a value greater than control (~0.2 pH units). The NaCl induced alkalinization occurred over the same time course as did the RVI. To evaluate the roles of Na^+ and Cl^- in TRC alkalinization osmolarity was increased with *N*-methyl-D-glucamine (NMDG)-Cl or with NMDG-sulfate. Alkalinization occurred with both test substances, indicating that the alkalinizing mechanism(s) is independent of Na^+ and Cl^-. In hypotonic solutions TRCs swelled and rapidly lost calcein and BCECF, indicating the activation of a large anion pathway. In conclusion, TRC volume changes occur during salt taste transduction. TRCvolume compensatory mechanisms involve Na^+- and Cl^--independent transport pathways, including a pathway for large anions.

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180. Genetic dissection of biotin and acetate induced membrane currents in *Paramecium* chemoresponse

W.E. Bell[1,4], R.R. Preston[2], J. Yano[1], J.F. Fiekers[3] and J.L. Van Houten[1]

[1]*Department of Biology, University of Vermont, Burlington, VT 05405,* [2]*Department of Physiology, Allegheny University of the Health Sciences, Philadelphia, PA 19129,* [3]*Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405 and* [4]*Department of Biology, Virginia Military Institute, Lexington, VA 24450, USA*

Paramecium tetraurelia respond to attractant stimuli with fast, smooth swimming caused by membrane hyperpolarization. Swimming out of an attractant results in a reduction in speed and increased turning associated with depolarization and action potentials. This behavior allows for populations of cells to accumulate in attractants, which signal the presence of their bacterial food source. Biotin and acetate hyperpolarize the cells to the same extent, however, voltage clamp and mutant analysis show that the mechanisms are quite different. Cells exposed to biotin show an outward conductance consistent with plasma membrane Ca^{2+} pump activity. Removal of biotin results in a depolarizing I_{Ca}, which is magnified by Ca^{2+} regulated Na^+ and Mg^{2+} currents. The depolarization causes an abrupt change in ciliary orientation and an accompanying turn in swimming direction. Under some conditions it also activates a Ca^{2+} regulated $I_{K(d)}$, a hyperpolarizing conductance which causes a rapid increase in swimming speed. Acetate initially hyperpolarizes cells probably by employing an outward $I_{K(Ca)}$. The hyperpolarization may be maintained by Ca^{2+} pump activity. A small I_{Ca} on removal from acetate is insufficient to activate the Na^+, Mg^{2+} and K^+ currents associated with the biotin off response. Measurements of Fura-2 loaded cellsindicate that Ca^{2+} levels increase by ~20 nM during the biotin off response. Ca^{2+} measurements on cells stimulated by other attractants are ongoing.

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181. Proton-activated currents in taste cells of rat vallate papilla

W. Lin[1,2] and S.C. Kinnamon[1,2]

[1]*Colorado State University, Fort Collins, CO 80523 and* [2]*Rocky Mountain Taste and Smell Center, University of Colorado Health Center, Denver, CO80262, USA*

Protons elicit a sour taste. Several proton-gated cation channel subunits involved in acid sensing in neurons have been cloned recently (García-Añoveros *et al.*, 1997, Proc. Natl Acad. Sci. USA, 94: 1459–1464; Waldmann and Lazdunski, 1998, Curr. Opin. Neurobiol., 8: 418–424). Among them, mammalian degenerin-1 (MDEG1, also known as BNaC1) is expressed in rat taste cells of vallate papilla, suggesting a possible role in sour taste (Ugawa *et al.*, 1998, Nature 395: 555–556). We used giga-seal whole-cell recording to examine properties of proton-activated currents in single taste cells of isolated rat vallate taste buds. Citric acid was used to lower the pH in bath solutions. Stimulation with citric acid could elicit an inward current in most cells when the pH was reduced from 7.4 to 6.5 at a holding potential of –80 mV. The current showed a sharp pH-dependence, with increasing amplitude at lower pH values. At pH 5, ~80% of the cells (n = 39) showed increases in holding current and membrane conductance. The proton-activated current usually consisted of a combination of two kinetic components, a rapidly inactivating current followed bya sustained current. The peak current reversed at ~30 mV, indicating that the proton-gated channel is permeable to cations. Amiloride (500 μM) partially suppressed the transient component with no apparent effect on the sustained component. These results suggest that proton-gated cation channels are involved in sour taste transduction. Further studies will be needed to determine the contribution of specific acid-sensing channels to proton-activated currents in vallate taste cells.

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182. Additive effects of fatty acids and denatonium or saccharin in isolated taste receptor cells

J.D. Boughter Jr[1], R.C. Christy[1], D.V. Smith[1] and T.A. Gilbertson[2]

[1]*Department of Anatomy & Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201 and* [2]*Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808, USA*

Fatty acids inhibit delayed rectifying K^+ currents in taste receptor cells (TRCs), providing a mechanism for transduction of fats

(T.A.Gilbertson, *et al.*, 1997, Am. J. Physiol., 272: C1203). K^+ channels are also a direct or indirect target for stimuli that humans describe as sweet- or bitter-tasting, suggesting the possibility that fatty acids may also enhance the response of TRCs to these depolarizing taste stimuli. Interestingly, psychophysical data suggest that there may be perceptual interactions between fats and stimuli representing these other taste qualities. In the present study,we sought to determine if additive effects between fatty acids and sweet or bitter stimuli occurred at the level of the TRC.We used whole cell patch clamp methods to record currents from TRCs in isolated rat fungiform taste buds in the presence ofbath-applied stimuli: 10 μM linoleic acid (C18:2), 500 μM denatonium benzoate, 20 mM Na-saccharin, plus mixtures of these compounds. Individually, these stimuli significantly inhibited outward K^+ currents in TRCs. In the presence of C18:2, there wereresidual outward currents that were eliminated by theaddition of denatonium. Saccharin and C18:2 also had a smalladditive effect. These results indicate that saccharin and denatonium may block other types of K^+ channels in addition tothe fatty acid-sensitive channel and suggest a possible mechanism for interactions between fats and sweet- or bitter-tasting stimuli.

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183. Effects of zinc and other metal ions on delayed rectifying K^+ channels in rat taste cells

J.T. Schilleci and T.A. Gilbertson

Pennington Biomedical Research Center, LSU, Baton Rouge, LA 70808, USA

Zinc deficiency has long been associated clinically with hypogeusia and dysgeusia and zinc supplementation has been show to ameliorate these conditions in many cases. We used whole cell patch clamp techniques to determine if zinc had specific effects on taste receptor cell activity. Zinc, cadmium, nickel and manganese applied extracellularly (5 μM to 5 mM) all reduced outward K^+ currents but had markedly different efficacies. Zn^{2+} inhibited >90% of the delayed rectifying K^+ (DRK) current with an IC_{50} = 1.5 mM. Cd^{2+}, Ni^{2+} and Mn^{2+}, which only partially inhibited the DRK currents (~10–30%), had much weaker effects (IC_{50} = 14.3, 28.0 and 60 mM respectively). Because the significant effects appeared to be limited to Zn^{2+}, we examined its effects in detail on the kinetics of potassium channels in rat taste cells. External Zn^{2+} (5 mM) produced an ~3-fold slowing of K^+ current activation and caused a –15 mV shift in the inactivation curve. Zn^{2+} did not significantly affect tail currents or use-dependent inactivation. In contrast to Zn^{2+}, the other metal ions showed no significant effects on K^+ channel kinetics. The evidence from these studies is consistent with the existence of a specific binding for Zn^{2+} at a site closely associated with the regulation of DRK channel function. The moderate reduction in outward K^+ current associated with the other metal ions studied may be explained by simple ion-surface charge interactions.

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184. The role of delayed rectifying K^+ channels in chemosensory fat signaling in the gut

I. Kim, B. Keller, L. Liu, S. Liou, D.A. York and T.A. Gilbertson

Pennington Biomedical Research Center, LSU, Baton Rouge, LA 70808, USA

Our previous work has shown that a subtype of delayed rectifying K^+ (DRK) channel, a Shaker Kv1.5-like channel, in taste cells is inhibited by essential fatty acids (*cis*-polyunsaturated; PUFAs), which we believe represents a gustatory cue for dietary fat. Preliminary immunocytochemical work from our lab has shown that several fat-responsive organs, including the liver, pancreas and duodenum, contain these channels as well, suggesting that they may play a role in fat sensing. To explore this point, we have begun work to investigate fatty acid effects in a pancreatic β cell line (HIT-T15) and a duodenal enteroendocrine (cholecystokinin-secreting) cell line (STC-1). Reverse transcription polymerase chain reaction reveals the presence of mRNA for Shaker Kv1.5 channels in HIT and STC-1 cells, consistent with previous immunocytochemistry. Sequencing reveals that the Shaker Kv1.5 channels in these two targets are >90% identical to those cloned from heart. As in taste receptor cells, patch clamp recordings show that PUFAs inhibit DRK channels in a concentration-dependent fashion. Palmitoleic acid (C16:1), a monounsaturated fatty acid, also inhibited DRK channels in STC-1 and HIT cells. Insulin radioimmunoassays were performed on HIT cells to determine the ability of fatty acids to cause insulin release. With the exception of lauric acid (C12:0), the same fatty acids that inhibited DRK channels acids that caused a significant release of insulin (~3-fold), while others were ineffective. We are currently performing ELISAs to determine the ability of fatty acids to release cholecystokinin from STC-1 cells.

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185. Sequential activation of basic helix–loop–helix and repeat helix–loop–helix transcription factors during olfactory placode development

C.J. Burns, O. Pozzoli[1], G. Consalez[1] and M.L. Vetter

Department of Neurobiology and Anatomy, University of Utah, Salt Lake City, UT 84132 and [1]Department of Biological and Technological Research (DIBIT), San Raffaele Scientific Institute (HSR), Via Olgettina 58, I-20132 Milano, Italy

The molecular mechanisms guiding olfactory neurogenesis are incompletely understood. Recent work has found that basic helix–loop–helix (bHLH) transcription factors play a role during this process. We investigated the presence of bHLH and repeat helix–loop–helix (rHLH) transcription factors during olfactory placode development in *Xenopus laevis* using whole mount *in situ* hybridization with a panel of digoxigenin-labeled probes.

We have defined four successive stages of gene activation during olfactory placode development. Within the presumptive placode the *Xenopus* bHLH gene *Neurogenenin Related-1* (*XngnR-1*) is expressed first at stage 15. Second, the rHLH gene, *EBF-2*, is expressed at stage 16. Third, the *Xenopus Atonal Homologues 3* (*Xath 3*) and *5* (*Xath 5*) are expressed at stage 17. Fourth, *NeuroD*, another bHLH gene, shows spotty expression in the placode a few

hours after onset of *Xath 3* and *Xath 5* expression. Coincident with earliest *NeuroD* detection the rHLH gene, *EBF-3*, is strongly expressed. By stage 20 *NeuroD* expression increases to fill the placode. This coincides with the onset of β-*III tubulin* expression indicating the presence of mature neurons. Interestingly, *NeuroD* and *XngnR-1* expression are restricted to the placode perimeters at later developmental stages.

The early expression of *XngnR-1* and *EBF-2* may specify early neural fate, whereas subsequent expression of *NeuroD*, *Xath 3*, *Xath 5* and *EBF-3* may regulate differentiation of olfactory receptor neurons. Future studies will use overexpression and dominant negative constructs to determine the function of these genes during olfactory neurogenesis.

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186. TGF-α overexpression modulates terminal differentiation of olfactory receptor neurons: involvement of TGF-β receptors

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

[1]Department of Physiology, [2]Sanders-Brown Center on Aging and [3]Division of Otolaryngology—Head & Neck Surgery, Department of Surgery, University of Kentucky College of Medicine, Lexington, KY 40536, USA

Olfactory receptor neurons (ORNs) die and are replaced by proliferation of progenitor cells in the olfactory epithelium (OE) that subsequently differentiate into mature ORNs that express olfactory marker protein (OMP). In transgenic (T) mice in which the TGF-α gene is driven by the keratin-14 promoter, there is a 73% increase in TGF-α in nasal-olfactory tissues compared with nontransgenic (NT) littermate controls as determined by radioimmunoassay (T.V. Getchell *et al.*, submitted for publication). InWestern blots of nasal-olfactory mucosal homogenates, OMP isreduced by 59% in T compared with NT mice. Immunostainingshows substantially decreased OMP immunoreactivity inTs compared with NTs, suggesting that the higher level of TGF-α is modulating terminal differentiation of ORNs. *In vitro*,terminal differentiation of ORNs is promoted by TGF-β (Mahanthappa and Schwarting, 1993, Neuron, 10: 293). In other proliferative epithelia, TGF-β receptor (TGF-βR) expression is reduced by TGF-α overexpression. To determine if TGF-βR expression differs in T and NT mice, sections of OE together with skin and palate as positive controls from littermate pairs of Ts andNTs were immunostained concurrently with an antibody to TGF-βR type II. Immunoreactivity for TGF-βR II was localized in ORNs. In Ts, the intensity of TGF-βR II immunoreactivity in ORNs was considerably reduced compared with NTs, as it was in cells of control proliferative epithelia. These results suggest that TGF-α overexpression modulates terminal differentiation of ORNs through down-regulation of the expression of TGF-β receptors.

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187. Expression of biotransformation enzymes in human fetal olfactory mucosa

J. Gu[1], T. Su[1,2], Y. Chen[1], Q.-Y. Zhang[1] and X. Ding[1,2]

[1]Wadsworth Center, New York State Department of Health, Albany, NY12201 and [1]School of Public Health, State University of New York at Albany, NY 12201, USA

High levels of cytochrome P450 are present in the olfactory mucosa (OM) in mammalian animals and contribute to the known tissue-selective toxicity of numerous chemical compounds. Olfactory toxicity in perinatal period may have greater impact on behavior, growth, and development than in adults. To establish a molecular basis for determining the risk of developmental toxicity in OM, the expression of a number of P450 isoforms and other biotransformation enzymes was examined in hepatic and nasal microsomes prepared from aborted human fetus at gestational days 91–125 (G91–G125). The relative tissue levels of various biotransformation enzymes were determined on immunoblots. Expression of CYP2A, CYP2B6, CYP2J, NADPH-P450 reductase and microsomal epoxide hydrolase was detected in both OMand liver at as early as G91. The level of expression in OM is lower than that in liver, except for the CYP2A-related protein, which was expressed in OM at much higher levels. OM expressionof CYP2A6, CYP2A13, CYP2B6 and CYP2J2 mRNA was confirmed using RNA-PCR. The prenatal expression of biotransformation enzymes in human OM may have detrimental consequences upon transplacental exposure to drugs and other foreign compounds.

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188. Sonic hedgehog signaling in rodent tongue cultures

J.M. Hall, T.E. Finger, D.K. MacCallum[1] and C.M. Mistretta[2]

Rocky Mountain Taste and Smell Center and Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262, [1]Anatomy and Cell Biology, Medical School, University of Michigan, Ann Arbor, MI 48109 and [2]Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA

Lingual taste buds form within papillae in mammals. We are studying molecular signals that participate in papilla and taste bud development. Genes for the developmental signaling molecule Sonic hedgehog (*Shh*) and its receptor, Patched (*Ptc*), are expressed within developing fungiform and circumvallate papillae in rat and mouse embryos. To learn whether *Shh* and *Ptc* expression proceeds *in vitro* as *in vivo*, we examined the expression patterns of *Shh* and *Ptc* in rat and mouse embryonic tongue organ cultures. In embryonic rat tongue cultures, papillae develop in their characteristic patterned array at times comparable to those *in vivo*.

Tongue or lower jaw explants were taken from gestational day 13 and 14 rat embryos and day 12, 13 and 14 mouse embryos, and maintained at the liquid–gas interface in conventional organ cultures. Explanted tongues were collected after 0–5 days in culture and assayed for *Shh* or *Ptc* expression by whole mount *in situ* hybridization. In E12 mouse explants, *Shh* is initially broadly expressed in the developing tongue and, as *in vivo*, becomes restricted to developing fungiform and circumvallate papillae. *Shh* is also expressed in papillary precursors in rat tongue cultures. *Ptc* expression mirrors *Shh* expression as in intact embryos. Thus,

expression of *Shh* and *Ptc* in embryonic tongue cultures mimics that observed *in vivo*. Explanted tongue cultures may prove valuable models in which to manipulate relevant intercellular signaling systems.

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189. BDNF and NT-3 mRNA expression patterns in the developing and adult human tongue

I.V. Nosrat, A. Seiger[1] and C.A. Nosrat

Department of Neuroscience, Karolinska Institutet, S-171 77, Stockholm and [1]Department of Clinical Neuroscience and Family Medicine, Karolinska Institute, S-141 86, Huddinge, Sweden

BDNF and NT-3 mRNAs are expressed in developing and adult rodent tongue, and are important for the proper development of the lingual gustatory and somatosensory innervation in rodents. Here, we wished to determine whether the findings in rodents apply to humans. Using *in situ* hybridization, distinct and specific, and in some instances overlapping patterns of BDNF and NT-3 mRNA expression patterns were found. BDNF mRNA was expressed in the developing fungiform and circumvallate papillae, and in the subepithelial mesenchyme. In adult fungiform papillae, BDNF mRNA was found in taste buds, and in restricted areas in the non-gustatory lingual epithelium. NT-3 mRNA was found in the developing lingual epithelium, in gustatory papillae, and in lingual mesenchyme and muscle. In the adult tongue, NT-3 mRNA was observed in taste buds of fungiform papillae, overlapping with BDNF mRNA. NT-3 mRNA was additionally found in restricted areas in filiform papillae. Protein gene product 9.5 antibodies were used to investigate a possible correlation between lingual innervation and sites of neurotrophin gene activity in the adult tongue biopsies. Human tongue innervation differed from that of rodents possibly due to a different neurotrophin expression pattern in the human tongue. As shown in rodents, we suggest that BDNF and NT-3 are required for the initiation and maintenance of the gustatory and somatosensory innervation also in humans. The broader and somewhat overlapping expression patterns of BDNF and NT-3 mRNAs in humans, suggest additional and possibly somewhat overlapping roles for BDNF and NT-3, and also indicates differences between species.

190. Development of extra-oral taste buds in the rat

Z. Popovska, S. Jain and R.D. Sweazey

Department of Anatomy, Indiana University School of Medicine, Fort Wayne, IN 46805, USA

Previous investigations have described the development of extra-oral taste bud populations in several mammalian species including the cat, sheep and hamster. However, similar information is not currently available for the rat, a species widely employed in the study of taste development and upper airway reflexes. To quantify the development of rat extraoral taste buds we examined laryngopharyngeal tissue in postnatal rats aged 1–90 days. Rats were perfused with formalin and the laryngopharynx was removed, blocked, decalcified, and embedded in paraffin. Serial 8–10 μm sections were mounted onto slides and stained with hematoxylin & eosin. Taste buds were counted by drawing sections with a camera lucida and plotting both mature and immature taste buds. Extra-oral taste buds, absent at birth, first appear between 2 and 3 days postnatally. At this age, extra-oral taste buds were immature with the majority located on the palatopharyngeal eminence and smaller numbers located on the lateral edges of the epiglottis and surrounding aryepiglottal folds. Over the first month of life there was a rapid increase in the number of extra-oral taste buds, although many of these taste buds were immature. This increase was most pronounced on the epiglottis and aryepiglottal folds with smaller increases observed on the palatopharyngeal eminence and nasopharynx. The number of extra-oral taste buds continues to increase at a slower rate after the first month. Our results suggest that the development of rat extraoral taste buds is similar to that observed previously in other rodent species such as the hamster.

191. Nitric oxide may play a signaling role in olfactory development in *Manduca sexta*

A. Nighorn, N.J. Gibson, W. Rössler, J.G. Hildebrand and L.P. Tolbert

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

During development of the moth *Manduca sexta*, olfactory receptor axons grow into the antennal lobe of the brain and initiate the formation of olfactory glomeruli. Glomerulus formation involves interactions among the axons, glial cells and neurons of the antennal lobe. We have begun to examine the role of the nitric oxide (NO)–soluble guanylyl cyclase (sGC) pathway in mediating these interactions. Using *in situ* hybridization and immunocytochemistry, we have found that receptor axons express NO synthase before, during and after glomerulus formation. Perineurial cells also are intensely labeled, while glial cells and antennal-lobe neurons are not labeled at any stage of development. sGC, on the other hand, is expressed in many antennal lobe neurons. These neurons respond to exogenously applied NO with an elevation of cGMP levels. Injection of the NO synthase inhibitor L-NAME into developing pupae while olfactory receptor axons are growing into the brain causes abnormal development of glomeruli. The male-specific macroglomerular complex in many cases looks almost normal, but 'ordinary' glomeruli are small and misshapen, and some areas of the neuropil are devoid of glomeruli; glial cells do not form complete glomerular borders according to the normal timetable. Moreover, a unique serotonin-immunoreactive neuron branches abnormally within the glomeruli that form. One possible explanation for the L-NAME effect is that receptor axons normally release NO, which stimulates the NO-sensitive sGC present in antennal lobe neurons. Effects of NO on glial cells may be indirect, or via another intracellular signaling pathway.

192. A novel gene, olfactoregulin, implicated in axon guidance, synapse formation, and regeneration in the rat olfactory bulb

J.M. Otaki and S. Firestein

Department of Biological Sciences, Columbia University, New York, NY 10027, USA

Olfactory sensory neurons expressing a given olfactory receptor terminate in a few discrete targets in the olfactory bulb. Granule cells in the olfactory bulb and dentate gyrus have been known to regenerate throughout adult life. We have cloned a novel transmembrane gene, olfactoregulin, from rat olfactory bulb, that may play a role in synapse formation, axon guidance and regeneration.

The cDNA sequence analysis revealed that this novel gene encodes a long 2765 amino acid residues with a putative transmembrane domain and several other motifs including EGF-like repeats and tyrosine kinase phosphorylation sites. The N-terminal portion of olfactoregulin has a significant homology to one of the neuregulins, raising the possibility that it may play a role in synapse formation. The C-terminal portion has a significant homology to one of the pair-rule genes cloned from *Drosophila*, raising the possibility that it may play a role in morphogenesis. Multiple-tissue Northern blot shows this gene is expressed almost specifically in the brain. *In situ* hybridization further revealed that this gene is highly expressed in the olfactory bulb granule cells, aswell as in the hippocampal granule cells. Expression is also seenin other differentiating neurons but not in the mature or non-differentiating cells. In the developing main and accessory olfactory bulb, olfactoregulin is differentially expressed. This provides support for the hypothesis that olfactoregulin may be involved in the zonal projection of olfactory sensory neurons to the olfactory bulb.

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193. Neuromodulation of Kv1.3 by insulin receptor kinase in the olfactory bulb during sensory deprivation

K. Tucker, J.A. Simmen and D.A. Fadool

Zoology Department, College of Science and Mathematics, Auburn University, Auburn, AL 36849, USA

Insulin activates a number of signaling pathways that regulate cellular metabolism, growth, and plasticity in the CNS. Because we have previously shown that acute insulin application to olfactory bulb neurons (OBNs) causes current suppression of potassium channels (Kv1.3), insulin receptor (IR) kinase may play a secondary role in ion channel modulation via tyrosine phosphorylation in this area of the brain. We now show that in studies of co-transfected Kv1.3 and human IR cDNA in HEK 293 cells, bath application of insulin causes an increase in tyrosine (Y)-specific phosphorylation. The modulation appears to be reciprocal; expression of Kv1.3 protein will down-regulate the degree of IR phosphorylation. Kv1.3 site-directed mutagenesis revealed that current suppression and concomitant phosphorylation can be reversed by removal of the Y-phosphorylation recognition motifs in the ion channel—residues YYY111–113, Y137 and Y479 are all important targets. We demonstrate by Western analysis that the β subunit of the IR is developmentally expressed in the rat olfactory bulb (OB) across postnatal (P) ages ranging from P1 to P16 and persists in the adult OB (P60). IR is localized to the outer nerve, external plexiform, and granular cell layers, but its expression is reduced in all but the granular cell layer after 30 days of naris occlusion. We are presently immunoprecipating OB proteins that demonstrate a reduction in Y phosphorylation with odor/sensory deprivation. In summary, tyrosine phosphorylation of Kv1.3 and the IR kinase may be involved in the modulation of OBN excitability.

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194. Focal denervation alters olfactory bulb development

J.M. Couper Leo and P.C. Brunjes[1]

Program in Neuroscience and [1]Department of Psychology, University of Virginia, Charlottesville, VA 22903, USA

Several lines of research indicate that the olfactory epithelium plays an essential role in the development of central olfactory structures. For example, reducing receptor function results in a 25% decrease in olfactory bulb size as well as a constellation ofother changes. Surgical deafferentation, accomplished by removing the olfactory placode or by destroying the olfactory epithelium, has even more drastic effects, including widespread forebrain malformations. We have devised a means by which only small portions of the bulb can be denervated in order to compare the consequences of normal axonal contact and denervation in adjacent pieces of tissue. Adapting a technique described by Costanzo, small Teflon strips were inserted between the cribriform plate and olfactory bulb of P1 rat pups. The Teflon insertion severs existing olfactory connections and prevents in-growth of new axons in the 'shadow' region behind it. The manipulation has substantial effects on several aspects of bulb development. Nissl stains, OMP immunocytochemistry and DiI labeling indicate that the olfactory nerve and glomerular layers are dramatically reduced or even absent. Reductions in calretinin and tyrosine hydroxylase staining indicate compromised function or number of periglomerular (PG) cells. MAP2 and TuJ1 immunocytochemistry suggests modified mitral (M) and PG morphology. Semithin sections also indicate disrupted M and PG organization in the Teflon shadow. These results indicate that neural contact is vital for normal bulb circuitry, and provide a new technique for examining the role of the first nerve in development.

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195. Sonic hedgehog expression in the glomeruli during rat olfactory system development

Q. Gong and A.I. Farbman

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

Sonic hedgehog (*Shh*) is a secreted signaling molecule. It has been shown to mediate many important short and long range patterning processes during vertebrate development. *Shh* signal is transduced through its membrane-bound receptor *patched*. To explore whether *Shh* is involved in the development of the olfactory system, we have used immunohistochemistry to examine the expression pattern of *Shh*. *Shh* is first detected in the olfactory bulb at E20. It is present in the earliest appearing glomeruli in the rostral part of the olfactory bulb. At P0, we observe *Shh* restricted within the glomeruli which are more abundant. *Shh* continues to be present in the glomeruli until adulthood although the level seems to decline after P40. *In situ* hybridization revealed that the transcripts of *sonic hedgehog* are detected in the mitral and tufted cell bodies. Therefore, *Shh* protein in the glomeruli is localized in the mitral and tufted cell dendrites. *patched* transcripts are found in the ordorant receptor neurons (ORN), indicating that ORN axons may have the ability to receive *Shh* signal from the mitral and tufted cell dendrites in the glomeruli. The correlation of the

onset of *Shh* expression in the first glomerulus and the ability of ORN axons to receive *Shh* signal suggest that *Shh* is involved in helping the convergence of ORN axons into the glomeruli or it may function as a 'stop' signal for olfactory axon growth. Further studies are in progress to test these hypotheses.

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196. Phosphodiesterases and calcium signaling in mouse olfactory receptor neurons

G. Liu and B. Talamo

Department of Neuroscience, Tufts University School of Medicine, 136 Harrison Ave, Boston, MA 02111, USA

To investigate the roles of various phosphodiesterases (PDEs) in regulating intracellular Ca^{2+} levels ($[Ca^{2+}]_i$) in olfactory receptor neurons (ORN), PDE inhibitors were utilized. $[Ca^{2+}]_i$ in individual ORNs loaded with fura-2 AM was monitored using fluorescence ratio imaging. Application of various PDE inhibitors to the cellsresulted in the different response profiles. Among cells which responded to high K^+, ~41% responded to IBMX, a non-selective PDE inhibitor. 8-Methoxymethyl-IBMX, an inhibitor selective for the PDE1 subtype, elevated $[Ca^{2+}]_i$ in 33.3% of the cells, all of which also were activated by IBMX. However, rolipram, an inhibitor for the PDE4 subtype, elevated $[Ca^{2+}]_i$ in only nine of 149 cells; seven of these nine cells responded to IBMX. PDE4 is reported to be expressed primarily in ORN cell bodies and axons. These data suggest that PDE1C2, which is localized in the cilia, plays a primary role in somatic $[Ca^{2+}]_i$ elevation in the majority of ORNs. However, rolipram-sensitive cells may represent a subset ofORNs employing different transduction pathways. Further, theincrease of $[Ca^{2+}]_i$ evoked by IBMX required extracellular calcium, and some ORNs were sensitive to as little as 1 μM IBMX. Our data suggest that adenylyl cyclase is tonically active in mouse ORNs and that variations in adenylyl cyclase and PDE levels may affect ORN sensitivity. PDEs, especially PDE1C2, appear to play a significant role in the cAMP-mediated transduction pathway in ORNs.

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197. Intracellular Ca^{2+} stores control the waveform of odor-induced Ca^{2+} transients in the dendrite and soma but not in the cilia of olfactory receptor neurons

F. Zufall, T. Leinders-Zufall, G.M. Shepherd[1] and C.A. Greer[1]

Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD 21201 and [1]Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510, USA

Previously, we found that odor stimulation causes the generation of a characteristic Ca^{2+} wave spreading through the entire ORN starting in the cilia and eventually leading to a global Ca^{2+} rise in the dendrite and soma (Leinders-Zufall *et al.*, 1997, 1998). Because Ca^{2+} is a key signal for odor adaptation, it is necessary to understand the molecular mechanisms underlying this Ca^{2+} rise. Here, we have used a combination of electron microscopy, confocal imaging and perforated patch recording to provide insight into the role of intracellular Ca^{2+} stores in this process. Several main findings emerge from this work. Salamander ORNs contain intracellular Ca^{2+} stores in their soma, dendrite and knob, but not in their cilia. Even at rest, the stores contain a releasable pool of Ca^{2+} that can be discharged applying the SERCA pump inhibitor thapsigargin or the ryanodine receptor agonist caffeine. SERCA pumps mediate the refilling of caffeine-sensitive stores. There is evidence for the presence of capacitative Ca^{2+} entry but it is notinvolved directly in odor transduction. Ca^{2+} released from thapsigargin-sensitive pools serves to amplify and boost odor-induced Ca^{2+} transients in the knob, dendrite and soma, but not in the cilia; this effect underlies Ca^{2+} wave propagation from the cilia to the soma. There is substantial Ca^{2+}-induced Ca^{2+} release. Store-operated Ca^{2+} release can be sufficient to gate Ca^{2+}-activated K^+ currents and hyperpolarize the membrane potential of ORNs. Thapsigargin-sensitive Ca^{2+} stores are not necessary for generation of an electrophysiological odor response. We suggest that store-operated Ca^{2+} release provides a molecular signal that can spread from the knob to the nucleus of an ORN.

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198. Odor-elicited intracellular calcium changes in cultured human olfactory cells

G. Gomez, D. Restrepo[1] and N.E. Rawson

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104 and [1]University of Colorado Health Sciences Center, Denver, CO 80262, USA

An important step in establishing a cell culture system for the *in vitro* study of olfaction is assessing whether the cultured cells possess similar physiological properties to those of mature olfactory neurons (ORNs). Various investigators have successfully established proliferating cell lines from olfactory tissue, but few have demonstrated odor sensitivity of these cells. We have successfully established cultured cell lines from adult human olfactory tissue obtained using an olfactory biopsy procedure and measured their ability to respond to odor stimulation using calcium imaging techniques. Under specific growth conditions, these cultured cells respond to odor mixes that have been previously shown to elicit intracellular calcium ($[Ca^{2+}]_i$) changes in mature human ORNs (N.E. Rawson *et al.*, 1997, J. Neurophysiol., 77: 1606–1613). As in the human ORNs, these $[Ca^{2+}]_i$ changes were reversibly blocked by inhibitors of the olfactory signal transduction cascades. In addition, the cultured cells also responded to stimulation with single odorant mixtures. Interestingly, some of these $[Ca^{2+}]_i$ responses were insensitive to the signal transduction inhibitors, indicating that that these odors elicit an increase in $[Ca^{2+}]_i$ through a nonspecific mechanism or that there may be alternate biochemical pathways for odor signaling in these cultured cells. Our results demonstrated that cultures of adult human olfactory neurons could respond to odors with changes in $[Ca^{2+}]_i$.

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199. Both external and internal calcium reduce the sensitivity of the olfactory cyclic-nucleotide-gated channel to cAMP

S.J. Kleene

Department of Cell Biology, Neurobiology, and Anatomy, University of Cincinnati, Cincinnati, OH 45267-0521, USA

In vertebrate olfaction, odorous stimuli are first transduced into an electrical signal in the cilia of olfactory receptor neurons. Many

odorants cause an increase in ciliary cAMP, which gates cationic channels in the ciliary membrane. The resulting influx of Ca^{2+} and Na^{+} produces a depolarizing receptor current. Modulation of the cyclic-nucleotide-gated channels is one mechanism of adjusting olfactory sensitivity. Modulation of these channels by divalent cations was studied by patch-clamp recording from single cilia of frog olfactory receptor neurons. In accord with previous reports, it was found that cytoplasmic Ca^{2+} at >1μM made the channels less sensitive to cAMP. The effect of cytoplasmic Ca^{2+} was eliminated by holding the cilium in a divalent-free bath and was restored by adding calmodulin (CaM). External Ca^{2+} could also greatly reduce the sensitivity of the channels to cAMP. Increasing $[Ca^{2+}]_{out}$ from 0.1μM to 3mM increased the $K_{1/2}$ for cAMP at–50mV from 1.3 ± 0.1μM (n = 7) to 10.6 ± 1.4μM (n = 7). Thisreduction was seen when $[Ca^{2+}]_{out}$ exceeded 30μM and was not affected by the divalent-free bath, by CaM or by the level of cytoplasmic Ca^{2+} buffering. Thus the effects of cytoplasmic and external Ca^{2+} are apparently mediated by different mechanisms. There was no effect of CaM on a Ca^{2+}-activated Cl^{-} current that also contributes to the receptor current. Increases in Ca^{2+} concentration on either side of the ciliary membrane may contribute to olfactory adaptation. This work was supported by NIH grant R01DC00926.

200. Electrical communication among olfactory receptor neurons by peripheral waves

J.M. Parker, B. Lindemann[1] and J. Caprio

Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA and [1]Department of Physiology, Saar University, D-66421 Homburg, Germany

Olfactory receptor neurons (ORNs) transmit action potentials to the olfactory bulb and are also members of a population of cells forming the olfactory epithelium. The distribution and conductance of the ORNs within the sensory epithelium have functional consequences as indicated by the phenomenon of peripheral waves (PWs), odorant-induced synchronized oscillations recorded from the olfactory sensory epithelium in all classes of vertebrates. We recorded PW activity (16–31 Hz) to potent amino acid stimuli in *in vivo* recordings from sensory regions of olfactory lamellae in the channel catfish, *Ictalurus punctatus*. We hypothesize that PW activity is initiated by odorant-activated ORNs that drive loop currents through neighboring cells, thereby increasing the response thresholds of neighboring ORNs that possess molecular receptors for the odorant. Once the ORNs that were first activated become adapted, the direction of the current inverts during stimulus presentation, due to the activation of those ORNs which were previously inhibited by the loop current. In this manner, a bi-stable current pattern may be maintained during odor presentation. Thecurrent loops can extend over distances of millimeters and synchronize a large number of ORNs. Although neither the specific pattern nor frequency of the PWs appear related to stimulus quality in the channel catfish, the hypothesized result of the oscillations is to synchronize transmitter release (i.e. act as a logic gate) at the terminals of the ORNs within their specific target glomeruli of the olfactory bulb.

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201. Expression of mRNA encoding for gap junctional proteins in mouse olfactory epithelium

C. Zhang and D. Restrepo

Department of Cellular and Structural Biology. University of Colorado Health Sciences Center, Denver, CO 80262, USA

Chemical transmission and electrical coupling are the two basic mechanisms for cell-cell communication in the nervous system. Intercellular channels clustered at gap junctions mediate electrical coupling. Gap junctions represent an important mode of intercellular communication. For example, in the visual system gap junctions between cone photoreceptors are believed to couple at low luminance where spatial averaging would improve contrast sensitivity without cost to spatial acuity (Tsukamoto *et al.*, 1992). However, little is known about the function of gap junctions in theadult olfactory system. In salamander, intracellular injection of vital dyes results in co-staining of adjacent cells in the olfactory epithelium (OE), suggesting the presence of gap junctions between a number of different epithelial cells (Schwartz Levey *et al.*, 1992). In addition, antibodies against connexin 43, one of the channel-forming proteins of gap junctions, label the apical portion of the mouse OE (Miragall *et al.*, 1992). Using degenerate polymerase chain reaction, we have cloned two kinds of connexins (43 and 45), from adult mouse OE. *In situ* hybridization indicates that connexin mRNA is expressed in a substantial fraction of cells with nuclei laying in a broad area centered half way between the lamina propria and the surface of the OE (presumably the globose basal cells and olfactory neurons). Expression of connexin mRNA was observed in a ventrolateral zone within the sensory epithelium. This is the first direct evidence showing zonal expression of gap junctions in a substantial fraction of olfactory sensory neurons. This work was supported by NIDCD grant DC00566.

202. Effect of inhibitors on cyclic nucleotide and nitric oxide activated potassium fluxes in the olfactory nerve of the garfish, *Lepisosteus platostomus*

G.R. Kracke, A. Krambeck and E.D. Speichinger

Department of Anesthesiology, University of Missouri, Columbia, MO65212, USA

Cyclic nucleotide gated channels are non selective cation channels directly activated by intracellular cAMP and cGMP. We have previously shown that the membrane permeant cyclic nucleotides, 8Br cGMP and 8CPT cAMP, and S nitrosocysteine, a nitric oxide (NO) donor, reversibly increase K efflux in the garfish olfactory nerve. This provided initial evidence for the presence of these channels in olfactory nerve axons. We now provide further evidence for these channels by testing cyclic nucleotide gated channel inhibitors on cyclic nucleotide and nitric oxide activated K efflux. Isotope fluxes were used as an indirect measure of channel activity since the submicron diameter of the axons precludes direct patch clamp analysis. K efflux from the axons increases with membrane depolarization and decreases with hyperpolarization. The nerves were removed from animals, mounted on wire frames, and incubated in a Ringers solution containing ^{86}Rb, a K analog. The rate coefficient of isotope efflux was measured in isotope-free, low divalent cation, bicarbonate-buffered perfusate at 28°C. Mg, Ca and the local anesthetic, tetracaine, reported cyclic nucleotide

gated channel inhibitors, were tested; 10 mM Mg, 9 mM Ca and 1 mM tetracaine inhibited the cyclic nucleotide activated K effluxes. None of the inhibitors blocked the NO activated K efflux, indicating that NO stimulates K efflux through an alternate mechanism. Overall, these results provide further evidence for the existence of cyclic nucleotide gated channels in the axons of vertebrate olfactory neurons.

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203. Effect of protein phosphorylation on the hyperpolarization-activated current, I_h, in rat olfactory receptor neurons

G. Vargas and M.T. Lucero

University of Utah, Department of Physiology, Salt Lake City, UT 84108, USA

The hyperpolarization-activated I_h channel is modulated by neurotransmitters acting through several second messenger systems. In particular, the second messenger cAMP binds to the channel producing a depolarizing shift in the voltage dependence of I_h activation (D. DiFrancesco and P. Tortora, 1991, Nature, 351). The channel can also be regulated by PKA-dependent phosphorylation (F. Chang *et al.*, 1991, J. Physiol. Lond., 440); however, the effects of I_h phosphorylation are less defined. Therefore, we used whole-cell patch-clamp techniques to study the effects of both cAMP and phosphorylation on I_h. Internal perfusion of 1 mM cAMP produced a 10 ± 2 mV depolarizing shift in the $V_{1/2}$ and a 2.6 ± 0.3 mV (mean ± SEM, n = 15) reduction of the slope of the conductance-voltage relationship. To determine if the cAMP effects are mediated through I_h phosphorylation, we tested two protein kinase inhibitors. 50 nM K252a (non-specific kinase inhibitor) produced a 65 ± 17% (n = 4) reduction of I_h current amplitude at –90 mV, an 8 ± 2 mV hyperpolarizing shift in $V_{1/2}$ and a 23 ± 7% reduction of I_h maximal conductance. 500 nM H-89 (PKA specific inhibitor) produced a 16 ± 3% (n = 4) reduction of I_h current amplitude at –90 mV, a 3.3 ± 0.2 mV hyperpolarizing shift in $V_{1/2}$ and, in two out of four cells, an 8±0% reduction of I_h maximal conductance. These results showthat, in rat ORNs, I_h can be modulated by PKA-dependent phosphorylation and may serve to explain some of the modulatory actions of dopamine on I_h in these neurons (G. Vargas and M.T. Lucero, 1999, J. Neurophysiol., in press).

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204. Reversible disruption of odor transduction by adenylyl cyclase inhibitors

S. Chen, T. Leinders-Zufall and F. Zufall

Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD 21201, USA

For many *in vitro* studies of olfactory function, it would be useful to have available a tool by which activity in olfactory receptor neurons (ORNs) can be disrupted in a reversible and relatively specific manner. Here, we show that two pharmacological inhibitors of adenylyl cyclase, MDL12330 and SQ22536, can serve this role. In isolated salamander ORNs, both drugs were potent inhibitors of odor-induced membrane currents elicited by cineole, acetophenone or *n*-amyl acetate, and the effects of both agents were fully reversible. Dose–response curves revealed that MDL-12330 was slightly more potent than SQ22536. To test whether the agents inhibited the transduction pathway at the level of adenylyl cyclase, odor receptor activation was bypassed with IBMX. IBMX-induced currents were blocked with the same K_i values as odor-induced currents. Moreover, there was no measurable effect of the drugs on the CNG channels themselves. Both these results establish MDL12330 and SQ22536 as inhibitors of olfactory adenylyl cyclase. We then tested the effects of the two drugs on awide variety of odorants. In the course of these experiments, wefound that lyral and lilial, which are usually considered as stimulators of $InsP_3$, also induced robust membrane currents in some salamander ORNs. The lyral- and lilial-induced currents occurred in cells that were also responsive to IBMX and their properties were indistinguishable from odor currents known to depend on the cAMP system. MDL12330 and SQ22536 inhibited lyral- and lilial-induced currents with the same potency as responses to cineole.

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205. Nitric oxide activates an outward current in olfactory receptor neurons from *C. caudiverbera* and *X. laevis*

O. Schmachtenberg and J. Bacigalupo

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

Various functions have been attributed to nitric oxide (NO) in theolfactory epithelium, but the underlying mechanisms have remained elusive. Here we report that exogenous NO, applied in pulses of 1.5 s through microperfusion with the NO-donor sodium nitroprusside (SNP), caused an outward current in V-clamped isolated olfactory neurons from the two anuran species. Co-perfusion with the NO-scavenger hemoglobin diminished the current reversibly. Lowering external potassium significantly enhanced the effect, but low external calcium reduced it. Nanomolar concentrations of charybdotoxin (CTX), a blocker of Ca^{2+}-activated K^+-channels, diminished the SNP-effect, but did not completely abolish it. These observations and the I–V relation suggest that SNP somehow activates a Ca^{2+}-dependent K^+-conductance. Experiments with localized stimulation indicated that a CTX-sensitive part of the SNP-induced current dominates in the ciliary region of the cell, whereas the insensitive part localizes to the soma. NO is a potent activator of soluble guanylyl cyclase (sGC), therefore we used ODQ and LY83583, selective inhibitors of sGC, to investigate if the effect is mediated by cGMP. Neither of them altered the SNP-induced current. However, co-application of nifedipine, a Ca^{2+}-channel blocker, with SNP abolished its effect. Together, these results indicate that NO causes the opening of Ca^{2+}-activated K^+-channels in a $[Ca^{2+}]_o$-dependent, cGMP-independent manner. Interestingly, we did not observe the effect in the rat (n = 11).

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206. Odor suppression of V-gated currents contributes to the net odor-induced response in vertebrate isolated olfactory neurons

M. Sanhueza and J. Bacigalupo

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

We recorded odor-induced currents from isolated olfactory neurons (ONs) of the Chilean toad and the rat under whole-cell conditions. Odor pulses of 0.3 and 1.5s induced a suppression current (I_S), whose polarity was always opposite to the net V-dependent current activated by voltage pulses (4 s). Under physiological ionic conditions, I_S cannot be explained by the opening of ion channels. Considering that the latency of I_S (~20ms) is much shorter than that of the transduction current (I_T), and that I_S is larger and faster when odorants are applied tothe soma as opposed to the cilia, we conclude that the phenomenon can only be explained as an odor suppression of the somatic V-dependent currents. Chronic bath application of high odorant concentrations produces a reversible suppression of voltage-gated currents (Kawai *et al.*, 1997, J. Gen. Physiol., 109: 265). In our work, however, we applied odorant pulses of similar duration and concentration as those usually utilized to induce regular odor responses. We recorded I_S in 95% of the neurons, while I_T was observed in 30% of them. I_S overlaps with I_T, but it can be observed in isolation at the I_T reversal potential (0 mV) or in neurons that do not display a transduction current, including those missing their cilia. Under I-clamp, suppression can by itself modulate the spontaneous discharge of action potentials. Our results demonstrate that, in isolated ONs, transduction and suppression occur under similar conditions, suggesting that suppression may participate in olfactory coding.

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207. Mixture suppression and odor suppression of cAMP-induced current in olfactory receptor neurons

H. Yamada and K. Nakatani

Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Various studies in vertebrate olfactory receptor neurons (ORNs) show that mutual suppression between odorants can be observed when the odorants are applied simultaneously (D.G. Laing *et al.*, 1988, Perception of Complex Smell and Tastes, Academic Press, Sydney). However, the underlying mechanisms of this mutual suppression are unknown. Here we investigated the nature of suppression of the transduction current by odorants in newt (*Cynops pyrrhogaster*) ORNs. To investigate the odor suppression, we recorded from the cyclic AMP-introduced ORN using a whole-cell patch clamp technique. Cineole, isoamylacetate, anisole, and limonene (0.5 mM each) suppressed cyclic AMP-induced current in all 12 cells we tested. Furthermore, the calculated Pearson's *r* correlation coefficients for the responses of ORNs to each pair of the odorants were found to be >0.85, well above the significance threshold ($P < 0.01$). This means that the inhibitory effect of odorants had little specificity to the type of odorants or cells. Accordingly, it is expected that if an odorant does not cause an excitatory response, the odorant will suppress the transduction current induced by the other odorants. We have observed such cases; excitatory responses to a mixture of anisole and isoamylacetate (0.5 mM each) were smaller than that to 0.5 mM isoamylacetate when 0.5 mM anisole did not elicit excitatory response. The suppression was also observed with electro-olfactogram (EOG) when a mixture odorant was applied.

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208. Electrophysiology of OMP-null ORNs and rescue with an OMP-IRES-GFP adenovirus

L. Ivic, M. Pyrski[1], L. Richards[1], S. Firestein and F.L. Margolis[1]

Columbia University, Department of Biological Sciences, New York, NY10027 and [1]University of Maryland, School of Medicine, Department of Anatomy and Neurobiology, Baltimore, MD 21201, USA

ORNs were isolated from control and OMP null mice. Cell responses to the application of an odor mixture (amyl acetate, acetophenone, cineole and citralva) or the phosphodiesterase inhibitor IBMX were measured under the whole-cell patch-clamp configuration. Response latency to odor or IBMX application was the same for ORNs from control and null mice. The amplitude of these responses was very variable from cell to cell in both groups, although the ranges of the amplitudes were similar in both groups. Comparable response amplitudes of up to 700 pA were observed, which were comparable in ORNs from both groups. The recovery kinetics of the odor and IBMX responses were also very variable in both groups, but more so for OMP nulls.

To further test involvement of OMP in signal transduction we generated an adenoviral vector containing a bicistronic expression unit including the OMP coding sequence, an internal ribosomal entry site (IRES) and a coding sequence for the enhanced green fluorescent protein (EGFP). OMP nulls were anesthetized and the epithelium of the right nostril was perfused with the virus for 15 min on three successive days, resulting in local infection rates of >50%. EOGs were recorded 4 days after the last viral infection. Green fluorescence facilitated placement of recording electrodes atthe highly infected sites. EOGs showed fast recovery kinetics, similar to those recorded in the controls, demonstrating rescue ofthe OMP-null phenotype. This result indicates that OMP participates in olfactory signal transduction and that its role does not require long-term developmental cellular processes.

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209. Biophysical properties of feline olfactory receptor neurons

F. Lischka, G. Gomez, M. Haskins[1] and N.E. Rawson

Monell Chemical Senses Center, Philadelphia, PA 19104 and [1]Department of Veterinary Medicine, University of Pennsylvania. Philadelphia, PA 19104, USA

Many basic elements of olfactory neurophysiology have been found to be remarkably similar across diverse species, but species differences have also been discovered that suggest evolutionary changes have occurred that may optimize function. Remarkably, virtually nothing is known of olfactory physiology in any obligate carnivore species. As a representative of this class, we have begun studies of feline olfactory receptor neurons (ORNs) isolated from

the nasal septum and turbinates of domestic shorthair kittens and cats. Electrophysiological and calcium imaging techniques were used to examine the response characteristics of ORNs using procedures similar to those used previously to study rodent and human ORN function. Electrophysiological recordings revealed that odorant stimulation could elicit activation of a cation current or suppression of a voltage-activated potassium conductance. Thephosphodiesterase inhibitor IBMX also activated a cation conductance in some cells. Calcium imaging studies using the ratiometric fluorescent indicator fura-2 revealed increases in $[Ca^{2+}]_i$ in response to elevating extracellular potassium and to odorant mixtures in some cells. In addition, some cells responded to IBMX or forskolin with an increase in $[Ca^{2+}]_i$. As in rat and human ORNs, odorant-stimulated increases in $[Ca^{2+}]_i$, were dependent on extracellular calcium. Feline ORNs also occasionally exhibited odorant-stimulated decreases in $[Ca^{2+}]_i$, like those of human, but unlike rat ORNs studied with similar procedures and stimuli. These cells may therefore provide a viable model to study the mechanism and role of this type of ORN response.

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210. Pheromone-sensitive olfactory receptor neurons in the moth *Manduca sexta*: circadian changes in the spontaneous activity and adaptation of the olfactory response

K. Bittmann[1], J. Dolzer[1,2] and M. Stengl[1,2]

[1]*University of Regensburg, Biology I, Department of Zoology, D-93040 Regensburg and* [2]*University of Marburg, Biology, Animal Physiology, D-35032 Marburg, Germany*

The activity of olfactory receptor neurons (ORNs) changes due to internal cues, like a circadian clock, or to external cues, such as previous strong stimulation. Using the tip recording technique, the pheromone-sensitive ORNs innervating long trichoid sensilla on the moth antenna were examined.

The spontaneous action potentials (APs) of ORNs are distributed in bursts with a main frequency of 30–100 Hz. Therefore, it appears that ORNs themselves can act as oscillators, modulating or even partly causing odor-dependent oscillations in the CNS (G.Laurent, 1996, Trends Neurosci., 19: 489). While investigating the AP activity during a 16:8 h light:dark cycle it was found that the total spontaneous activity of the nocturnal moth's ORNs is higher during the light phase, while the number of APs per burst appears to be reduced. When the ORNs were stimulated with different concentrations of bombykal (BAL), two populations ofsensilla with significantly different thresholds in their AP responses were detected. Adapting pheromone stimuli caused significant reduction of several response parameters. The amplitude of the sensillar potential, as well as its rising phase were shifted to higher stimulus intensities for at least 1 log unit, while itsdecline was faster. Moreover, the dose–response curve of the adapted AP frequencies exhibited a plateau at about half the maximum of the unadapted response. Currently, we concentrate on patch clamp studies and examine, whether changes of intracellular cGMP concentrations might underlie the observed long-term variability in the neuronal activity of moth ORNs due to internal and external cues.

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211. Odor-evoked, chloride-mediated conductance in lobster olfactory receptor neurons

R.E. Doolin and B.W. Ache

The Whitney Laboratory, 9505 Ocean Shore Blvd, St Augustine, FL 32086, USA

The extent to which odors inhibit olfactory receptor neurons (ORNs) is unclear. Understanding the importance of odor-evoked inhibition requires further understanding of the cellular mechanisms by which odors inhibit ORNs. A cyclic nucleotide-mediated increase in potassium conductance has been linked to hyperpolarizing receptor potentials in lobster ORNs (Michel and Ache, 1992, J. Neurosci., 12: 3979). In culture, lobster ORNs express an odor-suppressible steady-state chloride conductance (Zhainazarov and Ache, 1995, Chem. Senses, 20: 808) that potentially could be inhibitory. We now report that odors suppress a steady-state chloride conductance in these neurons *in situ*. Whole-cell, current-clamp recordings were obtained from lobster ORNs in a semi-intact nose preparation that maintained the normal polarity of the receptor cells. Applying L-proline (100 μM) to the outer dendrites (cilia) hyperpolarized 37 out of 83 the ORNs tested. The change in membrane potential was associated with a decrease in membrane conductance (n = 6). The underlying current had a negative voltage relationship between –90 and –50 mV, which extrapolated to a reversal potential of –40 mV (n = 22). The peak magnitude was reversibly suppressed to 39% of control magnitude by the chloride channel blocker 9-AC (n = 2). These findings, although still preliminary, are consistent with the idea that L-proline suppresses a steady-state chloride conductance in lobster ORNs *in situ.* If so, at least two different mechanisms appear to contribute to odor-evoked hyperpolarizations in these cells.

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212. Regulation of a Na^+-gated nonselective cation channel by PI(4,5)P_2 and PI(4)P

A.B. Zhainazarov[1] and B.W. Ache[1,2]

[1]*The Whitney Laboratory, University of Florida, St Augustine, FL 32086 and* [2]*Departments of Zoology and Neuroscience, University of Florida, Gainesville, FL 32610, USA*

Olfactory receptor neurons in the lobster express a nonselective cation channel activated by intracellular Na^+ (J. Neurophysiol., 1995, 73: 1774). This channel has been implicated in the odor activation of the neurons *in situ* (J. Neurophysiol., 1998, 79: 1349).We now show that phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2] and phosphatidylinositol 4-phosphate [PI(4)P] applied to the intracellular face of excised patches activate the channel in the absence of Na^+. Aluminum, which tightly binds to PI(4,5)P_2, and an antibody against PI(4,5)P_2 irreversibly inhibit channel activity evoked by PI(4,5)P_2. An antibody against PI(4)P also inhibits the activation of the channel by PI(4)P. Applying 4 μM PI(4,5)P_2 or PI(4)P in the presence of Na^+ to the intracellular face of the patch decreases the half-effect concentration of Na^+ from 74 to 22 mM with PI(4,5)P_2 and to 29 mM with PI(4)P. Na^+-gated channel activity was irreversibly inhibited by monoclonal antibodies against PI(4,5)P_2 and PI(4)P in patches never exposed to exogenous phosphatidylinositols, suggesting that endogenous inositol phospholipids are required for the activation of the

channel by intracellular Na^+. The ability of PI(4)P and PI(4,5)P_2 to regulate the activity of the channel suggests that membrane inositol phospholipids may potentially serve as intracellular signaling molecules in these primary sensory neurons and may also provide a general mechanism to explain how the sensitivity of Na^+-gated channels to Na^+ could be much greater in intact cells than in excised membrane patches.

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213. An electrogenic Na^+/Ca^{2+} exchanger in squid olfactory neurons

J.P. Danaceau and M.T. Lucero

University of Utah Department of Physiology, Salt Lake City, UT, USA

Certain odors, such as L-glutamate and dopamine, increase internal Ca^{2+} in olfactory receptor neurons (ORNs) from the squid, *Lolliguncula brevis* (Lucero *et al.*, 1995, Chem. Senses, 19: 509). These two odors respectively depolarize and hyperpolarize squid ORNs. To further study the effects of increasing internal Ca^{2+}, we used 10 mM caffeine to release Ca^{2+} stores in nystatin-patched ORNs. Caffeine elicited two types of responses; the most noticeable was a large, depolarizing cation-selective current. This cation-selective current was abolished by removal of external Na^+ and a smaller, chloride-selective component was uncovered. The Cl^- component was reversibly blocked by 100 μM niflumic acid. The cation component was not blocked by external Cd^{2+} or amiloride, suggesting that it was not due to a cyclic nucleotide-gated channel. The strict sodium dependence of this cation component indicates that it is due to an electrogenic sodium/calcium exchanger. This exchanger could play a role in odor responses. In the case of hyperpolarizing responses, such as those activated by dopamine, the depolarizing currents generated by Na^+/Ca^{2+} exchange would oppose and therefore attenuate hyperpolarizing responses. In addition, the removal of internal Ca^{2+} would terminate a Ca^{2+}-dependent odor response. By contrast, a depolarizing odor response, such as that generated by glutamate, could be augmented by the depolarizing Na^+/Ca^{2+} exchanger current.

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214. Sustaining olfaction at low salinities: evidence for a dynamically maintained ionic microenvironment associated with the olfactory sensilla (aesthetascs) of the blue crab, *Callinectes sapidus*

R.A. Gleeson[1], K. Hammar[2] and P.J.S. Smith[2]

[1]*The Whitney Laboratory, University of Florida, St. Augustine, FL 32086;* [2]*The BioCurrents Research Center, Marine Biological Laboratory, Woods Hole, MA 02543, USA*

Several lines of evidence reported in previous studies suggest that, in low salinity conditions, the structural and functional integrity of the dendrites of the blue crab's olfactory receptor neurons is sustained by an ionic/osmotic microenvironment dynamically maintained within the aesthetascs (Cell Tissue Res., 1996, 284: 279–288; J. Exp. Biol., 1997, 200: 445–456). We hypothesize that a continuous diffusion of ions from the hemolymph, driven by an actively generated concentration gradient between the hemolymph and the external environment, produces this microenvironment within the sensillar lymph that bathes the dendrites. Morphological studies using the extracellular tracer, lanthanum, indicate that a paracellular pathway through the aesthetascs allows for direct passive diffusion of ions from the hemolymph to the sensillar lymph and ultimately to the external environment (Chem. Senses, 1998, 23: 551). In this study we used self-referencing, ion-selective microelectrodes to directly measure the profiles of Ca^{2+} and K^+ flux in the vicinity of the aesthetascs of crabs acclimated to low salinity conditions (15% seawater). The results clearly show an outward flux gradient extending from the aesthetasc tuft for both ions. Measurements at control locations on the antennule yield flux that is well below that associated with the aesthetascs. Calculations of the per aesthetasc flux give 2.03–5.36 fmol/s for K^+ and 0.78 fmol/s for Ca^{2+}. Assuming a passive diffusion model, these levels are comparable to that expected based on Na^+ flux measurements determined previously using ^{22}Na isotope. These findings further support the hypothesis of an ionic/osmotic microenvironment that is dynamically maintained within the aesthetascs at low salinities.

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215. Survey of ionic channels in identified chemosensory neurons of the nematode *Caenorhabditis elegans*

W.T. Nickell, R.Y.K. Pun[1] and S.J. Kleene

Department of Cell Biology, Neurobiology, and Anatomy and [1]*Department of Molecular and Cellular Physiology, University of Cincinnati, Cincinnati, OH 45267, USA*

Nematode neurons lack fast Na^+ action potentials; communication between neurons apparently involves graded depolarization and electrotonic conduction. Thus, it is probable that these neurons possess a novel array of channels. We used inside-out excised patch recordings to investigate the voltage-regulated conductances in olfactory neurons AWA and AWC. With divalent-free solutions on both sides of the membrane, two distinct channels were usually present. Steps from a holding potential of 0 mV to positive potentials opened distinct channels with a unit conductance of 43 pS and a reversal potential of –63 mV; these currents are presumably carried by K^+. Steps to negative potentials greater than –50 mV produced inward currents. In contrast with the discrete conductance states of the outward channels, this presumed Na^+ channel exhibited distinct periods of 'flickery' conductance. If the normal resting potential of *Caenorhabditis elegans* neurons is similar to that of neurons in the nematode *Ascaris* (–30 mV), these channels would serve to bound the membrane potential of *C.elegans* neurons between 0 and –50 mV. In the voltage range between these two limits, the membrane appears to have an extremely low conductance, which is probably necessary for long distance communication in the absence of regenerative action potentials.

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216. Functional neurogenesis and odotopic mapping of olfactory neurons in aesthetasc sensilla of the spiny lobster

P. Steullet, H.S. Cate, V. Ngo, W.C. Michel[1] and C.D. Derby

Department of Biology, Georgia State University, Atlanta, GA 30303 and [1]Department of Physiology, University of Utah Medical School, Salt Lake City, UT 84108, USA

The antennule, which is the olfactory organ of the spiny lobster *Panulirus argus*, possesses on the distal part of its lateral flagellum >1000 aesthetasc sensilla, each innervated by ~300 olfactory receptor neurons (ORNs). At each molt, new aesthetascs and their ORNs are born in the proximal part of the aesthetasc region of the lateral flagellum and old aesthetascs and their ORNs are lost from the most distal segments of the flagellum, with an overall net addition. The ORNs associated with new aesthetascs have high intracellular levels of taurine, and these levels decrease with age of the ORN such that mature ORNs do not contain taurine levels detectable with immunocytochemical techniques. Using agmatine, a channel-permeant cation, as a marker for activity-dependent labeling, we have found that the odor-dependent activity labeling is systematically weaker in ORNs of new aesthetascs than in those of old aesthetascs and is inversely correlated with the intracellular level of taurine. This suggests a slow and gradual maturation of ORNs that can last weeks to months. In mature aesthetascs, ~1–2% of the ORNs are labeled by a single odorant such as AMP, ammonium, cysteine, glycine, proline or taurine. Mature aesthetascs tend to be functional repetitive units, each containing representatives of the various functional types of ORNs, although there is some variability in ratios of different types of ORNs innervating aesthetascs in some flagella.

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217. Olfactory responses to hostplant volatiles recorded from sensory cells of long trichoid sensilla on the antennae of the female sphinx moth *Manduca sexta*

V.D.C. Shields and J.G. Hildebrand

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

The antennae of the female *Manduca sexta* are equipped with chemoreceptors responsible for detecting hostplant volatiles (Shields and Hildebrand, 1998, Soc. Neurosci. Abstr., 24: 2098). We are interested in understanding how *M. sexta* detects and processes olfactory information about these volatiles by sensory cells housed in particular antennal olfactory sensilla. We have recorded extracellularly from the sensory cells of the tallest, single-walled, multiporous trichoid sensilla using a panel of >100 different odorants from eight chemical classes (terpenoids, aromatics, fatty acids, alcohols, aldehydes, alkanes, ketones and esters), including floral and vegetative host-plant volatiles.

In the majority of sensilla tested, only one of the two sensory cells responded to any of the compounds tested. Based on the response spectra, we found three categories of cells. One subset showed a clear excitatory response to compounds belonging to several chemical classes (broadly 'tuned' cells), while the second subset responded to only a few selected compounds within a single chemical class (narrowly 'tuned' cells). A third subset of cells did not respond to any of the compounds tested, even though spontaneous activity was recorded.

A majority of sensilla responded best to aromatics and, to a lesser degree, to terpenoids. Weak or no responses were observed with selected alkanes, esters and sex-pheromone components.

The results suggest that some olfactory sensory cells housed in these sensilla are narrowly tuned to only a few, chemically related plant odors. This finding is similar to the well-known 'tuning' of pheromone responsive sensory cells.

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218. A comparison of GC olfactometry Charm^{TM} to a static headspace method for measuring odor detection thresholds

A.B. Marin

International Flavors and Fragrances, R&D, 1515 Hwy 36, Union Beach, NJ07740, USA

Published odor detection thresholds for human subjects have been reported as measured by air dilution olfactometry or by sensory methods where the odor is delivered in the headspace above a solution. In this study, detection thresholds were measured and compared for four subjects and eight compounds delivered by two methods: (1) stimuli were presented separately in a stream of humidified air and thresholds measured by gas chromatography olfactometry (GCO) Charm^{TM}; and (2) each stimulus was presented in the headspace above a series of dilute solutions and each subjects = threshold was estimated from their dose response curve (A.B. Marin *et al.*, 1991, J. Sens. Stud., 205). Threshold estimates for each odor stimulus were repeated three times by both methods, then the methods were compared by ANOVA. For twostimuli, geraniol and galaxolide, triplicate threshold estimates were also measured for three of the four subjects by a third method: a single staircase method where stimuli were presented in polyethylene squeeze bottles (R.L. Doty *et al.*, 1987, Brain Res. Bull., 18: 597). For most of the odor stimuli and subjects, average threshold values measured by GCO Charm^{TM} and from dose–response curves were within one order of magnitude and were not statistically different. Also, thresholds for geraniol and galaxolide by GCO Charm^{TM}, from dose–response curves and the single staircase method were the same. However, thresholds estimates for geraniol, coumarin and galaxolide for the four subjects in this study were more than an order of magnitude lower than published values.

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219. Unconscious odor discrimination, detection and quality thresholds

T. Radil[1,2] and C.J. Wysocki[1,3]

[1]Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA [2]Institute of Physiology, Czech Academy pf Sciences, Prague, Czech Republic and [3]Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Weak odors can be detected without subjects being conscious of them. We wished to determine whether discrimination between two odors also was possible without the subject's awareness. First, detection thresholds (the lowest concentration at which a stimulus could be detected six times in a row) were obtained for eugenol and

pyridine in an ascending, binary, forced-choice (odor versus blank) paradigm. The subjective threshold for perceiving odor quality was next obtained by presenting the individual's detection threshold concentration as the reference stimulus and increasing the concentration of the odorant in the paired bottle (as above). Subsequently, each subject was presented, in pair-wise fashion, two randomly sequenced bottles containing eugenol and pyridine, either at the subject's quality threshold, detection threshold, each of two neighboring concentration steps above or each of four concentrations below the detection threshold. Each of the eight stimuli was repeated 10 times, in a random order. The incidence ofcorrect discrimination was then expressed. Furthermore, in eachof the 80 discrimination trials, we utilized a continuous certainty/guessing scale, with 'absolutely certain' and 'absolutely guessing' at the extremes. Results revealed that at concentrations corresponding to quality thresholds, discrimination was perfect and the subjects were certain of their judgements; however, correct odor discrimination also was possible at weaker concentrations, corresponding to the detection thresholds, where the subjects typically reported basing their responses on guesses. Thus, discrimination of weak odorants, about which subjects seem unaware, could play a role in olfactory perception.

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220. Synthesis, olfactory properties and molecular modeling of aliphatic ketones identified from solitary bees

A. Finke, S. Sonnenberg[1] and P. Weyerstahl

Technical University Berlin, Institut für Organische Chemie, D-10623 Berlin and [1]Haarmann & Reimer GmbH, D-37601 Holzminden, Germany

Solitary bees, contrary to the well-known honey bees, do not live in big communities but as single individuals. During intraspecific and interspecific competition females of many *Nomada* spp. secret a particularly pleasant smell from their cephalic glands, which can be easily perceived by the human nose. W. Franke *et al.* (1989, Pure Appl. Chem., 61: 539–542) identified several terpenoid ketones from those secrets. The ketones vary in chain length and position and number of double bonds. Therefore, they are of interest for a systematic odor evaluation and structure odor correlation. Starting from citral and citronellal, several alcohols were synthesized by the Grignard and Barbier reaction with different bromides and reaction with directly metalated isoprene. Subsequent oxidation and isomerization yielded the corresponding ketones. The ketones possess pleasant fruity and floral odors. Relative odor detection thresholds for 27 compounds were determined by GC–olfactometry. The derived values were related to the molecular structures using modern computational QSAR-techniques like MFA and Receptor. Several correlations between the threshold values and structural elements were found.

221. Formulation of a standard odorant mixture to test human sniffers for specific anosmia

J.E. Friedrich and T.E. Acree

Department of Food Science & Technology, Cornell University, New York State Agricultural Experiment Station (NYSAES), Geneva, New York 14456, USA

Gas chromatography–olfactometry (GC/O), commonly used to identify odor-active chemicals in extracts and headspaces, can be used to study the differences in human sniffers. Normal olfactory acuity measured as thresholds is usually defined as responses less than two standard deviations from a population mean or the mean of the most sensitive group in a bimodal distribution. Further deviation is defined as specific anosmia. The objective of this research is to formulate a standard odorant mixture to test individuals for specific anosmia.

To incorporate all potential olfactory modalities, odorant selection for the standard mixture was based on descriptive aroma categories. An aroma genus of 26, which includes the 23 food aroma categories defined by ASTM DS66 plus three non-food aroma categories, was used. Compounds with known specific anosmia, which gave baseline separation (RI OV101), and were stable, commercially available and low in toxicity were also included. The standard odorant mixture was formulated and analyzed by a reference individual to create a benchmark for further GC/O studies. Using the benchmark values for the aroma categories, we are able to quantitatively measure the individual acuity of each sniffer. Using this standard odorant mixture we can screen individuals for specific anosmia, obtain coefficients of response to specific aroma categories and eliminate sniffers with general anosmia.

222. Sniffing longer rather than stronger to maintain olfactory constancy

N. Sobel[1], C.A. Hartley[2], R. Khan[3], E.V. Sullivan[1,4] and J.D.E. Gabrieli[1,3]

Departments of [1]Neuroscience, [2]Symbolic Systems, [3]Psychology and [4]Psychiatry and Behavioral Science, Stanford University, Stanford, CA 94305 USA

Air flow resistance is usually greater in one nostril than in the other, resulting in different air flow-rates between nostrils. Greater flow-rate improves odorant detection thresholds. Thus, one expects odorant detection to be superior in the nostril with greater flow-rate (GFR nostril) relative to the nostril with lesser flow-rate(LFR nostril). Previous research, however, has found odorant detection thresholds in the GFR and LFR nostrils to be equal. Considering the expected positive effects of flow-rate on detection thresholds, this equivalence was paradoxical.

We propose that perhaps subjects compensate for the reduced air-flow in the LFR nostril by sniffing for greater duration whenusing that nostril. To test this, we combined uni-nostril ascending-staircase threshold testing and forced-choice detection of vanillin and propionic acid, with anterior rhinomenometry, in 30 volunteers. Detection thresholds were equal for the GFR and LFR nostrils. Sniff duration was on average 16% longer in the LFR than in the GFR nostril ($P = 0.002$). As the difference in air flow-rate between the nostrils increased, so did the difference induration of sniff ($P = 0.002$). By preventing increase of sniff duration in the LFR nostril, we induced a marked advantage in detection for the GFR over the LFR nostril ($P = 0.02$). This compensatory mechanism enables the maintenance of olfactory constancy despite different air flow-rates in the two nostrils. Furthermore, this finding implies that during each sniff, each nostril receives a slightly different image of the olfactory world.

223. Odor identification in mixtures: is olfactory working memory the ultimate limitation?

D.G. Laing and A. Jinks

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

Humans have substantial difficulty in identifying three odorants in a mixture (D.G. Laing and G. Francis, 1989, Physiol. Behav., 46: 809). This limitation was not overcome using different test paradigms or different odorants, or with extensive training, suggesting that the limitation is physiologically based. During a further series of experiments that investigated the role of temporal coding in mixture perception, the data suggested that humans could identify the components of binary mixtures and their order of perception, but that with ternary mixtures most could not determine temporal order or identity above chance levels. Using a unique test paradigm, other experiments indicated that the latter limitations were probably due to slow processing and subsequent retrieval of information from olfactory working memory. The data indicated that if neural information about a third component enters memory <600–900 ms after entry of information about the initial two odorants, that the identity and order of processing of all components will not be discerned. It is proposed that a massive reduction in the amount of information occurs early in the olfactory system when mixtures are processed, to provide olfactory memory with a manageable task as regards the identification of salient mixture components.

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224. The effect of human axillary odors on memory recollections

D. Chen and J. Haviland[1]

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104 and [1]Psychology Department, Rutgers University, 53 Ave E, New Brunswick, NJ 08854, USA

Most studies on the relation between odor and memory examined either odor identification and recognition memories in learning tasks or the effectiveness of olfactory cues for verbal recalls as compared with other cues. The present study explores: (1) whether axillary odors from different developmental stages would differentially impact on the characters mentioned, the emotional quality, the content and the length of autobiographical memories of young adult men and women; and (2) whether the impact, if any, would be contingent upon how the odors were perceived by the observers. Seven groups of odor stimuli were collected from: (1) pre-pubertal boys, (2) pre-pubertal girls, (3) college-aged men, (4) college-aged women, (5) older men, (6) older women and (7)homes of the donors. Three hundred and eight young adult observers recalled a dream and an event after smelling one of the seven groups. Regression analyses show that axillary odors from different developmental stages significantly impacted on the characters mentioned and the emotional quality of the recalls after the perceived intensity of the odors was controlled for. In particular, male and young adult odors elicited more negative emotion words than female, children or older adult odors. Older adult odors elicited more family references than young adult odors for female subjects. Young adult odors elicited more friend references in women than in men.

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225. Psychological effects of musky compounds: comparision of 4,16-androstadien-3-one, androstenol and muscone

S. Jacob and M. McClintock

Department of Psychology, University of Chicago, 5730 S. Woodlawn Ave, Chicago, IL 60637, USA

Recently, others have claimed that 4,16-androstadien-3-one is a human pheromone. Before the term pheromone can be applied, itis necessary to determine whether its effects are compound specific. Our studies have shown that androstadienone modulated the psychological state but did not release behavior. In order toinvestigate whether these modulatory effects are unique to androstadienone, we determined whether similar responses are produced by androstenol and muscone. These compounds share androstadienone's musky odor and are also social chemosignals in pigs and musk deer respectively. In a controlled laboratory setting, we conducted a double-blind, within-subject, repeated measures experiment that was counterbalanced for treatment. In 19 women and 19 men, a nanomolar concentration of each musky compound was presented within a strong odor carrier of clove oil in propylene glycol, minimizing any detectable olfactory differences among them. Subjects were given a psychological battery at baseline, 0.1and 1.0 h after initial exposure. Initial results show that subjects had some differential psychological responses for specific compounds. Further work is required to clarify the mechanism by which these chemicals produced psychological effects, whether effects are unique from olfactory processing, if they are enhanced, limited or altered by specific social or sexual contexts, and if humans naturally release adequate concentrations into the air sothat recipients can access and process that specific chemical information.

230. Taste reactivity to sucrose after taste aversion conditioning is unaffected by glossopharyngeal nerve transection

S. Eylam, M. Garcea and A.C. Spector

Department of Psychology, University of Florida, Gainesville, FL 32611, USA

Glossopharyngeal nerve (GL) transection in rats is known to markedly reduce the number of gapes to intraorally delivered quinine. In this experiment we tested whether gapes to a palatable stimulus conditioned to be aversive would be reduced by GL transection. Ten naive male Sprague–Dawley rats were implanted with two intraoral (i.o.) cannulae. Five received bilateral GL transection (GLX) and five served as sham-operated controls (CON). One-bottle 0.3 M sucrose intake was measured, followed immediately by an injection of 0.15 M LiCl (1.33 ml/100 g body wt), during three 15 min morning conditioning sessions. There were no significant differences in intake between water-deprived GLX and CON groups; however, sucrose intake did significantly decrease across sessions ($P < 0.05$), indicating that a conditioned taste aversion to this stimulus was acquired in both groups. Rats were then habituated to the taste reactivity chamber and i.o. fluid infusion for 3 days, and tested on the fourth day with a 1 ml

infusion (1 min) of 0.3 M sucrose. No significant differences were found between the two groups for total aversive or ingestive scores, or for individual oromotor responses, including gapes ($P > 0.1$). These results show that the GL is not a necessary afferent limb for normal taste-induced gape responses for all taste compounds. Thus, the well-established decrease in quinine-elicited gaping in GL-transected rats must depend on the interaction between the specific stimulus properties of the alkaloid and the neurotomy.

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231. Gustatory responses of common marmoset to compounds sweet in humans: conditional taste aversion test

V. Danilova, G. Hellekant and T. Roberts

Department of Animal Health and Biomedical Sciences, University of Wisconsin—Madison, Madison, WI 53706, USA

Previously we demonstrated that in common marmosets both chorda tympani and glossopharyngeal nerves consist of three major clusters of taste fibers characterized by predominant sensitivity to sweeteners (S cluster), bitter compounds (Q cluster) or acids (H cluster). The purpose of this study was to investigate the relationships between the activity in different types of taste fibers and the gustatory behavior in marmosets.

Conditional taste aversion (CTA) test was used to investigate the taste of 29 compounds, described as sweet by humans. Conditioned stimulus was 0.2 M sucrose and unconditioned stimulus was the sickness induced by the injection of LiCl. Eight conditioned and 10 control animals were used to investigate to what extend marmosets generalize the acquired aversion from sucrose to the other stimuli.

In the control group the consumption of the sweeteners differed from 600% for sucrose to 30% for D-phenylalanine (water consumption was 100%). In the conditioned group the consumption of 21 sweeteners was significantly suppressed, while six stimuli were consumed at the same level as water. Finally, stevioside and D-phenylalanine were rejected in control group as well as in conditioned.

Comparing these data with responses of single chorda tympani and glossopharyngeal fibers reveals a correlation between the response in a particular cluster of taste fibers and the reaction in CTA test. Marmosets generalized taste of sucrose only to those sweeteners which stimulated the S-cluster fibers. The results show that, by combining single fiber nerve recordings and CTA tests, it possible to gain incite on how an unnknown compound may taste to an animal species.

232. Use of the cytosensor microphysiometer to study hamster taste bud cell responses to sweet compounds

S. Khare, K. Gokulan, R. McGregor[1] and D.S. Linthicum

Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843 and [1]Linguagen Corp., Nutley, NJ, USA

A silicon-based biosensor microphysiometer (Cytosensor) can be used to measure real time cell responses to a variety of chemical substances, including ligands for specific receptors. The microphysiometer measures the rate of extracellular pH changes of living cells as a reliable index of the integrated functional response to receptor activation.

We isolated taste buds (circumvallate, fungiform, foliate) from hamster tongue and made a taste cell/tissue preparation suitable to use in the microphysiometer. The cell preparation was mixed with low-melting agrose and immediately spotted on to the membrane of the Cytosensor cell capsule. The cells were perfused with bicarbonate-free DMEM containing 0.5 mg/ml BSA. We tested the effect of sweet compounds (SC45647, thaumatin, alitame, cyanosuosan, NC174, Na-saccharin and NHDHC). The taste bud cells were exposed to sweet compounds for 30 s. Taste bud cell preparation showed an increase in the extracellular acidification rate in response to SC45647, cyanosuosan, NC174 and Na-saccharin. Thaumatin, alitame and NHDHC did not show any detectable increase in the acidification rate above the basal value. Muscle tissue was used as a negative control and it showed no response to the tested compounds. The results demonstrate a response pattern to sweetener compounds as reported by Danilova *et al.* (1998, J. Neurophysiol., 80: 2102–2112) using the single fiber response recordings for various sweetener compounds. Effect of denotonium benzoate (DB) was also tested on the taste bud cells and exposure of also resulted in an increase in the acidification rate. This study highlights an alternative method for evaluating the responses of taste bud cells.

233. Adenovirus mediated gene transfer of GFP into cultured rat taste cells

L.M. Stone[1,2], C.L. Wilcox[3], C.J. Ruiz[1,2] and S.C. Kinnamon[1,2]

[1]Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523, [2]Rocky Mountain Taste and Smell Center, Denver, CO and [3]Department of Microbiology, Colorado State University, Fort Collins, CO 80523, USA

To investigate the possibility of using gene transfer to study taste cell biology and to assay isolated taste bud cultures, we infected taste buds with an adenoviral vector containing GFP. The expression cassette of the replication-defective vector contained GFP driven by the cytomegalovirus immediate early promoter and the adenoviral portions of the vector were derived from the human adenovirus type 5 (Ad 5). Taste buds were isolated from rat lingual epithelium, placed into modified Pixley medium and infected immediately, or after various time periods in culture. Following application of the virus, taste buds were incubated at 37°C for 24–48 h, then assayed for GFP fluorescence. Taste buds infected immediately after isolation displayed strong GFP labeling in the majority of centrally located cells. However, increasing times in culture resulted in a decreasing numbers of labeled taste cells. Preliminary studies indicated that immunocytochemical analysis of isolated, infected taste buds is possible following fixation in 4% paraformaldehyde. The taste buds remained immobilized on poly-D-lysine-coated slides, and the GFP signal was retained throughout processing. In conclusion, adenoviral vectors can be used to transfer foreign genes into taste cells and infected cells can be further manipulated to address specific questions.

234. Physiological recordings from gustducin expressing taste cells in GFP-tagged transgenic mice

T. Ogura[1,3], W. Lin[1,3], J.A. Kozak[2], Z. Zheng[2], R.F. Margolskee[2] and S.C. Kinnamon[1,3]

[1]Colorado State University, Fort Collins, CO 80523, [2]Howard Hughes Medical Institute, The Mount Sinai School of Medicine, New York, NY 10029 and [3]The Rocky Mountain Taste and Smell Center, Denver, CO 80262, USA

Taste transduction of sugars, amino acids and most bitter compounds is initiated when these compounds bind to specific receptors that are coupled to G-proteins and second messenger systems (Kinnamon and Margolskee, 1996, Curr. Opin. Neurobiol., 6: 506–513). Previous studies suggest that the chemoreceptor-cell-specific G-protein, gustducin, is expressed in a subset of taste cells and is involved in bitter and sweet taste transduction (Ruiz-Avila *et al.*, 1995, Nature, 376: 80–85; Wong *et al.*, 1996, Nature, 381: 796–800). Recently, transgenic mice have been developed in which the gustducin promoter has been linked to green fluorescent protein (GFP). Thus, taste cells which express gustducin will also express GFP, so that they can be targeted for physiological recording (Zheng, Ruiz-Avila and Margolskee, unpublished data). Immunoreactivity for α-gustducin colocalized with GFP fluorescence, which suggests that the transgene is expressed in the gustducin-lineage of taste cells. Whole-cell voltage-clamp recording showed voltage-gated currents are present in GFP-labeled taste cells of circumvallate papillae. Further, Ca^{2+}-imaging experiments using fura-2 showed that a subset of GFP-labeled cells in circumvallate papillae increased $[Ca^{2+}]_i$ in response to an artificial sweetener, SC45647, and/or a bitter compound, denatonium. These mice should be useful for examining the role of gustducin in sweet and bitter taste transduction.

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235. Molecular cloning and characterization of genes specifically expressed in gustducin-positive taste receptor cells

L. Huang, Z. Zheng and R.F. Margolskee

Department of Physiology and Biophysics, Howard Hughes Medical Institute, Mount Sinai School of Medicine, 1425 Madison Ave, Box 1677, New York, NY 10029, USA

Taste receptor cells vary ultrastructually, immunochemically and physiologically, and use multiple signal transduction pathways to respond to tastants. To gain insight into the components of different taste transduction pathways, we set out to clone genes that are specifically expressed in particular taste receptor cell subtypes. Transgenic mice expressing green fluorescent protein (GFP) from the gustducin promoter conferred green fluorescence on the gustducin expressing cells, enabling their ready isolation from the circumvallate papillae of GFP-transgenic mice. Gustducin/GFP-negative taste receptor cells, identified by their bipolar shape and lack of green fluorescence, were also isolated. RNAs from 19 GFP-positive and five GFP-negative cells were individually reverse transcribed with oligo(dT) primers to generate first strand cDNAs, tailed with dATP by terminal transferase, and amplified by the polymerase chain reaction (PCR) using oligo-(dT) adaptor-primers and Taq polymerase. cDNA libraries from individual taste receptor cells were constructed by subcloning the PCR products into bacteriophage vectors. Differential screening of a gustducin-positive taste receptor cell cDNA library resulted in the isolation of 300 clones which hybridized more strongly to the self-probe than to a nonself-probe from a gustducin-negative taste receptor cell.

By their nucleotide sequence we classified the clones into the following categories: housekeeping genes, marker genes, development- and differentiation-related genes, transcription factors, signal transduction genes and novel/unknown genes. Profiling the pattern of expression of these clones showed that some were co-expressed with gustducin in the gustducin/GFP-positive subset of taste receptor cells, and may be novel signal transduction or differentiation factors.

236. Partial rescue of gustducin null mice by transgenic expression of transducin

W. He and R.F. Margolskee

Department of Physiology and Biophysics, Howard Hughes Medical Institute, Mount Sinai School of Medicine, 1425 Madison Ave, Box 1677, New York, NY 10029, USA

The transduction of responses to bitter and sweet compounds utilizes guanine nucleotide binding proteins (G-proteins) and their coupled receptors. Gustducin, a transducin-like G-protein, and rod transducin are selectively expressed in taste receptor cells. Gustducin knockout mice have profoundly diminished behavioral and electrophysiological responses to many bitter and sweet compounds, although these mice retain residual responses to these compounds. Gustducin and rod transducin are biochemically in distinguishable in their *in vitro* interactions with phosphodiesterase, rhodopsin and G-protein α subunits. To gain insights into the gustducin-independent mechanisms underlying the residual responses to bitter and sweet compounds and to compare the function of gustducin versus transducin in taste transduction *in vivo*, we generated transgenic mice that express transducin in place of gustducin.

The gustducin promoter was used to drive high levels of expression of rod transducin in the gustducin lineage of taste receptor cells in gustducin knockout mice. Forty-eight-hour two-bottle preference tests showed that transgenic expression of rod transducin rescued, at least partly, responses to denatonium benzoate, sucrose and the artificial sweetener SC45647. However, expression of the transducin transgene did not restore responses to quinine sulfate. These results imply that transducin and gustducin differ, at least in part, in their function in taste receptor cells, and that transduction of responses to quinine may differ from those to denatonium. To determine the respective roles of gustducin and transducin in taste transduction, gustducin/transducin double knockout mice are being generated and will be compared with mice in which either G-protein is singly knocked out.

237. Glutamate chemoreception in *Paramecium*

A. Bergeron, Y. Sun and J.L. Van Houten

University of Vermont, Department of Biology, Burlington, VT 05405, USA

Glutamate is an attractant stimulus for *Paramecium tetraurelia*. There are at least two specific binding sites for glutamate on the cell surface membranes; one of the binding sites is involved in the repellent effects of IMP and the second appears to mediate

attraction to glutamate. Cells hyperpolarize in glutamate (~7 mV in 5 mM K-L-glutamate), most likely by an initial K conductance, and sustained conductance from activation of the plasma membrane calcium pump. Glutamate also rapidly elevates intracellular cAMP levels, to 3-fold in 30 ms and to 7-fold in 250 ms. We haveused polymerase chain reaction (PCR) to amplify sequences from the Paramecium genome that may correspond to glutamate receptors. An ~1 kb piece of DNA has been found using conserved degenerate primers based on ionotropic glutamate receptor sequences from other species. A 600 bp piece can be further amplified from the 1 kb piece using nested primers. Sequence analysis is ongoing. (Primers for metabotropic receptors did not successfully amplify DNA in earlier attempts with PCR.) In another approach, we have found a peripheral protein of ~21 kDa and an integral membrane protein of 37 kDa that consistently elute with glutamate from glutamate–agarose columns.

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238. Functional expression of taste-mGluR4 in Chinese hamster ovary (CHO) cells indicates a taste receptor activated by monosodium glutamate (MSG)

A.M. Landin, H. de Carvalho, I. Junaid and N. Chaudhari

Department of Physiology and Biophysics,University of Miami School of Medicine, Miami, FL 33101, USA

Based on molecular and behavioral evidence, we have previously postulated that a metabotropic glutamate receptor, mGluR4, plays a role as a taste receptor for monosodium glutamate (MSG). In taste tissue, the mGluR4 gene is expressed as an abbreviated mRNA lacking the normal 5′-end and the first 5′ 850 translated nucleotides. The corresponding mGluR4 protein is predicted to lack approximately half of the extracellular N-terminus, including a portion of the putative glutamate binding domain. To examine whether this novel mRNA encodes a functional receptor, we have cloned both the brain- and taste-mGluR4 into pcDNA vector and established stably transfected lines of CHO cells. Transfected cellswere screened for mGluR4 expression using Western blots. Brain-mGluR4 transfectants contain an immunoreactive band of 100–110 kDa while taste-mGluR4 transfectants contain a 65–68 kDa band, as predicted from the DNA sequence. To address whether taste-mGluR4 is a functional receptor, we asked if stimulation with MSG leads to a decrease in cellular cAMP, as hasbeen shown for brain-mGluR4. Cells in 96-well plates were pretreated with 1 mM IBMX for 20 min followed by 10 min in1mM IBMX and 10 μM forskolin, with or without MSG (1μM–30 mM). Cells expressing either brain- or taste-mGluR4 showed decreases in cAMP concentration when stimulated with MSG. Extracts of vector-transfected cells did not show decreased cAMP. The dose response for taste-mGluR4 is right-shifted by >2orders of magnitude relative to brain-mGluR4. The higher concentrations of MSG necessary to activate taste-mGluR4 strongly support its postulated role as a taste receptor for MSG.

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239. Development of glutamate receptors in rat taste buds

K.N. Kim[1,2], A. Caicedo[1] and S.D. Roper [1]

[1]*Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL, USA and* [2]*Department of Oral Physiology, College of Dentistry, Kangnung National University, Kangnung, Korea*

Ongoing studies from our laboratory suggest the presence of non-NMDA type synaptic glutamate receptors in rat taste buds (Caicedo *et al.*, this meeting). However, the function of those receptors is not known yet. Developmental changes in the glutamate receptors in taste cells may provide clues of their functional role.

We used the cobalt technique by Pruss *et al.* (1991) to visualize the presence of glutamate receptors. This technique is based on theability of glutamate to stimulate synaptic receptors that allow cobalt to enter a cell. Intracellular cobalt is visualized by precipitation with ammonium sulfide. Five hundred tongue slices including foliate taste buds were incubated for 5 min in Tyrode buffer with 1 mM glutamate (GLU) or 100 μM CNQX.

The number of cobalt-stained cells in the GLU group was always larger than CNQX and the stained cells appeared to be correlated with synaptic, not taste, glutamate receptors. We found that the number of cobalt-stained cells changed during development. The first appearance of cobalt-stained taste cells occurred in 20-day-old rats. The number of cobalt-stained cells increased with age and reached a maximum at 45 days. The shapes of stained cells looked similar at all age groups.

Non-NMDA type glutamate receptors in taste cells may play arole in the modulation of taste transduction pathways or the control of taste bud development.

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240. Synaptic glutamate receptors in rat taste buds

A. Caicedo, K.N. Kim and S.D. Roper

Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33136, USA

Receptor cells in taste buds (TBs) form synaptic connections with sensory afferent fibers. Taste cells (TCs) may also synapse with other cells within the TB. Furthermore, there may be efferent inputs to TBs from the nervous system. The identity of neurotransmitter(s) at these synapses is not yet known. Glutamate, a major excitatory neurotransmitter in other sensory organs, might act at synapses in TBs. We used a cobalt staining technique to detect Ca^{2+}-permeable glutamate receptors in TBs.

When 500 μm slices of foliate and vallate papillae were briefly exposed to 1 mM glutamate in the presence of $CoCl_2$, a subset of TCs accumulated Co^{2+}. Cobalt-stained cells were spindle-shaped with thin processes extending to the apical and basal ends of the TB. Cobalt uptake showed dose dependency in the range from 10μM to 1mM glutamate. Interestingly, glutamate concentrations >1 mM depressed cobalt uptake. This dose response relation for cobalt uptake suggests that synaptic glutamate receptors, not receptors for glutamate taste, were activated. Sensory axons and adjacent non-sensory epithelium were not stained. Glutamate-stimulated cobalt uptake was antagonized by the non-NMDA receptor antagonist CNQX. Depolarization with 50 mM K^+ did not increase the number of stained cells. Also, applying NMDA

(300 μM) did not induce cobalt uptake. This pharmacological characterization of the cobalt uptake suggests that non-NMDA receptors are present in TCs.

Receptors on TCs might be autoreceptors at afferent synapses, postsynaptic receptors of a putative efferent system, or postsynaptic receptors at synapses with other TCs.

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241. Taste stimuli induce transmitter release at rat taste bud synapses *in vitro*

M.S. Jafri and S.D. Roper

Department of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33136, USA

Taste buds (TBs) are chemical detectors that respond to gustatory stimuli. Chemosensory input to TBs is transmitted synaptically to primary afferent neurons. The transmitter(s) at TB synapses have not yet been identified. We employed cyclic voltammetry to study the release of neurotransmitter(s) from TB cells. Responses consist of stimulus-dependent increases of oxidizable substrate(s), such as biogenic amines.

Applying depolarizing stimuli (50 mM KCl in Tyrode's solution) focally to TBs in 200 μm slices of rat vallate or foliate papillae elicited reproducible responses in 5 μm carbon fiber electrodes (CFEs) positioned in TBs. Reducing extracellular Ca^{2+} abolished the response. We did not see any responses to focal application of Tyrode's solution alone. Furthermore, KCl produced no responses when the CFE was positioned in areas where TBs were absent. The responses presumably reflect the release of synaptic transmitter(s) from TB cells. Applying 20 mM saccharin (SAC) or 1 mM denatonium (DEN) also elicited Ca^{2+}-dependent responses exclusively from TBs. The origin of these responses was confirmed by recordings from acutely isolated rat TBs. Calcium-dependent responses to KCl, SAC or DEN from isolated TBs were similar to those in slice preparations. Responses to other taste stimuli are currently being explored.

Typically, CFE recordings allow one to identify certain neurotransmitters, especially biogenic amines; however, responses to date do not explicitly implicate a conventional transmitter. Moreover, the responses to KCl, SAC or DEN differ qualitatively from each other. The identity of the transmitter(s) released from TB cells is currently under investigation.

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242. Migration of BrdU-labeled cells in rat vallate taste buds during cell renewal

Y.K. Cho, O. Ndubuizu and D.V. Smith

Department of Anatomy & Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Taste bud cells arise from epithelial cells that enter the taste bud and live for ~10 days. During this time, it is generally believed that the cells migrate from the periphery to a more central position within the taste bud. We pulse-labeled dividing cells using BrdU (50 mg/kg) and sampled taste buds from the vallate papilla 2.5–10.5 days later. Taste buds were cut transversely to their long axis and sections were immunoreacted with an antibody against BrdU. The position of every labeled cell within each taste bud was determined by measuring its location between the center of the bud and the perimeter. Over these survival times, 3514 cells were quantified, with at least 250 at each time point. Although there was large variation in the position of the cells within the taste buds, a one-way ANOVA showed a significant effect of time on this position [$F(6,3507) = 14.51$, $P < 0.001$]. Cells were significantly closer to the center of the taste bud at 3.5 days than at 2.5 days and their position was monotonically closer to the center up to 6.5 days, where the number of labeled cells per taste bud reaches its maximum. As the number of labeled cells declined beyond 6.5 days, the mean position of the labeled cells at 8.5 and 10.5 days was significantly closer to the periphery (Bonferroni test, $P < 0.05$), suggesting that older cells die near the center of the taste bud.

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243. Proliferation of taste receptor cells is lower during early postnatal rat development as compared with adults

S.J. Hendricks and D.L. Hill

University of Virginia, Charlottesville, VA 22903, USA

Distinct changes in function and morphology of the peripheral taste system occur during early postnatal development of rat; including formation of the taste pore, increased taste bud size, and increased neural responsiveness to sodium stimuli. In an effort to determine if cell cycle dynamics also change during development, fungiform taste receptor cell proliferation was assessed in rats at three ages: day of birth (neonates), 21 days (juveniles), and +60 days (adults). Animals were injected with bromodeoxyuridine (BrdU) and tongues were collected 2 h later. Circadian effects on cell proliferation were measured; the time producing the greatest rate of BrdU labeling was individually determined for each age group and thus used as the injection time for that group. In adults, there is an average of 2.55 ± 0.43 labeled cells per bud, which agrees with previous work (A.I. Farbman, 1980, Cell Tissue Kinet., 13: 349). In contrast, neonates and juveniles show less proliferation as compared with adults (1.01 ± 0.03 and 1.31 ± 0.06 labeled cells per bud respectively). These data suggest that increases in taste bud volume are not solely due to increased proliferation during development.

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244. Molecular cloning of novel type calpains from catfish barbel epithelium

T. Ookura, E. Koyama[1], J.G. Brand[1,2] and Y. Kawamura

National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan, [1]University of Pennsylvania, Philadelphia, PA 19104 and [2]Monell Chemical Senses Center, Philadelphia, PA 19104, USA

Calpain, a calcium-activated neutral protease found in the cytoplasm, may play important roles in intracellular signal transduction cascades that are regulated by calcium. Previously, we reported data on a novel type calpain from catfish taste epithelium (T.E. Ookura *et al.*, 1997, Chem. Senses, 22: 764). With the above cDNA as a probe, we have screened a lambda screen-cDNA library constructed from catfish barbel epithelium. Of the several positive phage clones, we characterized a 2.6 kb calpain cDNA. This clone (IP-nCL4b), encoding a polypeptide of 649 amino acids, shares 90% identity with the previously reported clone (IP-nCL4a). The most diverse segment is located at the N-terminal region. Both calpains share 61% identity with the human digestive tract calpain (human nCL4). Northern blot

analysis revealed IP-nCL4a expressed in barbel and intestine in catfish. Considering that an anti-IP-nCL4a antibody recognized the apical region of taste buds in catfish barbels, these calpains could be taste and intestine specific. In some taste cells from catfish barbels, the potent taste stimulus, L-arginine, evokes an increase in intracellular calcium. This calcium increase may be sufficient to activate taste cell calpains. Their roles in taste transduction and adaptation, will be discussed.

245. Localization of ENaC in taste buds

A.K. Vinnikova[1,2], J.A. DeSimone[2], J.M. McCarty[1,2], G.M. Feldman[1,2] and D.J. Benos[3]

[1]Department of Veterans Affairs Medical Center, Richmond, VA 23249, [2]Virginia Commonwealth University, Richmond, VA 23298 and [3]University of Alabama, Birmingham, AL 35294, USA

The epithelial sodium channel (ENaC) mediates salt taste perception in rat. Immunolocalization of ENaC in anterior rat tongue was initially performed utilizing antibodies to the bovine renal amiloride-sensitive sodium channel (R.E. Stewart *et al.*, 1995, Acta Anat., 153: 310; J.A. DeSimone *et al.*, 1995, Chem. Senses, 20: 684). Subsequently, the presence of α, β and γ subunits of ENaC has been demonstrated in rat taste tissues (O. Kretz *et al.*, 1999, J. Histochem. Cytochem., 47: 51; W. Lin *et al.*, 1997, Chem. Senses, 22: 735). In the current study, we used affinity-purified polyclonal antibodies against the bovine αENaC to further define the αENaC distribution in taste receptor cells of fungiform and circumvallate papillae by laser scanning confocal microscopy. Immunoreactivity to αENaC was present in many cells of the fungiform taste buds but only in relatively few cells of the circumvallate taste buds. As seen on 3-D reconstructed images, the densest staining was observed in the perinuclear region of taste cells. Labeling could also be detected throughout cytosol and on the apical and basolateral membranes. Perinuclear staining was especially evident in the circumvallate taste cells. In addition, we confirmed αENaC mRNA expression in isolated fungiform taste buds by RT-PCR. The current study extends previous findings. The smaller fraction of αENaC-positive cells within the circumvallate taste papillae compared with that of fungiform papillae is consistent with the relative amiloride insensitivity of whole nerve responses to NaCl from the posterior tongue.

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246. Immunocytochemical markers for light and dark cells in mouse taste buds

R.C. Christy, C. Yu, J.M. Pardo[1], J.D. Boughter Jr and D.V. Smith

Department of Anatomy & Neurobiology and Program in Neuroscience and [2]Division of Otolaryngology—Head and Neck Surgery, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Taste bud cells can be classified ultrastructurally into dark (type I) and light (type II) cells. Immunocytochemical studies have shown that subsets of taste cells express various molecular markers, including NCAM, gustducin, several of the human blood group antigens, keratin and other molecules. We processed taste buds of C57BL/6J mice for immunoreactivity to antibodies against gustducin, NCAM, the Lewis^b blood group antigen and the H blood group epitope. Tissue was embedded in polyester wax, cut into 3 μm sections transversely to the long axis of the taste buds and processed for immunocytochemistry. Double-labeling experiments showed that separate subsets of cells expressed NCAM and gustducin, with no overlap in the expression of these two markers. These cells were round in transverse section, a distinctive characteristic of light cells. In transverse section, the extensive cytoplasmic processes of dark cells are clearly evident, making them easily differentiated from light cells; dark cells expressed both of the blood group antigens, which were never expressed by light cells. In order to confirm the ultrastructural identification of these cell types, additional tissue was further processed for electron microscopy (EM). Correlative EM and immunocytochemistry demonstrated directly the relationship between cell type and molecular expression. These molecular markers make it possible to identify taste cell type at the light microscopic level, which will facilitate future studies relating cell morphology to function.

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247. Are neuron specific enolase, serotonin and protein gene product 9.5 present in 'type iii' cells of rat taste buds?

C.L. Yee, B. Böttger and T.E. Finger

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

Different types of taste cells exist in taste buds and can be defined morphologically or histochemically. Murray defined type III cells in rabbits according to morphological criteria; the presence of dense cored vesicles and synapses being defining features of type III cells (R.G. Murray, 1973, in Friedmann (ed.), Ultrastructure of Sensory Organs). Histochemically, rabbit type III cells contain serotonin. In rodents, morphological cell types are not as clearly defined. Careful examination of mouse taste buds failed to reveal a distinct type III cell. However, a population of taste cells in rodents can accumulate serotonin and possess synapses. Accordingly, the serotonin containing taste cells in rodents began to be referred to as type III cells. More recently, PGP9.5 and NSE also have been reported to be present in type III cells of rodents (S. Yoshie *et al.*, 1988, Arch. Histol. Cytol., 51: 379–384; T. Iwanaga *et al.*, 1992, Biomed. Res., 13: 225–230).

In the present study double-label immunocytochemistry has been performed on rat tongues for serotonin, NSE and PGP9.5. The goal of this study is to determine if PGP9.5, NSE and serotonin are present in the same taste cells in the rat.

Results show that PGP9.5 and serotonin generally do not colocalize. The majority of the time, NSE and serotonin also do not colocalize, whereas PGP and NSE generally do. PGP 9.5 and NSE containing taste cells represent a different cell population than serotonin containing taste cells.

248. Relation of the Lewis^b carbohydrate epitope to functional markers in rat taste-bud cells

D.W. Pumplin

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

Receptor cells of taste buds are continuously replaced during the life of the animal, but their afferent axons respond primarily to stimuli belonging to a single taste quality. To maintain constancy of taste responses, a newly formed receptor cell that is sensitive to one of the four taste qualities (salt, sour, sweet and bitter) must

synapse with an axon carrying information about that quality. This requires cell-cell recognition which is likely to be mediated by molecules present on the surfaces of the receptor cell and axon. During differentiation, taste receptor cells acquire: (1) a mature morphology with an apical process exposed to tastants at the taste pore; (2) ion channels and/or components of second-messenger systems for excitation in response to a particular group of tastants; and (3) presynaptic proteins (syntaxin, SNAP-25, synaptophysin and synaptobrevin) involved in neurotransmitter release. The carbohydrate epitope Lewis^b occurs on rat taste-bud cells that possess an apical process and contain alpha-gustducin, a G-protein involved in the detection of sweet and bitter substances. Lewis^b does not appear elsewhere in the rat digestive tract. More cells express Lewis^b in taste-bud populations that are more sensitive to sweet or bitter substances (nasoincisor duct and vallate papillae) than in taste-bud populations sensitive to salts and acids (fungiform papillae). Taste cells that express Lewis^b do not express presynaptic proteins, while taste bud cells that express both alpha-gustducin and presynaptic proteins do not express Lewis^b. Since both alpha-gustducin and presynaptic proteins should be required for responses to sweet and bitter tastants and communication with afferent axons, these observations suggest that Lewis^b is expressed by taste receptor cells prior to synapse formation, and that the carbohydrate group is removed or masked once a synapse has formed. This supports a role for Lewis^b in recognition between taste receptor cells and axons. These findings indicate that expression of the glycosyltransferases responsible for synthesizing the Lewis^b epitope is both cell-specific and developmentally regulated.

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249. Colocalization of serotonin-like immunoreactivity with synaptic proteins in taste buds of the rat

J. Bourne[1,2] and J.C. Kinnamon[1,2]

[1]Department of Biological Sciences, University of Denver, Denver, CO80208 and [2]The Rocky Mountain Taste and Smell Center, Denver, CO,USA

Localization of serotonin-like immunoreactivity (IR) in taste cells of rodents has long fueled the speculation that serotonergic taste cells are probable candidates as taste receptor cells. The goal of the current project is to determine whether serotonin-like IR colocalizes with synaptic proteins thought to be involved in the regulation of neurotransmitter release at synapses from taste receptor cells onto sensory nerve fibers. After pretreating Sprague–Dawley rats with 5-hydroxytryptophan (5-HTP, the intermediate precursor between L-tryptophan and serotonin), the tissues were prepared for immunohistochemistry. Confocal microscopy revealed the colocalization of serotonin-like IR with synaptotagmin, but no colocalization of serotonin-like IR with syntaxin. These results suggest that synaptotagmin, the calcium sensor thought to be necessary for the exocytosis of synaptic vesicles, is present in the serotonergic cells. However, the failure of serotonin-like IR to colocalize with syntaxin, a protein associated with the priming anddocking of synaptic vesicles, argues against a specific 'active zone' on the presynaptic membrane of the serotonergic cells. Our results support previous postulations that serotonergic cells may actually function in a paracrine-like manner. We are currently investigating this possibility by conducting experiments to determine if serotonin-like IR colocalizes with SNAP-25, another protein associated with the docking of synaptic vesicles to the presynaptic membrane.

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250. Taste cells with synapses express SNAP-25-like immunoreactivity

R.B. Yang[1,2], H.H. Crowley[1,2] and J.C. Kinnamon[1,2]

[1]Department of Biological Sciences, University of Denver, Denver, CO 80208 and [2]The Rocky Mountain Taste and Smell Center, Denver, CO, USA

We hypothesize that taste bud synapses utilize synaptic proteins similar to those found in many synapses of the central nervous system. One of those proteins is SNAP-25, a 25 kDa protein that is thought to be associated with the presynaptic active zone. We have used >20 antisera directed against various synaptic proteins in circumvallate taste buds of the rat. To date, our results indicate that SNAP-25 immunoreactivity (IR) is present in a subset of taste cells and most intragemmal nerve processes, with intense IR also associated with the nerve plexus located below the basal lamina ofthe taste bud. Of a total of 15 taste cells with synapses, all exhibited SNAP-25 IR. Both macular and finger-like synapses were present, similar to those observed by Kinnamon *et al.* (1985, 1988) in the mouse. Our data suggest that SNAP-25-IR cells may be actively functioning gustatory receptor cells and that SNAP-25 may play a role in the synaptic vesicle cycle in taste bud receptor cells. We believe that the ability of SNAP-25 antisera to label taste cells with synapses may serve as a useful tool for future studies correlating structure with function in the taste bud.

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251. Immunohistochemical analysis of synaptic proteins in foliate taste buds of the rat

D. Marzulli and J.C. Kinnamon

Department of Biological Sciences, University of Denver, Denver, CO80208 and Rocky Mountain Taste and Smell Center, Denver, CO80262, USA

In the central nervous system numerous synaptic proteins are found which are associated with synaptic vesicles, the presynaptic active zone and the postsynaptic membrane. We hypothesize that these synaptic proteins are present at synapses from taste cells onto afferent nerve fibers in the taste bud. Using confocal microscopy, we have investigated the distribution of four synaptic vesicle proteins in foliate taste buds of the rat: syntaxin, synaptobrevin, synaptotagmin and SNAP-25. Using combinations of monoclonal and polyclonal antibodies, we have observed that these four synaptic proteins are present in subsets of taste cells. Syntaxin-like immunoreactivity (IR) appears in a punctate pattern in taste cells as well as in afferent nerve fibers. Synaptobrevin IR is present in a larger subset of cells as well as the afferent nerves. Synaptotagmin IR and SNAP-25 IR appear predominately in nerves and in small subsets of taste cells. Colocalization experiments have demonstrated that synaptobrevin and synaptotagmin colocalize in a small subset of taste cells. Likewise, there is some colocalization of synaptotagmin IR and syntaxin IR in taste cells. Colocalization ofsynaptobrevin IR and syntaxin IR appears to be in the intragemmal and subgemmal nerve processes, but not in any taste cells.

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252. Localized beta radiation causes isolated loss of taste receptor cells

Nelson, G.M., D. Mellenburg[1] and M.E.C. Robbins[2]

Departments of Anatomy and Cell Biology, [1]Radiology and [2]Radiation Biology, University of Iowa, Iowa City, IA 52245, USA

Taste loss is a common side effect of radiation therapy for head and neck cancers. Loss of taste compounds a patient's inability to eat and contributes to decreased nutritional status during therapy. Quality and quantity of taste loss is variable and its measurement is complicated by xerostomia. The site of radiation damage in taste receptor cells is thought to correlate with DNA proliferation, but symptomatic taste loss does not correlate exactly with taste cell turnover. Focal radiation damage to taste receptor cells can be achieved with localized beta radiation without causing significant salivary gland damage. Beta radiation is delivered to Sprague–Dawley taste cells with a tongue-irradiator, designed to deliver beta radiation to the surface of the tongue. Graded damage to taste cells (% of taste buds lost) can be achieved by varying the dose of radiation administered. A single dose of 18 Gy results in nearly complete ablation of taste buds of both fungiform and circumvallate papillae. In addition, no significant damage to the parotid or minor salivary glands occurs. Other regions of the tongue show infiltration of mast cells into the muscle tissue, damage to blood vessels and lingual salivary glands, and sloughing and regeneration of the surface epithelium. This method of radiation damage is similar to that observed by whole mouth methods, with the exception of producing a more localized, controlled lesion. Using this procedure, it will be possible to measure taste changes caused by radiation of the taste cells independently from significantly decreased salivary flow.

253. Degeneration of fungiform papillae after selective denervation of the lingual nerve in 10-day-old rats

N.A. Guagliardo, S.I. Sollars and D.L. Hill

Department of Psychology, University of Virginia, Charlottesville, VA22903, USA

In adult rats, transection of either the lingual nerve or the chorda tympani nerve (CTX) results in minimal morphological disruption of fungiform papillae. However, combined CT and lingual nerve transection alters normal papillae structure. In contrast, CTX in 10-day-old rats mimics the disruption of papillae morphology noted in combined adult lingual/CTX. In the present study, 10-day-old rats received unilateral transection of the lingual nerve proximal to the CT/lingual nerve juncture. Care was taken to avoid injury to the CT. Tongues were removed 17 days after transection. The dorsal epithelium was stained with methylene blue and observed under darkfield microscopy. Counts were made of fungiform papillae with a pore, papillae without a pore, and filiform-like papillae. In contrast to 76 ± 3 (mean ± SEM) fungiform papillae with a pore on the intact side, there were only 19.7 ± 9 papillae with a pore on the transected side. There were no filiform-like papillae on the intact side, but there were 20.2 ± 5 filiform-like papillae on the cut side. The total from all categories of fungiform papillae was different between cut (44 ± 7.8) and intact (78 ± 3) sides of the tongue. These results indicate that in neonatal rats, the CT alone is not able to support taste bud and papillae structure. This is in contrast to the ability of either the CT or the lingual nerve to support papillae structure in adult rats. In combination with previous studies, the present results suggest that the CT and lingual nerves interact to support fungiform papillae morphology during a sensitive period of development.

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254. Evolution of the vertebrate olfactory receptor gene family

J. Freitag, G. Ludwig, L. Von Buchholtz and H. Breer

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

In vertebrates, the ability of the olfactory system to recognize and discriminate odorous compounds is supposed to depend on the multiplicity and diversity of olfactory receptors. Comparative studies showed that in mammals the receptor repertoire is extremely large, but in fish considerably smaller. Furthermore, mammalian and fish receptors exhibit only moderate sequence conservation and form separate, non-overlapping receptor families, although both groups share some highly conserved sequence motifs indicating common origin from ancestral genes. Comparing the primary structures of olfactory receptors from aquatic, semiaquatic and terrestrial vertebrates representing different levels of vertebrate evolution revealed that olfactory receptors can be categorized in two classes; each class seems to be specialized for the recognition of odorous ligands in either an aquatic or a terrestrial environment. Furthermore, the studies gave first insights concerning the phylogenetic evolution of the vertebrate olfactory receptor gene family; the data suggest that adaptation of vertebrates to novel environments has coincided with structural and numerical changes of the olfactory receptor gene repertoire. As a contribution to elucidate the phylogenetic origin of the olfactory receptors, attempts were made to identify receptors of river lampreys, which are descendants of the earliest craniates. The results indicate that lamprey receptors indeed represent the most ancient family of the hitherto identified vertebrate olfactory receptors and that the agnathostome genes diverged from the gnathostome receptor genes before those split into the two classes.

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255. Small EST projects on male and female antennae of the moth *Manduca sexta* reveal a diversity of insect odorant binding proteins

H.M. Robertson, R. Martos, C.R. Sears, E.Z. Todres, L.A. Schmidt, C.M. Brakebill, P. Mostafavipour, S.J. Rovelstad, K.K.O. Walden and J.B. Nardi

Department of Entomology, University of Illinois at Urbana—Champaign, Urbana, IL 61801, USA

We undertook small-scale Expressed Sequence Tag projects of ~350 clones each on the antennae of male and female *Manduca sexta* in search of the elusive olfactory receptor proteins of insects. The only serpentine or seven-transmembrane G-protein-coupled receptor identified is a distant relative of the patched protein of

Drosophila melanogaster that is likely to be involved in developmental processes. The ortholog of Snmp-1 (sensory neuron membrane protein 1) from *Antheraea polyphemus* (M.E. Rogers *et al.*, 1997, J. Biol. Chem., 23: 14792) was identified in the female library, while another member of this two-transmembrane family related to CD36 of mammals was identified in the male library. The role of this family of membrane proteins in insect olfaction is unclear. Three members of the tetraspanin family, which has ~20 members each in mammals (e.g. CD63 and CD9) and *D. melanogaster*, were found, but again their possible role in insect olfaction is unclear. Instead the major finding of these projects was the diversity of insect odorant binding proteins (IOBPs), with the discovery of two proteins related to the pheromone binding protein and four that are allied with the 17 members now known from *D. melanogaster*. We conclude that insects probably have scores of IOBPs expressed in subsets of their olfactory and other chemosensory sensilla, where they might serve to selectively present odorants to the still elusive insect olfactory receptor proteins.

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256. YAC transgenic approach to clarify the mechanisms of odorant receptor gene expression and specific projection of olfactory neurons

A. Tsuboi, S. Serizawa, T. Ishii, H. Nakatani, M. Asano[1], S. Yoshihara, S. Sengoku, M. Suzuki[2], Y. Iwakura[1], F. Nagawa and H. Sakano

Department of Biophysics and Biochemistry, University of Tokyo, Tokyo 113-0032, [1]Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Tokyo 108-8639 and [2]Instute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, Kumamoto 862-0976, Japan

We have characterized a murine odorant receptor gene cluster including *MOR28*, *MOR10* and *MOR83* genes on chromosome 14. We introduced a 460 kb DNA region containing the *MOR28* cluster into the mouse genome using the YAC transgenic system. The expression of transgenic and endogenous *MOR28* genes was distinguished by tagging the genes with different marker genes, *lacZ* and *gap-GFP* respectively. Interestingly, the transgenic *MOR28* gene was rarely co-expressed with the endogenous alleles in each olfactory neuron, generating a distinct subset of neurons. These new subsets of neurons were found to project to glomeruli distinct from but, adjacent to those projected by neurons expressing the endogenous alleles. Surprisingly, segregation within the targeting glomeruli was also found between two subsets of olfactory neurons expressing either the paternal or maternal allele of *MOR28* gene. These findings argue that each OR allele is regulated independently for the choice of gene and specific projection upon the development of the olfactory neurons.

257. The expression of a cluster of highly homologous of odorant receptor genes

H. Cai[1,2], I. Griff[1,3] and R.R. Reed[1,2,3]

[1]Howard Hughes Medical Institutes, [2]Department of Neuroscience and [3]Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA

The organization and their physical linkage of closely related odorant receptor (OR) genes in the genome suggest that receptor gene clusters may function as a single regulatory unit. To examine this hypothesis, we have characterized a family of highly homologous OR genes, *nM4.1*, *nM4.2* and *nM4.3* displaying over 96% nucleotide and amino acid sequences identity. Three *nM4* receptor genes are encoded in a 180 kb region on mouse chromosome 17 in close proximity to another related OR gene, *M*4. Interestingly, two isoforms of the *nM4.2* and n*M4.3* genes, arising from alternative splicing within the coding regions, predict proteins with different NH_2 terminals. *In situ* hybridization studies revealed that the *M4* and *nM4* receptors are restricted to neurons within zone II of the olfactory epithelium and cells expressing *nM4* receptors project their axons to a single pair of glomeruli. Moreover, the majority of neurons expressing *M4*, *nM4.1*, and *nM4.2/3* are located in distinct domains along the basal/apical axis of the pseudostratified olfactory epithelium, and this layered expression corresponds to the order of these OR genes on the chromosome. Two splice variants of *nM4* receptor genes co-exist in the same cell, even though a given olfactory sensory neuron only expressed *nM4.2* or *nM4.3* at transcription level. The expression pattern and the genomic organization of this cluster of receptor genes support a *cis*-regulation model, in which a locus control region near the receptor gene cluster may temporally or spatially regulate the mutually exclusive expression of clustered OR genes in olfactory sensory neurons.

258. Identification of conserved sequence motifs in olfactory receptor proteins which may participate in upstream and downstream signal transduction

E. Skoufos[1,2] and G.M. Shepherd [1]

[1]Section of Neurobiology and [2]Center for Medical Informatics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA

Olfactory receptors (ORs) are thought to be the largest eukaryotic gene family including ~1000 different genes in the mouse. Computational analysis has given some insight into possible interactions of ORs with odor ligands by showing that TM segments have hypervariable residue sites that could interact with a large number of odor molecules. The external and internal segments of the ORs may contain conserved regions that could interact with the receptors' upstream and downstream signaling partners. To test this hypothesis, a comprehensive analysis using the Multiple EM for Motif Elicitation discovery tool was performed in all the full-length OR clones deposited in the public section of the Olfactory Receptor Database. Ten motifs have been identified that are present in all the olfactory receptors, in the same order. These motifs are concentrated either in the extracellular-most (three motifs) or the intracellular-most (seven motifs) parts of the receptors. This ten-motif structure is exclusive for ORs, since it is not present in other G-protein coupled receptors. The existence and localization of the motifs suggest that they may be involved in the interactions of the receptors with conserved olfactory binding proteins or direct axon guidance and with G-proteins.

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259. Probability considerations in the study of olfactory receptor tuning

J. White, T.C. Bozza[1] and T.K. Alkasab

Department of Neuroscience, Tufts Medical School, Boston, MA 02111 and [1]The Rockefeller University, New York, NY 10021, USA

A fundamental question regarding olfactory function concerns the specificity of individual receptor neurons and proteins. Recent studies in mammalian systems (e.g. Bozza and Kauer, 1998, J. Neurosci., 18: 4560; Krautwurst *et al.*, 1998, Cell, 95: 917; Zhao *et al.*, 1998, Science, 279: 237) have approached this question by testing the physiological responses of defined cells or receptor types. In these studies, responses were observed to one or a restricted number of odorants in a test set. On the surface, such findings seem to imply a high degree of specificity (or selectivity) in olfactory receptors.

However, this initial intuition may be incorrect. As an example: consider a test set of 100 monomolecular odorants (a large set for physiological recordings) drawn randomly from a much larger set of possible odorants (for example, the value '10 000' has found its way into the literature). If olfactory receptors were highly specific, each responding to a single odorous molecule, then the probability of a randomly selected olfactory receptor responding to one of the 100 odorants is 1%, a rather low chance of success. Decreasing receptor specificity increases this probability, but the receptor would have to be responsive to 70 different odorants to produce a better than 50% chance of responding to at least one of the test stimuli. Thus apparent specificity could result from the sampling problem inherent in odorant/receptor studies. These and other probability considerations may therefore provide guidance in the design, analysis, and interpretation of experiments examining receptor specificity.

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260. Characterization of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and *Xenopus laevis* oocytes

C.H. Wetzel, M. Oles, C. Wellerdieck, M. Kuczkowiak, G.Gisselmann and H. Hatt

Ruhr-University of Bochum, Department of Cell Physiology, D-44780 Bochum, Germany

Here we provide the first documentation of functional expressionof a human olfactory receptor protein (OR17-40), and that recombinant olfactory receptors can be functionally expressed inheterologous systems. Application of a mixture of hundred different odorants (Henkel 100) elicited a transient increase in intracellular [Ca^{2+}] within HEK293 cells stably or transiently transfected with the plasmid pOR17-40. By subdividing the odorant mixture into smaller groups we identified a single component that represented the only effective substance: helional. Only the structurally closely related molecule heliotroplyacetone also activated the receptor. Other compounds including piperonal, safrole and vanillin, were completely ineffective. Mock transfected cells and cells transfected with other receptors showed no change in intracellular [Ca^{2+}]. In addition we were able to functionally express the OR17-40 in *Xenopus laevis* oocytes. Coexpression ofa'reporter' channel allowed measurement of the response of oocytes injected with the cRNA of the human receptor to the odor mixture Henkel 100. The effective substances were the same (helional, heliotropylacetone) as those identified byfunctional expression in HEK293 cells. The dose–response curve for helional had an EC_{50} of ~1 μM. These findings open the possibility to now characterize the sensitivity and specificity of many (if not all) ofthe hundreds of different olfactory receptors in humans, and hence to answer the important but still open question of whether olfactory receptors are specialists or generalists.

261. A novel olfactory sensory neuron line, *odora*, properly targets olfactory proteins and exhibits odorant responses

J.R. Murrell[1] and D.D. Hunter[1,2]

[1]Program in Cell, Molecular and Developmental Biology, Departments of Neuroscience, and Anatomy and Cellular Biology and [2]Department of Ophthalmology, Tufts University School of Medicine, Boston, MA 02111, USA

The setting for interactions between the nervous system and much of the chemical world is in the neurons within the olfactory epithelium. A large family of odorant receptor proteins (ORPs) has been postulated to mediate these interactions. However, there are currently limited data to support a direct role for ORPs: expression of many of the proteins has not been demonstrated *in vivo*, and no efficient system has been described for expression of ORPs *in vitro*.

As a first step in designing an effective *in vitro* model for olfactory neurons, we created cell lines from progenitor cells within the olfactory epithelium. We named one of these lines *odora*, for *o*lfactory epithelium-*d*erived, *o*dorant *r*eceptor *a*ctivatable cells. These oncogenically transformed cells divide rapidly in culture, and express markers of the progenitor cell. Upon differentiation, the cells express several neuronal and olfactory markers, suggesting that this line is indeed representative of the olfactory neuron lineage.

Odora cells were transfected with constructs encoding several ORPs; interestingly, the expressed proteins are correctly trafficked to the plasma membrane. In particular, expression of one ORP, the rat U131, confers typical olfactory neuron responses to specific odorants: a marked increase in intracellular calcium in response to enanthic and pelargonic acids. We are currently testing other ORPs in *odora* cells.

Together, our data suggest that the lines that we have created are good models for olfactory sensory neurons. Use of these lines should help to define the role of ORPs in olfactory detection.

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262. Expression and molecular characterization of the olfactory receptor gene OR-Z6

M.M. Pyrski and F.L. Margolis

Department of Anatomy & Neurobiology, University of Maryland at Baltimore, MD 21201. e-mail: mpyrski@umaryland.edu

This study deals with the characterization of *OR-Z-6*, a putative olfactory receptor gene that we recently cloned. We present new data regarding (1) tissue specific mRNA expression and (2) molecular features of the *OR-Z-6* gene.

OR-Z-6 was discovered in the context of characterizing the transgenic mouse line H-lacZ-6 that was generated to study the function of the olfactory marker protein (OMP) promoter. The site

of transgene insertion maps close to the locus for the *OR-Z-6* gene. Using X-gal staining and *in situ* hybridization in tissue ofH-lacZ-6 mice we have shown that the expression of the *OMP-lacZ* transgene and the *OR-Z-6* receptor gene is primarily restricted to zone 2 of the olfactory epithelium (Pyrski *et al.* AChemS 1998).

We now characterize the expression of both genes in the olfactory bulb to determine whether axonal projections of both ORN subpopulations (*X-gal* positive and *OR-Z-6* positive) terminate in identical or neighbouring glomeruli. Furthermore, the strong sequence homology between *OR-Z-6* and the human testicular receptor gene *HTPCRX06* (Parmentier *et al.*, 1992) prompts us to investigate the expression of *OR-Z-6* in testicular tissue.

The second aspect of this study is the characterization of the molecular features of the *OR-Z-6* gene. Whereas abundant information exists about olfactory receptor mRNA expression in olfactory tissue, much less is known about the molecular features of this large gene family. Using molecular techniques including RACE and primer extension we analyze the *OR-Z-6* gene to address mechanisms regulating the expression of olfactory receptor genes.

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263. Molecular models of aldehyde interactions in the I7 olfactory receptor

M.S. Singer and G.M. Shepherd

Section of Neurobiology, Yale University School of Medicine, 236 FMB, 333 Cedar Street, New Haven, CT 06510, USA.
http://habibi.med.yale.edu/mike

We have previously reported potential aldehyde interaction sites in olfactory receptors (Singer and Shepherd, 1997). The rat olfactory receptor I7 has been shown to bind *n*-octanal preferentially (Zhao *et al.*, 1997). Mouse I7, which shares 95% identity, prefers *n*-heptanal (Krautwurst *et al.*, 1998). To study the molecular basis for these differential aldehyde responses, we built 3D molecular models of I7 based on the 7.5 Å resolution density map of rhodopsin (Schertler, 1998, Eye, 12: 504–510). The models revealed a pocket between TMs 3–6, similar to the epinephrine binding poocket in the beta-adrenergic receptor. Lysine 164 (TM4) was the most prominent residue in the pocket. This lysine is identical to histidine 159 (alternatively 430) in OR5. The importance of this residue has been supported by correlated mutation analysis and independent molecular models. The lysine may bind the aldehyde moiety of heptanal and octanal. The lysine has been suggested as apotential partner for interactions as a Schiff base (R. Reed, personal communication). Nearby, aspartate 204 (alternatively 509, TM5) acted as a counterion to the lysine. This arrangement resembles rhodopsin, where a lysine (TM7) forms a Schiff base with the aldehyde of retinal, and a glutamate (TM3) serves as counterion. Hydrophobic residues lined the rest of the pocket andmay contact the aliphatic portion of octanal and heptanal. The results provide new leads for site-directed mutagenesis.

We are grateful to Randall Reed for the mouse I7 sequence. Supported by grants from NIDCD; NIDCD, NIA, NIMH, NIAAA and NASA (Human Brain Project); and the Yale MSTP.

264. Guanidinium-based arginine analogs are detected by multiple odorant receptors (OR) in the zebrafish (*Danio rerio*) olfactory system

D.L. Lipschitz and W.C. Michel

Department of Physiology, University of Utah School of Medicine, Salt Lake City, UT 84108, USA

In fish, olfactory detection of basic, acidic and neutral amino acids is mediated by their interactions with distinct ORs (Friedrich and Korsching, 1997, Neuron, 18: 737). We used electro-olfactogram techniques to examine structure–activity relationships of guanidinium-based arginine analogs presumed to interact with basic amino acid ORs in the zebrafish olfactory epithelium. The relative stimulatory effectiveness (at 0.1 mM) was L-arginine methyl ester (AME) > agmatine (amino-4-guanidobutane = AGB) > 1-ethylguanidine sulphate (EGS) > L-α-amino-β-guanidino-propionate (AGPA) > L-arginine (L-Arg). Structural features conferring odorant qualities include L-isomerization, an unblocked α-amino group, or an esterified or removed carboxyl group. We tested these odorants in a cross-adaptation protocol to estimate the number of ORs that they activate. In this procedure, elimination of an odorant response in competitor odorant background (complete cross-adaptation) indicates the odorants interact with one or a few shared ORs. No cross-adaptation indicates that the odorants are detected by independent ORs. All test/competitor odorant combinations yielded partial cross-adaptation (40–>100% of the unadapted responses). These results are consistent with detection of guanidinium analogs by multiple ORs. Activity-dependent immunocytochemical labeling of olfactory receptor neurons (Michel et al., submitted for publication) supported this conclusion. By itself, the ion channel permeant probe (and odorant), AGB labeled 5% of sensory epithelium, while the addition of L-Arg, EGS, L-AGPA or L-AME to AGB resulted in a significant elevation of labeling (9–14%). Collectively, our results indicate that five or more distinct ORs may be involved in the detection of guanidinium-based substances.

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265. Localization of olfactory-type (ORs) and vomeronasal-type (V2Rs) receptors in different olfactory receptor neurons of goldfish

K.T. Anderson, A. Hansen and T.E. Finger

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

Molecular biological studies of goldfish epithelium reveal two multigene families of putative odorant receptors (Y. Cao *et al.*, 1998, Proc. Natl Acad. Sci. USA, 95: 11987) consisting of OR type(GFA) and V2R type (GFB) G-protein-coupled receptors. Previous *in situ* hybridization studies have shown GFB-positive receptors cells are more superficially located than GFA-positive receptor cells. With the hydrolyzed probes utilized by those investigators an unexpectedly large number of cells appeared labeled. In order to examine this situation in more detail we utilized full length RNA probes derived from cDNA kindly provided by Y. Cao and L. Stryer.

To analyze the differential expression of OR-type and V2R-type

genes in various cells, and their relative location in the goldfish epithelia, we performed two color *in situ* hybridization experiments on 6–15 μm cryostat sections, and electron microscopical (EM) examination of hybridized epithelia from wholemount preparations of olfactory lamellae. Under stringent hybridization conditions and using two full-length probes from the GFB family, wefound relatively fewer cells per linear region of olfactory epithelia than previously reported. GFA gene family members arelocalized to a deeper population of elongated cells in the epithelium, with dendritic processes often reaching the apical surface. Ultrastructural analysis shows that the probe V2R-8 reacts with microvillous receptor cells situated high in the epithelium whereas probe OR-28 reacts with ciliated cells situated deeper in the epithelium.

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266. Analysis of individual olfactory receptors within the expression zones of the rat olfactory epithelium

C.L. Iwema and J.E. Schwob

Department of Anatomy & Cell Biology, SUNY Health Science Center, Syracuse, NY 13210, USA

There is general agreement that the 1000+ olfactory receptors (ORs) are broadly distributed into one out of four zones within the olfactory epithelium. To date there has been little examination of the finer details of this configuration. For example, how rigid are the zonal boundaries? How is a particular OR organized within its zone in terms of its location (1) apically versus basally, (2) anteriorly versus posteriorly and (3) dorsally versus ventrally? Is a particular OR expressed in both mature and immature olfactory sensory neurons? To answer these questions we examined OR expression within the rat olfactory epithelium using non-radioactive *in situ* hybridization (ISH) and immunohistochemistry (IHC). We performed ISH using DIG-labeled riboprobes for eight different PORs corresponding to at least four different zones (dorsal to ventral): OR-14, OR-16 (zone I); OR-37 (zone I/II); OR-18 (zone II); OR-133 (zone III); and OR-107, OR-124, I7 (zone IV). In addition we double-labeled 4% paraformaldehyde-fixed olfactory epithelium that had been cryosectioned at 16 μm with a combination of DIG-labeled OR-riboprobes and IHC with OMP antibody (same sections) or GAP-43 antibody (adjacent sections) and determined that some ORs are present in both OMP(+)(mature) and GAP-43(+)(immature) sensory neurons. Spatial distribution of labeled neurons is being assessed via image analysis. This anatomical data complements the work of Mozell, Kent & Youngentob regarding odorant-induced patterns of activation in the rat olfactory system.

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267. Zone-specific differential regulation of mamFas/OCAM is maintained *in vitro*

J.A. Hamlin and J.E. Schwob

Department of Anatomy and Cell Biology, SUNY Health Science Center, 750 E Adams Street, Syracuse, NY 13210, USA

The mammalian homologue of fasciclin (mamFas), also known asthe RB-8 antigen or OCAM, is an NCAM-related member of the Ig superfamily, and is strongly expressed on olfactory axons originating from zones 2, 3 and 4 of olfactory epithelium *in vivo*. In contrast, there is light or no staining of zone 1 axons (from work in this laboratory and that of K. Mori). Previous work has shown that axons elaborated by olfactory epithelium *in vitro* also show differential staining for mamFas. However, it is unclear whether expression *in vitro* is regulated in an 'appropriate' manner, i.e. explants from ventral olfactory epithelium expressing mamFas and those from dorsal olfactory epithelium not expressing mamFas.

We cultured explants from specific zones of E16 and E17 rat olfactory epithelium on poly-L-lysine/laminin coverslips for 7 days, fixed the tissue, immunostained with the monoclonal antibody RB-8 and anti-NCAM, and labelled with fluorescent secondaries and tertiaries. All of the axons emanating from the explants were NCAM-positive, regardless of the zone of origin. Dorsomedial explants, taken from zone 1, had little or no staining with RB-8, while ventromedial explant culture axons stained densely for mamFas.

These findings demonstrate zone-specific expression of mamFas *in vitro*, making it unlikely that a retrograde signal from the olfactory bulb regulates expression. This suggest that the expression pattern is established earlier than E16 and is maintained by elements present in the explants, either by basal cells or other non-neuronal elements of the epithelium or mucosa.

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268. The role of O-CAM in establishing topographic projections between the olfactory neuroepithelium and the olfactory bulb

H.B. Treloar, Y. Yoshihara[1], K. Mori[2] and C.A. Greer

Department of Neurosurgury & Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510, USA, [1]Laboratory of Neurobiological Synapse and [2]Neuronal Function Research Group, Brain Science Institute, RIKEN, Japan

Olfactory receptor neurons that express the same odorant receptors (dispersed within zones of the olfactory neuroepithelium) target their axons to specific glomeruli in the olfactory bulb. Odor recognition is thus believed to be coded at the level of olfactory glomeruli, with odors eliciting responses in characteristic subsets of glomeruli. Therefore, guidance cues must be present within the olfactory pathway during development to establish the adult topography. We have investigated the role that the olfactory cell adhesion molecule, O-CAM, plays in establishing olfactory topography. It is expressed in a zone specific manner by olfactory receptor neurons and vomeronasal receptor neurons and may beinvolved in axon sorting and fasciculation. We examined expression of this potential axon guidance molecule in the developing olfactory bulb (when olfactory receptor cell axons areactively targeting the olfactory bulb) to determine whether O-CAM has a role in establishing the olfactory projection. Results indicate that O-CAM is expressed by both subsets of olfactory receptor cell axons and subsets of mitral/tufted cell dendrites in the developing olfactory bulb. This data suggests that the second order olfactory neurons (mitral/tufted cells) may also be chemically coded within the olfactory bulb. Furthermore, axonal

and dendritic expression of O-CAM are reciprocal within the olfactory bulb suggestive of O-CAM playing an active role in the targeting of axons to synaptic targets. As yet, the molecular mechanism controlling the process is unknown.

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269. NT-3 expression in olfactory receptor neurons and specific glomeruli of the olfactory bulb in adult mice

B. Böttger, A.J. Vigers[1], T.E. Finger and K.R. Jones[1]

[1]Rocky Mountain Taste & Smell Center and Department of Cellular & Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262 and [1]Department of Molecular, Cellular & Developmental Biology, University of Colorado, Boulder, CO 80309, USA

Neurotrophin-3 (NT-3), a member of a neurotrophin family, is present in some sensory neurons and serves as a survival factor for developing trigeminal ganglion cells. To investigate the expression of NT-3 in the olfactory system, we used a strain of transgenic mice, in which the *Escherichia coli lac-Z* gene is integrated into the NT-3 gene. So, all cells normally making NT-3 also make β-galactosidase (β-gal). Whole mount preparations of adult olfactory bulbs show that β-gal staining is heterogeneous. Strongly labeled punctate areas are present in the ventral anterior bulb, while moderate levels of expression occur in a band across the medial and lateral faces of the bulb. Cross sections show a few heavily labeled glomeruli, corresponding to the strongly label puncta in the whole mounts. Numerous more lightly labeled glomeruli also are evident. In addition, β-gal is present in olfactory receptor neurons in the dorsal zone of the olfactory epithelium. Double label immunocytochemistry, for olfactory marker protein (OMP) and β-gal, in cross sections of olfactory epithelium, show that all β-gal positive neurons are also OMP-positive. To test whether the β-gal expressing glomeruli receive heavy peptidergic trigeminal innervation, we double-labeled β-gal-stained tissue, using a CGRP antibody. Results show little correlation between the distribution of CGRP-irradiated fibers and β-gal reactive glomeruli or epithelium. Expression of NT-3 by olfactory receptor neurons appears related to intrinsic properties of the olfactory system and not to the trigeminal innervation of epithelium or bulb.

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270. Role for two messengers in bitter taste transduction

W. Yan[1], G. Sunavala[1], S. Rosenzweig[1], M. Dasso[1], J.G. Brand[2] and A.I. Spielman[1,2]

[1]Basic Science Division, New York University College of Dentistry, New York, NY 10010 and [2]Monell Chemical Senses Center, Philadelphia, PA19104, USA

Using the quench flow system we have investigated the mechanisms of bitter taste transduction in mouse gustatory and nongustatory tissue in response to denatonium and strychnine (for methods see Spielman, *et al.*, 1996, Am. J. Phys., 270: C926). Both denatonium and strychnine, individually and together (10 mM each), induced a gustatory tissue-specific increase in IP_3 production that peaked at 100 ms and declined to basal levels after 200ms. Strychnine response showed a slower return to basal levels. Nongustatory tissue demonstrated IP_3 production that was abouthalf of the gustatory tissue. The effect of strychnine and denatonium on cyclic nucleotides demonstrated the opposite effect. A mixture of strychnine and denatonium applied to taste tissue showed suppression of cGMP levels. A combination of denatonium and strychnine dropped cGMP levels by 46% with a maximum effect noticeable at 50 ms. A combination of both stimuli also suppressed cyclic AMP levels by 80%. Denatonium suppressed cAMP production by 45%, while strychnine reduced cGMP levels by 60%. We tested the possibility that gustducin may mediate the above mechanisms of cyclic nucleotide suppression. Preincubation of taste tissue with antibodies to gustducin, but not normal IgG, rescued the suppressive effect of denatonium. Antibodies to gustducin or normal IgG did not affect IP3 levels. These data suggest that denatonium and strychnine may act through a dual transduction mechanism in mouse taste tissue: activating IP3 and suppressing cyclic nucleotides through gustducin.

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271. Peripheral mechanisms for discriminating between different 'bitter' compounds in a caterpillar

J.I. Glendinning

Department of Biological Science, Barnard College, Columbia University, New York, NY 10027, USA. e-mail: jglendinning@barnard.columbia.edu

While there is clear evidence that most animal species can discriminate between different types of sapid stimuli, little is known about the peripheral mechanisms that underlie this process. I have been studying discriminative processes in the peripheral taste system of a caterpillar *Manduca sexta*. I focused on the population of identified taste cells that responds selectively to stimuli humans describe as bitter (e.g. salicin, caffeine, aristolochic acid and *Grindelia* extract). Electrophysiological studies have revealed that this population of bitter-sensitive taste cells is quite heterogeneous, containing taste cells with different molecular receptive ranges. I hypothesized that this heterogeneity might help caterpillars discriminate between different bitter compounds. Forexample, I predicted that *M. sexta* (1) should be able to discriminate between salicin and *Grindelia* extract because each stimulus activates different taste cells, but that (2) it should not be able to discriminate between salicin and caffeine because both compounds activate the same transduction pathway within the same taste cell. Using a stimulus generalization assay, I found clear behavioral support for both predictions. Unexpectedly, I found that *M. sexta* could also discriminate between salicin and aristolochic acid, which stimulate different transduction pathways within the same taste cell. Such discrimination could be based on a temporal and/or intensity code given that the transduction pathway activated by aristolochic acid exhibits a different temporal pattern of firing, and a 2-fold greater maximal firing rate, than the pathway activated by salicin.

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272. Rapid kinetics of receptor cell firing and second messenger modulation in an insect model system

K.D. Foster, A.I. Spielman[1] and L.M. Kennedy

Neuroscience Program, Department of Biology, Clark University, Worcester, MA 01610 and [1]New York University College of Dentistry, Basic Science Division, New York, NY 10010, USA

Biochemical events in taste transduction must occur within the time frame of behavioral responses to the stimuli. *Phormia regina* behavioral responses occur within 100 ms after sucrose contacts the taste sensillum, and in this species, the contact time and initial receptor cell firing response can be measured by sensillum tip-recording. We have been using this technique and a quench flow system (QFM5) to study the kinetics of receptor cell firing and second messenger formation in response to sucrose. Firing begins within 10 s; the rate begins decreasing by 50 ms and is significantly decreased at 75 and 100 ms. For the corresponding 100 ms of sucrose stimulation in sensillar homogenates, there were clear decreases in cAMP (to 23–39% of basal levels) after the first 25, 50 and 75 ms, and then an increase (311% of basal) after 100ms (K.D. Foster *et al.*, 1998, Chem. Senses, 23: 549). After 200ms, the cAMP decreased again (77% of basal). Levels of cGMP were decreased after 25, 50, 75 and 100 ms (55–71% of basal) and then increased (110% of basal) at 200 ms. A sucrose/IBMX mixture decreased cAMP at all time points and cGMP after 50, 75 and 100 ms. These results and a profound IBMX-induced suppression of receptor cell firing support our proposal that transduction involves cyclic nucleotide decreases (K.D. Foster and L.M. Kennedy, 1994, Chem. Senses, 19: 470), while cyclic nucleotide increases may mediate later aspects of responses to sucrose.

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273. Taste perception and responses to L-glutamate in the vagus nerve innervated into the alimentary organs in rats

K. Torii, M. Smriga and A. Niijima[1]

Ajinomoto Co., Inc. Central Research Laboratories, Kawasaki 210-8681 and [1]Niigata University School of Medicine, Niigata 951-8151, Japan

Monosodium L-glutamate (MSG) is popular as a food additive that produces umami taste (savoury) and improves taste quality. The specific L-glutamate binding using the taste membrane fraction from bovine circumvallate papillae was significantly (7-fold) enhanced by 5′-ribonucleotides (GMP). This synergistic effect is comparable to the results found in human sensory tests and to the electrophysiological observations in rodents, dogs, cats and monkeys. It is assumed that umami taste may serve as a marker for dietary protein intake, like saltiness is a marker for electrolytes. Sensors for L-glutamate in the oral cavity, gastrointestinal canal and hepatoportal region were studied. Effects of oral, gastric and intestinal infusions, as well as intraportal (i.p.) and intravenous (i.v.) administration of MSG on vagal gastric and vagal pancreatic nerve activities were also studied. Oral, gastric and intestinal infusion of 0.15 M MSG facilitated efferent activity of the gastric branch of the vagus nerve. Furthermore, 0.1 ml of 10nM MSG (i.p., i.v.) originated a reflex activation of the efferent discharges of the gastric branch of the vagus nerve. However, inhepatic vagomized rat injection of 0.1 or 0.5 ml of 100 mM MSG showed no effect on gastric vagal activity. Administration of MSG to its oral, gastrointestinal and hepatoportal sensors showed a similar effect on the activity of the pancreatic branch of the vagus nerve. Additionally, enhanced brawn adipose tissue (BAT) thermogenesis in rats receiving 2% MSG solution was observed, without any effects of glucose or NaCl. These results demonstrate the importance of the peripheral glutamate sensors for L-amino acid homeostasis and dietary protein intake.

274. Rapid induction of sodium appetite modifies taste-evoked activity in rat nucleus of the solitary tract

S.A. McCaughey and T.R. Scott[1]

Monell Chemical Senses Center, Philadelphia, PA 19104 and [1]University of Delaware, Newark, DE 19716, USA

Sodium-depleted rats develop an appetite for sodium salts and show changes in the gustatory neural response to NaCl in the periphery and brainstem. Most commonly, salt-sensitive neurons in depleted rats have given smaller responses to hypertonic NaCl than have corresponding cells in replete controls. A more powerful within-subjects experimental design is now available to assess therelationship between sodium appetite and taste. A need-free sodium appetite, induced by central administration of components of the renin–angiotensin system, can be generated quickly enough to permit us to monitor the activity of individual neurons in the nucleus of the solitary tract (NTS) before and after its creation. Subjects received pretreatment with deoxycorticosterone acetate followed during recording by a single intracerebroventricular infusion of renin. It was shown in a separate group of rats that this regimen consistently resulted in an elevated preference for hypertonic NaCl in <15 min. In rats used for neural recording, renin caused a significant decrease in responses to 0.3 and 0.5 M NaCl across all neurons. Infusion also resulted in dampened responding to iso- and hypertonic NaCl in the salt-oriented cells that were initially most active, while salt-cells that gave the smallest responses prior to infusion were unaffected. These results demonstrate that creation of a sodium appetite is associated with decreased responding to NaCl, even when created rapidly and independently of sodium deficiency.

275. Glossopharyngeal nerve regeneration re-establishes characteristic quinine-elicited gaping behavior and Fos-like immunoreactivity in the nucleus of the solitary tract

C.T. King, M. Garcia and A.C. Spector

Department of Psychology, University of Florida, Gainesville, FL32611-2250, USA

Gaping, in response to intraoral infusion of quinine, is severely attenuated by transection of the glossopharyngeal nerve (GLX), as are the number and distinct pattern of quinine elicited Fos-like immunoreactive (FLI) neurons within the gustatory NST (gNST). The present study was designed to examine concurrently the behavioral and functional neuroanatomical consequences of GL transection, regeneration and the prevention of regeneration, in

response to intraoral infusions of 3.0 mM quinine or water. Eighty-three male Sprague–Dawley rats were subjected to GLX (n = 58) or SHAM (n = 25) surgery. After 17, 52 or 94 days they were infused with 7 ml of quinine or water over 30 min (0.233 ml/min) through an intraoral cannula and videotaped for subsequent scoring of taste reactivity. Animals were then immediately sacrificed and brains were processed for FLI. Standardized sections throughout the rostrocaudal extent of the NST were selected forcounting of FLI-neurons. Nerve transection/regeneration was verified histologically by tallying taste buds in the circumvallate and foliate papillae. All tissue and behavioral analyses were conducted 'blind'. Thus far, based on the data of 42 rats (n = 3 per group), the most salient features are that (1) GLX dramatically attenuates the number of gapes and FLI-neurons in the gNST, particularly in the rostral central subdivision (RC), confirming previous reports; and (2) regeneration of the GL for 52 or 94 days appears to re-establish quinine-elicited gaping behavior and FLI expression in the gNST, especially within RC, to values comparable to quinine-stimulated SHAM rats.

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276. Taste-induced Fos expression in dopaminergic neurons in the nucleus of the solitary tract in the hamster

B.J. Davis and H.M. Smith

Department of Anatomy & Neurobiology and Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD 21201, USA

Tyrosine hydroxylase immunoreactive (TH-I) neurons (reflecting the presence of dopamine) in the nucleus of the solitary tract (NST) receive monosynaptic inputs via the chorda tympani (CT) and probably the glossopharyngeal (GN) nerves in the hamster. Itis likely that taste stimuli that preferentially activate either the CT or GN also excite populations of dopaminergic NST neurons that are mainly located in the respective NST target zones of each nerve. This was tested by plotting the distributions of Fos-anddouble-labeled Fos/TH-I neurons at the levels of the obex (caudal area postrema), obex + 0.5 mm (central canal enters IVth ventricle), obex + 1.3 mm (input via GN), obex + 1.9 mm (overlapping input via CT and GN) and obex + 2.3 mm (input via CT) following quinine (Q), sucrose (S) and water (W) stimulation. The numbers of Fos-I neurons was significantly different across cases (Q > S > W) and across the five levels, with the most Fos-I neurons located at obex + 0.5 mm and the least at obex + 2.3 mm. Although the numbers of TH-I neurons in the five levels were comparable across cases, the percentages of double-labeled Fos/TH-I neurons were significantly different across cases (S: 21.5% > Q: 16.7% > W: 11.5%). These findings indicate that dopaminergic neurons are functionally activated by taste stimulation, and that taste stimuli with markedly different hedonic properties induce Fos expression in populations of dopaminergic neurons that are located outside the anatomically defined target zones of the CT and GN.

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277. Human cortical activity for taste stimulation with MEG study

T. Kobayakawa[1], H. Ogawa[2], H. Kaneda[3], S. Ayabe-Kanamura[1,3] and S. Saito[1]

[1]*National Institute of Bioscience and Human-Technology AIST, MITI, Tsukuba, Ibaraki 305-8572,* [2]*Department of Physiology, Kumamoto University School of Medicine, Kumamoto, 860-0811,* [3]*Sapporo Breweries Ltd, Shizuoka 425-0013 and* [4]*Institute of Psychology, University of Tsukuba, Tsukuba, Ibaraki, 305-8572, Japan*

The gustatory-related regions of the cerebral cortex have not been identified precisely in humans. In this study we attempted to clarify the contribution of the SI to the first peak of gustatory evoked magnetic fields (GEMs), to determine whether the primary gustatory area (area G) was anterior or posterior to the central sulcus, and to trace the possible taste information flow after area G. We recorded the magnetic fields from the brain in response to two tastants, 1 M NaCl and 3 mM saccharin. Seven neurologically healthy volunteers (four males and three females) participated inthe experiments. For measurement of magnetic fields, we used a64-channel whole-head SQUID system (CTF Systems Inc., Canada). Forty trials were presented to each subject per session. Trials contaminated by eye movement were rejected. We estimated the location of areas activated sequentially after the onset of stimulation with magnetic source imaging. The central sulcus was activated less frequently than area G but with almost the same latency in cases of NaCl stimulation. We investigated area G precisely, and found it at the transition between the parietal operculum and the insular cortex. Following area G, we found activation in several cortical regions, e.g. both the frontal operculum and the anterior part of the insula, the hippocampus, the parahippocampal gyrus and the superior temporal sulcus.

278. Structural equation modeling of the relationship between olfactory functioning and cognitive functioning in non-demented younger and older adults

M.F. Dulay[1], K. Hattrup[1] and C. Murphy[1,2]

[1]*San Diego State University, Department of Psychology and* [2]*University of California Medical Center at San Diego, CA, USA*

Research using structural equation modeling indicates that shared variance exists between measures of sensory, sensory-motor, noncognitive, and cognitive functioning. This shared variance is hypothesized to reflect the relationship that these variables have with aging. For example, Baltes *et al.* (1994) postulated that shared variance among visual acuity, auditory acuity, and cognitive functioning in older adults reflects brain integrity. Furthermore, Anstey *et al.* (1997) hypothesized that shared variance among measures of vision, balance, and cognition reflects age-related effects on the central nervous system. The present study examined several a priori structural equation models to test the hypothesis that measures of olfactory and cognitive functioning are more strongly related in an old adult model (ages 55–83 years) compared with a young adult model (ages 18–45 years). Measures of verbal memory, fluency, reasoning, speed of information processing, vocabulary level and olfaction were combined into a second-order latent factor model using young adult sample data, old adult data and combined data. Results indicate that a structural equation

model using old adult data is more representative of the relationship between olfactory and cognitive functioning than a model with young adult data. These findings support the hypothesis that olfactory and cognitive functioning converge in old adulthood due to a 'common factor'. These results suggest that olfactory functioning is a good indicator of central nervous system functioning on old adulthood.

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279. Non-uniformity of olfactory loss with age

M.L. Pelchat

Monell Chemical Senses Center, Philadelphia, PA 19104, USA

Our purpose was to determine whether age-related olfactory loss is uniform across odorants. Results provided support for individualized patterns of non-uniform loss (e.g. some people lose sensitivity to a phenylethylalcohol first and others lose sensitivity to ethylvanillin first). This pattern of findings might be expected if age-related olfactory loss were due exposure to environmental toxins or to medication side-effects. The greatest non-uniformity was seen in the middle-aged participants. In contrast, in an older group, a more substantial degree of loss was present across all odorants. It is important to bear in mind that greater heterogeneity and greater loss of sensitivity are two different issues. These findings can help to enhance our understanding of how perception of flavors and fragrances (many of which are mixtures) changes as we age. For 'baby boomers' who are now middle aged, some components of mixtures may fade while others remain robust, leading to an imbalance in the fragrance. For very old individuals, with more pronounced and uniform losses, there might simply be a diminished intensity of a more balanced fragrance. The evidence for non-uniformity of loss also suggests that it may be more valid to use several odorants in tests of olfactory function than it is to use a single odorant. Finally, an understanding of whether loss isodorant-specific has the potential to shed light on cellular mechanisms of age-related change.

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280. Topical ephedrine administration and nasal chemosensory function in healthy human subjects

A.F.P. Temmel, C. Quint, J. Toth, A. Herneth[1] and T. Hummel[2]

Departments of Otorhinolaryngology and [1] Radiodiagnostics, University of Vienna, AKH Wien, Währinger Gürtel 18–20, A-1090 Vienna, Austria and [2]Department of Otorhinolaryngology, University of Dresden, Fetscherstraße 74, D-01307 Dresden, Germany

A placebo-controlled, randomized, double-blind study was performed to investigate dose-related effects of ephedrine on olfactory function in healthy subjects. Drug effects were assessed using olfactory and trigeminal psychophysical measures (intensity ratings, odor discrimination, butanol and formidic acid thresholds); nasal patency was assessed by means of anterior rhinoresistometry. The investigation was performed in 24 healthy volunteers; subjects were assigned to treatments A, B or C (three groups with eight subjects each; four women and four men per group). All subjects received placebo on the left side; on the right side, group A subjects received placebo, while group B and C subjects received 0.14 and 0.28 mg ephedrine respectively. Ephedrine produced a tendency towards an increase of nasal airflow. However, during the time of observation there was no significant difference between effects produced by the two dosages. Ephedrine had no systematic effect on measures of olfactory function. The only significant correlation to the nasal airflow wasfound for perceived intensity of the trigeminal stimuli which increased with increasing flow. Ephedrine appeared to have neither negative nor major positive effects on intranasal chemosensory function in healthy subjects. This indicates that ephedrine may be used as a decongestant in studies on olfaction.

281. Reduction in perceived, sulfurous malodor via cross-adaptation with ethyl esters

G. Preti[1,2], M.S. Gill[1] and C.J. Wysocki[1,3]

[1]Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, [2]Department of Dermatology, School of Medicine and [3]Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, PA 19104, USA

Odorant exposure produces adaptation to the stimulus and may influence, via cross-adaptation, the perception of other compounds that may utilize the same input channel. Earlier studies focused on cross-adaptation between structurally similar but perceptually different molecules, or between perceptually similar but structurally different molecules. The ethyl esters of E- and Z-3-methyl-2-hexenoic acid reduced the perceptual intensity of the corresponding, malodorous acid. This was hypothesized to result from a similarity in structure. The present study employed ethyl esters of 3-methyl-2-pentenoic acid (EE3M2PA) in an attempt to reduce the intensity of mercaptoethanol (MCAPOL), a sulfur-containing compound. EE3M2PA and MCAPOL are structurally and perceptually dissimilar. MCAPOL is similar in odor quality to many volatile sulfur compounds that play a significant role in the odor-bouquets of many environmental and human malodors. Results demonstrated that the fruity-smelling EE3M2PA, at comparable odor intensity, reduced the intensity of malodor from volatile sulfur compounds: E- and Z-EE3M2PA produced a 30 and 31% reduction in MCAPOL intensity respectively; however, cross-adaptation was asymmetric: exposure to MCAPOL did not affect sensitivity to EE3M2PA. This suggests that the esters have easier access to the olfactory receptors than does MCAPOL or that some olfactory receptor(s) have a high affinity for α,β-unsaturated esters. These esters may be incorporated into fragrances used in consumer products to reduce the perception of malodors that contain both volatile sulfur compounds and/or organic acids.

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282. Detection of L-menthol in the upper airways via olfaction

H. Nagata, P. Breslin, P. Dalton, N. Olver and I. Rodriguez

Monell Chemical Senses Center, Philadelphia, PA 19104, USA

In the upper airways, L-menthol can elicit sensations via three

different modalities: smell, taste and chemesthesis (both pungentand thermal sensations). Although humans can detect the presenceof L-menthol in both the nasal and the oral cavities, the relative sensitivity of each of these regions and the modality by whichabsolute detection of menthol occurs has not been well established.

In the first study, we obtained back-to-back nasal detection thresholds for L-menthol vapor in 100 subjects, using a two-alternative, forced-choice, up–down staircase procedure. Next, we measured the oral threshold for L-menthol dissolved in deionized water. However, L-menthol is a volatile compound and its vapor can reach the nasal cavity from the mouth. Thus, in study 3 we obtained oral thresholds with and without nose plugs in order to determine the effect of retronasal olfaction on the oral detection threshold for L-menthol. When retronasal flow was blocked, thresholds for oral menthol were significantly higher, indicating that retronasal olfaction can serve as an important sensory cue during testing of sensitivity for orally-presented L-menthol.

In the final study, we obtained nasal chemesthetic thresholds using the lateralization technique, which is based upon localization of trigeminal sensations in the nasal passages. The chemesthetic threshold was higher than the nasal detection threshold, indicating that menthol can be detected in the nose at concentrations that donot elicit irritation. Taken together, the results indicate that absolute detection of L-menthol in both the nasal and oral cavities is based upon olfaction.

283. Olfactory communication of emotion in humans

J. Haviland and D. Chen[1]

Psychology Department, Rutgers University, 53 Ave E, New Brunswick, NJ08854 and [1]Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA

Olfactory communication of fear has been demonstrated in rats. The present study examines the human ability to communicate happy and fearful emotions through their axillary odors. Young adult men (n = 11) and women (n = 14) watched on different occasions a happy and a scary video (13 min). Seventy-seven adults (37 men, 40 women), including the donors, completed three odor identification tasks. On task one, they were presented with three jars containing female happy, fearful, and blank odors. On task two, they were presented with three jars containing male happy, fearful, and blank odors. On task three, they were presented with all six jars. On the first two tasks, they were asked to pick a happy odor followed by a fearful one. On the third task, they picked two happy ones and two fearful ones. Binomial tests showed that female subjects identified happy female odors above chance-level on task one, identified happy male and fearful male odors above chance level on task two, and identified both happy odors and both fearful odors above chance level on task three. Examinations of the choice patterns based on frequencies showed that both female and male subjects correctly identified fearful odors as fearful, but while female subjects correctly identified happy odors as happy, male subjects identified both blank and happy odors as happy.

284. Cross-cultural variation in responses to malodors

D.D. Dilks, P. Dalton and G.K. Beauchamp

Monell Chemical Senses Center, Philadelphia, PA 19104, USA

The power of odors to modify human approach and avoidance behavior is well known. For example, the perception of a malodor can rapidly render almost any environment undesirable, suggesting that the selective dispersion of odorants might be an effective way to promote avoidance of a designated area.. However, the success of such a strategy would depend on the consistency of responses across individuals from different ethnic and cultural backgrounds. Unfortunately, the few published studies of ethnic and cultural differences in perception and preference for odors indicate that genetic differences between groups, differences in environment (e.g. experience or familiarity) and the interaction between these two factors can have a significant impact on the ability of any odorant to elicit a uniform response across individuals.

As a first step toward understanding any systematic bases for differences in odor perception across different ethnic and geographic groups, we have begun a cross-cultural investigation of the perception and responses to selected malodors. Our purpose is to determine if there is a universal core of sensory, social and psychological attributes for a variety of odorants, as experienced by a range of racial and ethnic groups in the USA and in selected non-US populations. With data from four US ethnic groups and one African group, our preliminary results have revealed both consistency and diversity. All groups scaled odors using the two dimensions of intensity and hedonic value, and all groups rated bathroom malodor as the most repellant. However, groups showed substantial variation in ratings of other odors.

285. The influence of verbal labeling on the perception of ambiguous odors

R.S. Herz and J.C. von Clef

Monell Chemical Senses Center, Philadelphia, PA 19104-3308, USA

The dramatic effect of context on the perception of odors in comparison to other sensory stimuli has long been noted. The general observation is that expectation can at times induce antithetical responses to an odor which maintains consistent chemical composition. It is proposed that the less an odor is obviously anchored to a particular source the more likely it will be susceptible to contextual influences. To empirically address this issue, we examined how verbal labels would influence the perception of five ambiguous odors. Ambiguous odors were defined as not having a readily determined source and as having potentially polarized hedonic anchors. The odorants were: violet leaf, patchouli, pine oil, menthol, 1:1 isovaleric + butyric acid mixture. Subjects individually sniffed each odor at two different sessions separated by one week. Odors were presented in jars without any visual cues. Subjects were handed each odor with a 'name'. At one session the subject was given the positive verbal label for the odor and at the other session they were given the negative label (e.g. 'parmesan cheese' vs 'vomit' for isovaleric + butyric acid). Results showed that hedonic evaluations, behavioral responses and memory associations made to an odor were dramatically influenced by the label provided for it. Some odors

were more influenced by the manipulation of labeling that others. Descriptions and explanations of particularly effective ambiguous odors are discussed.

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286. Latency, confidence and consistency as reflections of the stability of olfactory knowledge

P.M. Wise and W.S. Cain

Chemosensory Perception Laboratory, Department of Surgery, UC San Diego, La Jolla, CA 92093-0957, USA

When subjects seek to identify odors, they take longer to emit an incorrect than a correct answer (rule 1). This hardly means they have no answer to give for they commonly give a reasonable one. When incorrect, subjects also express low confidence, which means they can monitor their performance (rule 2). A study of 70 subjects revealed that latency of response separated distributions of correct from incorrect performance easily ($d' = 2.2$) and rated confidence separated them even more easily ($d' = 2.8$). In general, subjects also give inconsistent answers from one time to another when incorrect, as if they perceive odors differently from one presentation of the same item to another. Before we can decide whether this phenomenon of instability can be considered a rule, we need to know whether subjects can name items any more consistently when asked to do so than when merely asked to name them. The results showed that they cannot. Inconsistent answers are not therefore aresult of seeking a better answer on a second occasion. The inconsistency is apparently inherent, a fundamental instability in perceiving and retrieving. Successful identification comes from stabilizing this process, a matter achievable by various means. A particularly interesting outcome of the study was that latency of an answer and its rated confidence on first presentation predicted consistency of performance even in those cases where the answer was nominally incorrect. These variables do so well in accounting for performance that scoring of correctness tends toward the redundant.

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287. Semantic-free sorting of odor qualities by osmic, allosmic and anosmic subjects

D.A. Stevens[1] and R.J. O'Connell[1,2]

[1]*Department of Psychology, Clark University, Worcester, MA 01610 and* [2]*Department of Physiology, University of Massachusetts Medical Center, Worcester, MA 01655, USA*

We continue to explore individual differences in odor quality reports elicited by a large group of pleasant test odors and ask if different quality reports are related to the ability to perceive certain target odors. A non-verbal, semantic-free sorting method was used by 120 undergraduate volunteers. They sniffed odors from individual tubes, containing samples of one of 18 pleasant test odorants or a blank. Subjects sorted them into groups such that all members of a group had similar odor qualities. They then sniffed tubes containing individual target compounds, rated their intensity and provided a quality label from a list of standard descriptors. The subjects were then classified as osmic for a target substance, if their quality reports were modal, allosmic if another quality label was employed, and anosmic if no odor was reported. The frequencies with which each of the different test odorants was paired with others were then used as data for independent multidimensional scaling by MINISSA for each class of subject. The coordinates of the individual three-dimensional solutions were then used as input for joining cluster analyses by STATISTICA®. Several of the target compounds elicited cluster diagrams that showed reliable differences among subjects, both in the number of quality classes formed, and in their pattern of joining. Thus, the individual differences characteristic of osmic, allosmic and anosmic subjects for a target compound are readily apparent among another group of modally pleasant odorants.

288. Retronasal and orthonasal odorant identification without sniffing

D.A. Wininger[1,2] and B.P. Halpern[2]

[1]*Department of Psychology, Barnard College, Columbia University, New York, NY 10027 and* [2]*Psychology and Neurobiology & Behavior, Cornell University, Ithaca, NY 14853-7601, USA*

Veridical name identifications of eight food-grade liquid extracts of plant materials were learned to criterion (<5 errors for 32 randomized presentations, with corrections) during orthonasal presentations, then tested for identification with 16 retronasal and then 16 orthonasal presentations without corrections, using vapor phase presentations (Pierce and Halpern, 1996, Chem. Senses, 21: 529). Subjects had the veridical names of the eight odorants plus 12 other names. Retronasal sniffing was not used; orthonasal sniffing, not permitted. Subjects meeting the orthonasal criterion received the retronasal and next the orthonasal tests; then relearned the identifications to criterion with retronasal presentations and next received orthonasal and then retronasal tests. Results: 15 out of 17 subjects achieved the initial orthonasal criterion. Retronasal and orthonasal identification test errors did not differ significantly; overall error rates were <13%. Errors were rare (<2/16) or absent for banana, peanut, and wintergreen. Lemon odorant identification errors were intermediate for both retronasal and orthonasal routes, but the rate of orange orthonasal testing errors was twice the retronasal identification rate. Thirteen of the 15 subjects met the retronasal learning criterion. Orthonasal and retronasal identification test errors did not differ significantly; overall error rates were <9%. Errors were rare or absent for banana, chocolate, cinnamon, coffee and wintergreen; those for lemon and especially orange, more common for orthonasal than retronasal testing. Conclusions: In the absence of sniffing, accurate odorant identification and substantial transfer of identification information can occur for orthonasal and retronasal routes. Some odorants may be more easily identified retronasally.

289. Effects of different perceptual strategies during exposure to taste/odor mixtures

J. Prescott and J. Francis

Sensory Science Research Centre, University of Otago, Dunedin, New Zealand

It has been proposed that the ability of certain odors to enhance tastes is due to perceptual blurring between taste and odor qualities because of frequent pairing in, for example, food flavors. However, flavors, although usually perceived as a functional whole, are not indivisible synthetic entities. Thus, odor enhancement of sucrose sweetness is not observed when the intensities of all the components in a flavor are rated. As a result, odor induced

taste enhancement has been interpreted as a rating bias. To evaluate the alternative notion that cognitive strategies determine such interactions, we encouraged the adoption of synthetic or analytical approaches to odor/taste pairs, while keeping rating demands constant. Subjects who, pre- and post-exposure, rated the smelled and tasted sweetness (both in and out of 0.3 M sucrose) offour odours that varied (low/high) in smelled sweetness and familiarity, were allocated to one of three groups that received 12 exposures under different instructions: forced integration (FI, a synthetic approach in which taste/odour pairs were rated for overall mixture intensity); forced separation (FS, an analytical approach in which taste/odour pairs were rated for taste and odour intensity; exposure control, in which the tastes and odours were received and rated separately. As expected, initially unfamiliar odors paired with sweetness in a way that encouraged synthesis between the elements (FI group) increased in smelled sweetness, in contrast to the other groups. When tasted in sucrose, low familiarity or sweetness odors increased in sweetness, with concurrent changes in the extent to which they enhanced sucrose sweetness. Contrary to expectations, these changes were independent of group. These results are inconsistent with notions of odor/taste interactions as a ratings bias and tend to support a cognitive/perceptual explanation.

290. Right nostril superiority in odor discrimination of non-familiar but not familiar odors

I. Savic and M. Torper

Department of Neuroscience, Karolinska Institute, Stockholm, Sweden

Introduction: In a recent PET study on odor processing with non-familiar odors we observed that odors were discriminated better with right then left nostril, and that mainly the right cerebral hemisphere was activated. In the present study we investigated whether this asymmetry is valid also for the non-familiar odors, and whether any gender differences can be observed.

Methods: Fifty-three right-handed healthy subjects (age 21–45 years, 30 females), with normal vision and olfactory thresholds participated. Twenty pairs of familiar and 20 non-familiar odors were presented with 20 s in-between in the same different paradigm, alternating nostrils, and balancing the order. Odors were rated for familiarity. The subjects also discriminated pictures, using 20 pairs (10 on each side), alternating between the right and left visual fields. The number of errors was compared between: (a) right and left nostrils, (b) familiar and non-familiar odors, and (c) males and females (repeated measures ANOVA at $P < 0.05$).

Results: The odor discrimination performance was superior on the right side ($P = 0.007$), but only for non-familiar odors ($P = 0.02$ and $P = 0.23$ respectively). No significant gender difference was found, although females tended to outperform males in discriminating the familiar odors. Familiar odors were easier to discriminate then non-familiar ($P = 0.001$) and, as expected, the visual discrimination was superior on the right side ($P = 0.049$).

Conclusion: The present data suggest a semantic influence on odor processing. Odors seem, however, to be processed with a right-sided preponderance when the familiarity component is excluded. The odor-familiarity aspect should be taken into consideration when designing tests for detection of odor dysfunction.

291. Olfactory discrimination ability of human and nonhuman primates for 10 pairs of enantiomers

M. Laska and P. Teubner

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

Chiral recognition of substances is one of the most important andwidespread principles of biological activity. With regard to olfactory discrimination of enantiomers, however, the situation isstill unclear. Whereas a variety of optical antipodes has been described as having different odor qualities, few studies so far have directly tested the discriminability of (+)- and (–)-forms of such odorants, and even fewer have assessed conformities or differences in discrimination performance between species.

Using a behavioral conditioning paradigm, we investigated the ability of three squirrel monkeys to distinguish between 10 pairs of enantiomers.

We found that the animals were only able to significantly discriminate the enantiomers of alpha-pinene, carvone, limonene and fenchone, whereas they failed to distinguish between the (+)- and (–)-forms of menthol, rose oxide, camphor, alpha-terpineol, beta-citronellol and 2-butanol.

Using a triple-forced choice procedure, 20 human subjects were tested on the same tasks in parallel and—with the exception of fenchone—showed an almost identical pattern of discrimination performance compared with the squirrel monkeys.

Thus, the results of this study provide evidence of striking parallels in olfactory discrimination ability for enantiomers between human and nonhuman primates.

Further, our findings support the assumptions that(a) squirrel monkeys and humans may share common principles of odor quality perception, and (b) in both species enantioselective molecular odor receptors may only exist for some but not all volatile enantiomers and thus that chiral recognition of odorants may be restricted to some substances.

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292. Partial concordance between ratings of perceived odorant dissimilarity and latency to discriminate odorant pairs

J.W. Newlon, D.B. Kurtz, T.L. White, and P.M. Wise[1]

Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210 and [1]Department of Surgery, University of California at San Diego, La Jolla, CA 92039, USA

Understanding the perceptual similarities and dissimilarities between odorants is important to areas of study as diverse as the physiological quality coding of odorants and industrial product applications. Intuitively, it seems that some odorants are more alike than others. It is difficult, however, to determine which of a variety of techniques is appropriate to validly quantify these perceptual relationships between odorants. One method of evaluating validity is to determine the extent to which different methodologies produce similar results. The goal of this project was to compare two measures of perceived odorant dissimilarity: labeled dissimilarity scale (Kurtz, *et al.*, 1999, Percept. Psychophys., in press) and response latency (Wise *et al.*, 1997, Chem. Senses, 22: 823), i.e. the time necessary to respond 'same' or

'different' when comparing two odorants. Subjects evaluated the perceptual dissimilarity of a nine-odorant set with both methods. Results indicated that the measurement techniques were highly correlated ($r^2 = 0.65$, $P < 0.01$). Further, both methods can be used in conjunction with multi-dimensional analysis to produce odorant object spaces that are similar, though not identical. Thus, the extent to which the spaces are similar suggests that the measures are indeed measuring like components of perceived odorant dissimilarity.

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293. Influence of training on the evaluation of odor similarity

S. Ayabe-Kanamura[1,3], T. Kikuchi[1],T. Kawakami[2] and S. Saito[3]

[1]*Institute of Psychology, University of Tsukuba, Tsukuba, Ibaraki 305-8572,* [2]*Kyara Workshop, 6-14-1-201 Toyotama-Kita, Nerima, Tokyo 176 and* [3]*National Institute of Bioscience and Human-Technology, AIST, MITI, Tsukuba, Ibaraki 305-8566, Japan*

In general, verbal encoding is an important training process in which an expert perfumer or a wine taster learns various kinds of odors. How do these rich verbal encoding of odors in odor memory affect perceptual and cognitive aspects of olfaction? In this study, olfactory representation after olfactory training was examined by the evaluation of odor similarity. In experiment 1, evaluation of odor similarity using olfactory perceptions caused by odors were compared with those using olfactory imagery evoked by words in both trained and untrained subjects for everyday odors. The number of principal components needed to explain the real odors' similarities did not differ between two subject groups. However, the number of principal components for the imagined odors was bigger in the trained group than in the untrained group. These results suggest that the dimensions of the space representing the odors' similarities during olfactory sensation were not obviously changed through training. On the other hand, the space that depends on olfactory imagery seems to be structured with more dimensions after training to remember odors. In experiment 2, pre- and post-training odor similarities foressential oils were also compared within subjects, and no difference was found. These results suggest that representation of odors itself does not change after training to remember odors, but representation of odors linked with words is subdivided through olfactory training.

294. The recollective experience of odors and effects of level of processing: a comparison to memory for words

M.J. Olsson and E.B. Lundgren

Department of Psychology, Uppsala University, Box 1225, S-751 42, Uppsala, Sweden

Memory for odors and for visually presented words were compared in an episodic recognition experiment. In the study phase odors and words were presented during two conditions: one promoting deep encoding and one promoting shallow encoding. After a 20 min retention interval new and old items were presented according to a yes–no procedure. In addition to a positive recognition response, participants judged whether they had any explicit recollection of the encounter with the item (by saying *Remember*) or whether they rather had a feeling of knowing (*Know*). The results show that deeper processing at study did not significantly enhance recognition performance for odors, whereas the increase for words was substantial. Words were more recognized in the remember mode compared with odors. Remember responses for words increased with level of processing, whereas the proportion of know responses remained the same. For odors, remember responses also increased with level of processing, but the interaction pattern suggested that remember responses may be traded for know responses. The results are discussed in the framework of the system/process debate in theory of memory.

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295. Recollective experience in odor memory: influences of age and olfactory familiarity

M. Larsson,[1,2], C. Bjertsjö[1] and L. Bäckman[1,2]

[1]*Department of Clinical Neuroscience and Family Medicine, Division of Geriatric Medicine, Karolinska Institute, Stockholm and* [2]*Department of Psychology, Uppsala University, Sweden*

This study examined the nature of odor memory in young and old age. Subjects studied a set of familiar or unfamiliar odors. Later, a yes–no recognition test was given. When recognizing an odor, subjects indicated whether their decision was based on explicit recollection (*R*emembering), a feeling of familiarity (*K*nowing) or guessing (*G*). Preliminary data suggest that odor memory was poorer in older than in younger adults. When hit rates were broken down by R, K and G responses, an ANOVA showed that most of the recognized odors were categorized as R rather than K or G responses. Interestingly, age interacted with response type, such that young subjects generated more R responses than did older subjects and older adults generated more K responses than did theyoung adults. No age differences in guessing were observed. Ofmore interest, however, was the reliable triple interaction among age, odor familiarity and response type. The source of this interaction was that response type interacted with age such that older adults' experiences of explicit recollection, familiarity, and guessing were unrelated to olfactory familiarity. In contrast, younger adults reported more remember responses for familiar than for unfamiliar odor information, and more know and guess responses for unfamiliar than for familiar information. The absent effect of odor familiarity on response type in old age suggests that aging is associated with a more schematic processing of olfactory information.

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296. Distribution of tastant concentrations affects psychophysical functions: implications for taste mixture effects

D.A. Stevens

Clark University, Worcester, MA 01610, USA

Synergy and mixture suppression are defined in terms of observed intensities of mixtures relative to those predicted by combining the intensities of the components. These mixture phenomena can be due to an invalid combination rule, and/or by use of invalid predictors. The context in which the predictors' intensities are obtained affects the predictors' reported intensities and thus

finding synergy and suppression. With log-scaled concentrations, different mixture effects were found when the predicting intensities were determined along with those of the mixtures versus when in isolation, but not with equal-interval concentrations. This finding could be due to the contexts determined by the distribution of concentrations of the tastants. That possibility was tested in the present study by comparing the psychophysical slopes for sucrose obtained with positively- versus negatively skewed distributions of concentration. Using magnitude estimation, 43 volunteers determined the sweetness of sucrose at five concentrations ranging from 0.08 to 0.96 M. In the positive-skew condition, lower concentrations were more frequent than higher ones; the opposite held for the negative-skew condition. Each person served in both conditions, given at least 24 h apart in random order. The results showed the psychophysical slope was steeper for the positive-skew than for the negative-skew condition. Thus the distribution of concentrations can affect the predictors' intensities and thus affect apparent mixture phenomena.

297. Attentional mechanisms in taste detection

L.E. Marks[1,2] and S.P. Marshall[1]

[1]John B. Pierce Laboratory, New Haven, CT 06519 and [2]Yale University, New Haven, CT 06510, USA

An earlier study from this laboratory (Marks and Wheeler, 1998, Chem. Senses, 23: 19) reported that selective attention can modulate the detectability of weak taste stimuli. Sucrose thresholds were lower when subjects attended (expected) sucrose rather than citric acid, and citric acid thresholds were lower when subjects attended citric acid. Given the presence of analogous effects in vision, hearing and touch, a general principle may characterize selective sensory attention: selective attention may operate maximally when (a) attention is directed toward one channel among several within a given modality and (b) attended and unattended stimuli are detected in different channels. Attention could improve detectability by suppressing neural signals in unattended channels, thereby reducing background noise. In the present study, we measured forced choice thresholds for NaCl and KCl in six subjects using the procedure of Marks and Wheeler. While the subjects attended either NaCl or KCl, thresholds were tracked using a three down one up adaptive method, with tracks for attended and unattended stimuli selected randomly on 75 and 25% of trials respectively. Results showed a very small beneficial gain due to selective attention, roughly half that previously measured with sucrose and citric acid. This outcome is consistent with the hypothesis that the degree of attentional selectivity depends on the presence of multiple sensory channels and that, relative to sucrose versus citric acid, there is greater overlap in the channels responsible for detecting NaCl versus KCl.

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298. Taste matching among three bitter compounds

P.A.S. Breslin, A.J. Culotta, M.S. Kwon and G.K. Beauchamp

Monell Chemical Senses Center, Philadelphia, PA 19104, USA

We have initiated a series of studies to determine if stimuli falling into a common class (e.g. sweet; sour; bitter) are perceived as identical. We previously reported that moderate concentrations of three sugars (Nature, 1994, 369: 447) and several acids (Chem. Senses, 1994, 19) are indiscriminable when their concentrations are adjusted appropriately. Here, we present similar experiments to see if matches could be obtained among urea, quinine-HCl (QHCl) and sucrose octaacetate (SOA). The experiments consisted of a series of two alternative forced-choice (duo–trio) trials. Across sets of trials, the concentration of one compound was held while the concentration of the other was varied. For most subjects, we found concentrations of the test compounds QHCl and SOA that could not be discriminated from the standard, 0.2 M urea, $P_{(\text{discrim.})} \leq 0.58/0.50$. The remaining subjects were able to discriminate among the stimuli more easily, $P_{(\text{discrim.})} \leq 0.69/0.50$. In these latter subjects, discrimination among these compounds may have occurred due to temporal and spatial differences in their bitterness. Previous matches in sweetness and sourness occurred at the same test concentration for all subjects. In marked contrast, at the same standard urea concentration, individual matches for QHCl and SOA could be up to 2 log units different. This could reflect individual differences in the type or density of transduction sequences for these three compounds. We surmise that the tastes elicited by these bitter-tasting compounds are indistinguishable to most subjects because they act either at a common receptor cell type or at a higher level of signal integration to give rise to indistinguishable neural signals.

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299. PROP status does not predict sensitivity to all bitter compounds nor to suprathreshold bitterness ratings

E.M. Cubero and A.C. Noble

Department of Viticulture and Enology, University of California, One Shields Dr., Davis, CA 95616, USA

Detection thresholds were determined for propylthiouracil (PROP), sucrose octaacetate (SOA), naringin, caffeine, quinine, denatonium and limonin. Subjects were classified into three categories based on their sensitivity to PROP: 21 supertasters (threshold $<3.2 \times 10^{-5}$ M), 10 tasters (thresholds between 3.2×10^{-5} and 1.13×10^{-4} M) and 10 non-tasters (thresholds $> 1.13 \times 10^{-4}$ M). Non-tasters (NT) had a higher thresholds for SOA and naringin than PROP supertasters (ST) and tasters (T), whose thresholds did not differ. ST had a higher threshold for caffeine than NT, while neither differed significantly from T. Thresholds for quinine, denatonium and limonine did not vary significantly among PROP taster groups. When bitterness of suprathreshold concentrations was rated by time-intensity methods, ST gave higher maximum intensity (IMAX) ratings for limonin and naringin than NT. I_{max} for quinine and SOA did not differ among PROP status categories; T had lower I_{max} ratings for caffeine and denatonium than either NT or ST. Grouping Ss by thresholds for individual compounds showed significant differences in I_{max} only for caffeine (higher I_{max} for low threshold Ss than high) and naringin (lower I_{max} for low threshold Ss).

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300. Fungiform papillae anatomy: variation with sex, genetic taste variation, and pathology

Z.D. Cohen[1], L.M. Bartoshuk[1], K. Fast[1] and V.B. Duffy[1,2]

[1]Department of Surgery, Yale University School of Medicine, New Haven, CT 06520 and [2]School of Allied Health, University of Connecticut, Storrs, CT 06269-2101, USA

Taste function is affected by genetic taste status, hormonal variation and pathology. We describe anatomical correlates of taste variation in 124 healthy subjects, aged 18–59 years. Each subject received a spatial taste test (nine-point scale); concentrated NaCl, sucrose, citric acid and quinine hydrochloride solutions were unilaterally painted on tongue areas innervated by cranial nerves VII (anterior tongue) and IX (posterior tongue). Each subject also scaled (magnitude matching) 6-*n*-propylthiouracil (PROP) and NaCl. The ratio of PROP bitterness to NaCl saltiness was used to classify 30 nontasters, 66 medium tasters and 28 supertasters. Using videomicroscopy (Miller and Reedy, 1990), we measured the diameters of fungiform papillae as well as the density of fungiform papillae, ringed fungiform papillae and taste pores. Subjects with the largest numbers of papillae had the most ringed papillae and the smallest diameter papillae. The PROP ratio was related to density of fungiform papillae and taste pores (supertasters > medium tasters > nontasters). Females were most likely to be PROP supertasters, had highest taste pore densities and greatest variance in pores per papilla. Damage to VII releases inhibition on IX, thus producing a characteristic pattern: low perceived taste intensities on anterior, high on posterior tongue. Subjects who showed this pattern had the lowest number of pores per papilla (including poreless papillae). PROP status is clearly related to density of fungiform papillae and taste pores. Hormonal variation and pathology affect taste pore density without affecting fungiform papillae density.

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301. Spatial taste testing and genetic taste variation

J.M. Prutkin, K. Fast, L.A. Lucchina[1], D.J. Snyder[2] and L.M.Bartoshuk

Yale University School of Medicine, New Haven, CT 06520, [1]Unilever Research U.S., Edgewater, NJ 07020 and [2]Florida State University, Tallahassee, FL 32312, USA

Taste buds in fungiform papillae (anterior tongue) are innervated by the chorda tympani nerve (VII); those in foliate papillae (lateral edge of posterior tongue) by both the chorda tympani and glossopharyngeal nerves (IX); those in circumvallate papillae (posterior tongue) by the glossopharyngeal nerve; and those on the palate by both the chorda tympani and glossopharyngeal nerves. Genetic variation influences perceived bitterness of 6-*n*-propylthiouracil (PROP). When tasting with the whole mouth, supertasters perceive saturated PROP to be intensely bitter, medium tasters as moderately bitter and nontasters as barely perceptible. This experiment examined the perception of standard tastants as well as PROP on each area described above using a spatial taste test designed to assess clinical taste loss. Food or pharmaceutical grade solutions of NaCl (1 M), sucrose (1 M), citric acid (0.032 M) and quinine hydrochloride (0.001 M) were swabbed onto each side of the tongue on the loci noted above with a cotton-tipped applicator (n = 129). A water rinse preceded each application. Subjects rated perceived intensities (Green scale). Following this, to stimulate all taste receptors, subjects swished each solution, spat it out, swallowed the residual and rated the intensity. On a second day, the spatial test was repeated with 0.0032 M PROP (no swallow). On a third day, whole mouth PROP bitterness was scaled (sip and spit). The perceived intensities of NaCl, sucrose, citric acid and quinine applied to all loci positively correlated with whole mouth PROP bitterness with the single exception of quinine on the palate.

302. Functionality of taste localization in humans: selective expectoration of a target

J.F. Delwiche, M.F. Lera and P.A.S. Breslin

Monell Chemical Senses Center, Philadelphia, PA 19104, USA

We have previously demonstrated that people can localize a punctate gustatory stimulus on the lingual epithelium (Chem. Senses, 1997, 22: 650–651), and can use gustatory spatial information to selectively expectorate a target gelatin cube presented with somatosensorily identical distractor cubes (Chem. Senses, 1998, 23: 562–563). In the present study, we investigated whether people could expectorate a target flavored gelatin cube (1 cm^3) while retaining three flavored cubes in the mouth. Target/distractor combinations were sweet/neutral, salty/bitter, bitter/sweet and neutral/sweet. Subjects blindly placed four gelatin cubes in the nongustatory area under the tongue and, when instructed, pushed them forward and gently held them stationary against the inside of the teeth with the tongue tip. Subjects expectorated the suspected target into a cup as quickly as possible (~3 s); it was identified as the target by its color. Twelve subjects participated in 120 experimental trials each, with 30 trials conducted with each stimuli set. All subjects performed significantly above chance for sweet/neutral and salty/bitter target/distractor combinations. However, only 10 subjects were significantly above chance performance with bitter/sweet stimuli and only eight were above chance with neutral/sweet stimuli ($P < 0.05$). Results indicate that humans are capable of removing a gustatory target from a field of gustatory distractors via taste sensations. Detectability differences (d') found across stimuli sets ($P < 0.001$) indicate that somatosensory capture of gustatory stimuli may compete with localization of the target gustatory cue (e.g. neutral/sweet). Localization of taste sensations in the oral cavity thus can be used for the selective removal (or retention) of portions of a heterogeneous food bolus from the oral cavity in a manner paralleling the gustatory and ingestive behavior of other vertebrates (e.g. fish).

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303. Psychophysical evidence of monosodium glutamate enhancing effect on saltiness perception

M.E. Otero-Losada, M.P. Martínez and M.C. Zamora

Laboratorio de Investigaciones Sensoriales (LIS) CONICET, Marcelo T. de Alvear 2202 4 P, Buenos Aires, Argentina, (1122)

Monosodium glutamate (MSG) is a known flavor enhancer. We reported that MSG shortens reaction time to saltiness perception (SP) at low NaCl concentrations.

This work provides objective records of MSG-induced enhancement of SP using conventional psychophysical methodology (converging limits, CL). Stimuli: aqueous solutions of NaCl, NaCl + MSG, NaCl + KCl, NaCl + MSG + KCl, KCl, MSG and KCl +

MSG. Concentrations: NaCl 12.5, 25, 50, 100, 200 mM; KCl:NaCl ratio 2:1; MSG 4.32, 10.80, 27.00, 67.50, 168.75 mM (27 mM inmixtures). CL estimations were plotted against concentration and psychophysical slopes (β, Steven's coefficient) calculated. Results were analysed by two-way ANOVA followed by the least significant difference test (LSD).

β values, r^2 (regression coefficient) were: 0.80 ± 0.06, r^2 = 0.98 (NaCl); 0.60 ± 0.01, r^2 = 0.99 (NaCl + MSG); 0.69 ± 0.04, r^2 = 0.99 (NaCl + KCl); 0.65 ± 0.04, r^2 = 0.99 (NaCl + MSG + KCl); 0.49 ± 0.05, r^2 = 0.97 (MSG); 0.66 ± 0.02, r^2 = 0.99 (KCl); 0.58 ± 0.04, r^2 = 0.99 (Kcl + MSG). Concentration ($P < 0.0001$), panelist ($P < 0.0001$) and salt ($P < 0.001$) affected SP and salt interacted with concentration ($P < 0.0001$). KCl and (NaCl + MSG + KCl) solutions were different to MSG, NaCl and NaCl + MSG solutions (LSD, $P < 0.05$).

Thus βNaCl > βKCl > βMSG. MSG or KCl flattened response to NaCl. MSG specifically affected NaCl. MSG addition to NaCl + KCl mixtures was ineffective. βNaCl reduction by MSG or KCl resulted from increased SP at low NaCl levels (salt–concentration interaction) probably reflecting increased affinity of NaCl for its receptor sites.

The flavor-enhancing effects of MSG are NaCl specific. Proposed alternatives to reduce NaCl without reducing food flavor are: KCl or MSG combinations with NaCl. Qualitative differences in sensation usually turn consumers' acceptance towards MSG choice.

304. Increases in sensitivity for monosodium glutamate (MSG) after repeated exposures to MSG in food

C. Kobayashi and L.M. Kennedy

Neuroscience Laboratory, Biology Department, Clark University, Worcester, MA 01610, USA

Although an early study found no difference in MSG taste sensitivity between Japanese and Americans (S. Yamaguchi, 1991, Physiol. Behav., 49: 833), a later study did. The earlier results were attributed to a 'warming-up' effect, distinct from learning (R. Ishii *et al.*, 1992, Chem. Senses, 17: 365). This effect suggests that MSG sensitivity may involve an inducible taste mechanism, as was suggested for fructose or glucose by S. Eylam and L.M. Kennedy (1998, Olfaction and Taste XII, Ann. NY Acad. Sci., 855: 170; 1998, Chem. Senses, 23: 588). To test this hypothesis, we exposed 17 Americans to MSG in food and then compared their MSG sensitivities with those of two control groups (18 Americans without MSG exposure and 18 Japanese). Subjects overlaid their tongues with chocolate candies (controls) or shrimp crackers containing MSG (experimental group) briefly each day for 10 days.They then were tested with 0.925, 1.25 and 2.5 mM MSG, each paired with a NaCl concentration of equal taste intensity. First, 5 mM MSG was tasted and subjects were told, 'This is Ajinomoto'. Then the pairs were tasted in order of ascending concentrations, and subjects identified the Ajinomoto solution in each pair. There was a significant difference between all groups, and also between the two American groups, in correct identifications ($P < 0.001$, ANOVA and contrasts). Since subjects had been instructed not to be concerned with the taste of the treatment foods, the greater MSG sensitivity in the Americans exposed toMSG suggests an inducible taste mechanism, rather than a learning process.

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305. Contribution of interfacial properties of sapid substances in predicting their taste quality

M. Mathlouthi and F. Hutteau

Laboratoire de Chimie Physique Industrielle, Faculté des Sciences, Université de Reims Champagne-Ardenne, BP 1039, F-51687 Reims Cédex 2, France

The surface tensions, contact angles and adhesion forces (on a hydrophobic surface) were determined for water and aqueous solutions of sugars (D-glucose, D-fructose and sucrose), artificial sweeteners (aspartame, acesulfam-K, alitame, sodium cyclamate and sodium saccharin), sweetness inhibitors [sodium salt of 2-(4-methoxy-phenoxy) propionic acid and α-D-methyl 4,6-dichloro-4,6-dideoxygalactopyranoside] and bitter substances (caffeine and quinine sulfate), as well as for sugar/intense sweeteners mixtures.

While sugars at 10% (w/w) do not change the surface tension and contact angle of pure water, the artificial sweeteners were found to decrease these interfacial properties of water even at such low concentrations as 1% w/w. A linear correlation was found between the logarithm of relative sweetness and adhesion force for all sweet molecules investigated. The decrease in surface tension and contact angle of water in the presence of traces of bitter substances or sweetness inhibitors was appreciable. The relative sweetness of sugar/artificial sweeteners mixtures was linearly correlated to surface adhesion force with a negative slope. A positive value was observed for sweetness synergy and a negative for suppression.

Together with the apparent specific volume, interfacial properties prove to be useful tools for taste quality prediction. Inasmuch as sapid stimuli–receptor membrane interaction is an interfacial phenomenon, it is understandable that information on solvent cohesion and the affinity for a hydrophobic surface are of relevance for sweet response elucidation.

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306. Effects of gymnemic acid on taste stimulus intensity and identification

T.P. Hettinger, J.F. Gent, M.E. Frank and L.E. Marks[1]

Department of BioStructure & Function, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT 06030 and [1]J.B.Pierce Laboratory and Yale University, New Haven, CT 05419, USA

A sweet-taste intensity deficit was generated and its effects on taste identification measured in humans. The deficit was produced by a2 min, 5 ml, 0.5% gymnemic acid (GA) rinse, which reduced intensities of 300 mM sucrose and 3 mM aspartame to 14% of pre-rinse values ($P < 0.001$). Stimulus identification performance of 12 subjects who rinsed with GA was compared with that of 12 subjects who rinsed with water. Identification was measured in each subject for 10 replicates of 10 stimuli, resulting in a 10 × 10 'taste confusion matrix' (TCM). The stimulus set contained midrange concentrations of five primarily or partly sweet (sucrose and aspartame, and NaCl–sucrose, acid–sucrose and quinine–sucrose mixtures) and five nonsweet (NaCl, KCl, monosodium glutamate, quinine-HCl, citric acid) stimuli. The GA effect, which

dissipated linearly with time, was maintained throughout a session with GA rinses before every other replicate. Overall consistency of identification of the 10 stimuli was slightly poorer in the GA rinse group ($P < 0.03$) as quantified by T_{10}, a measure of transmitted information that is correlated ($r = 0.77$) with the percent correct. However, the sweet loss resulting from GA rinses led to a specific set of identification errors. Stimulus discriminability, quantified by T_2 (also derived from information theory), was impaired in the GA rinse group for sweet–nonsweet stimulus pairs ($P < 0.0001$) but enhanced for the aspartame/sucrose–NaCl pair ($P < 0.01$). GA had no effect on discriminability of nonsweet stimulus pairs. This TCM pattern of errors made by subjects with a GA-induced deficit could serve to identify sweet-taste loss in patient populations.

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307. Selective sweetness inhibitors and a biochemical mechanism for sweet water aftertaste

L.L. D'Angelo, G.A. King and G.E. DuBois

Corporate R&D, The Coca-Cola Company, PO Drawer 1734, Atlanta, GA30301, USA

Many sweetness inhibitors have been reported in the scientific literature. None of these inhibitors have been demonstrated to show selectivity against sweeteners of varied structural types. Some scientists have argued that this absence of selectivity is evidence for a single sweetener receptor. Here we report the first selective sweetness inhibitors. Our results support the idea of sweet taste mediation by several receptor subtypes of similar structure. In addition to our identification of the first selective sweetness inhibitors, our work in this field led us to an insight into the long-puzzling phenomenon known as 'sweet water aftertaste'. We propose a biochemical mechanism for 'sweet water aftertaste'.

308. The effect of L-lactic acid on solutions of D- and L-arabinose

R.W. Siertsema and G.G. Birch

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

As a complement to sensory studies of the effects of citric acid on sucrose sweetness intensity, solution properties of mixtures of D- and L-arabinose with L-lactic acid were determined. A chiral acid was chosen, so that any chiral discrimination between the arabinose enantiomers might be detected.

The partial specific volume of L-lactic acid (0.715 cm^3/g) was found to be higher than that of arabinose (0.619 cm^3/g), suggesting a sweet–bitter rather than sour taste for the acid. The high value may be explained by the low dissociation constant for lactic acid ($pK_a = 3.08$) (R.S. Shallenberger, 1993, Taste Chemistry, Blackie Academic & Professional, Glasgow).

No differences in solution properties were found between mixtures of lactic acid with either D- or L-arabinose, implying a lack of chiral discrimination between the enantiomers. The addition of lactic acid was found to have a significant effect on the solution properties of arabinose. At relatively high concentrations of arabinose, and low concentrations of lactic acid, the apparent specific volumes of the mixtures were found to be independent of the concentration of arabinose present. This implies that the effect of the acid on the sweetness of arabinose is independent of the concentration of the sugar at these concentrations. This is in accord with the findings of H.N.J. Schifferstein and J.E.R. Frijters (1990, Chem. Senses, 15: 87) for the sensory analysis of mixtures of sucrose and citric acid.

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309. Chlorhexidine affects anion taste in humans

J.F. Gent, M.E. Frank, A. Pepin and M. Nadeau

Department of BioStructure & Function, School of Dental Medicine, University of Connecticut Health Center, Farmington, CT 06030, USA

We examined taste-altering effects of chlorhexidine, a bis-biguanide antiseptic, on salts other than NaCl, and whether repeated rinses had greater effects than a single rinse. In one study, 10 subjects rated the intensity and named the quality of water, 0.1 M NaCl, 0.3 M Na acetate, 0.1 M MSG, 0.1 M KCl and 0.3 M sucrose mixed with NaCl or KCl, using a 'sip and spit' procedure. Ratings were made before and several times after a single 2 min rinse with 0.12% chlorhexidine (Peridex®). In a second study, 16 subjects, equally divided into repeat and single rinse groups, similarly evaluated NaCl, sucrose, 0.1 mM quinine-HCl and 3 mM citric acid. Both groups had pre-rinse taste tests followed by chlorhexidine rinse and post-rinse tests. The repeat rinse group rinsed twice a day for 3 days between tests. Effects on taste during the 30 min post-rinse test period included 50% reductions in intensities of chloride salts: NaCl, KCl and quinine-HCl ($P < 0.007$); enhancement in intensity of citric acid ($P < 0.002$); and change in the quality of NaCl from salty to bitter–sour ($P < 0.01$). Chlorhexidine did not affect the taste of the non-chloride salts Na acetate and MSG. Repeated rinsing did not enhance the effects and, with the exception of an enhancement in KCl intensity ($P < 0.007$), effects on taste disappeared after 4 h. Thus, the mechanism of action for chlorhexidine appears to involve the chloride anion, which, unlike the amiloride-sensitive sodium channel, may play an important role in human salt-taste transduction.

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310. Non-demented older adults with the APOE ε4 allele perform poorly on odor memory tasks

M.L. Sliger[1], M.F. Dulay[1], T.A. Lander[2], C. Kim[1], J. Ranzani[3], L. Thal[2] and C. Murphy[1,2]

[1]*San Diego State University,* [2]*UCSD Medical Center and* [3]*SDSU/UCSD Joint Doctoral Program in Clinical Psychology, San Diego, CA 92120-4913, USA*

The presence of the apolipoprotein E ε4 allele is considered a risk factor for Alzheimer's disease (AD), as well as multi-infarct dementia and heart disease. Individuals with at least one ε4 allele have a significantly greater risk for late onset sporadic and familial AD. Subjects with the ε4 allele show significantly lower scores on certain neuropsychological tests, especially those measuring recognition for faces and words, verbal recall, and visual attention compared with those without the ε4 allele. In addition, Murphy *et al.* (1998) found that persons who are heterozygous or homozygous for the ε4 allele show significantly poorer odor identification than those without an ε4 allele. The present study hypothesized that ε4 status is associated with decreased performance on the California odor learning test, which measures odor recall and odor recognition memory. Subjects consisted of three groups: AD patients diagnosed by two separate neurologists at the UCSD Alzheimer's Disease Research Center (ADRC), non-demented older persons

positive for at least one ε4 allele and non-demented persons negative for ε4 allele. Persons with the ε4 allele showed significantly poorer recall of odors than those without an ε4 allele. In addition, persons with the ε4 allele performed significantly better than the AD group. Results suggest that measurement of odor memory deficits can be an important tool in the diagnosis of pre-clinical signs of AD in non-demented older persons.

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311. Olfactory function and cirrhosis of the liver

S. Pabinger, A.F.P. Temmel, C. Quint, A.M. Herneth[1], P. Munda[2] and P. Ferenci[2]

Departments of Otolaryngology, [1]Radiology and [3]Gastroenterology, University of Vienna, Vienna, Austria

Although only few data on chronic liver disease and olfactory sensitivity are available, it has been reported that cirrhosis of the liver is occasionally accompanied by a reduced chemosensory ability. In the present study we examined olfactory sensitivity, odoridentification and odor discrimination in patients with compensated cirrhosis of the liver ($n = 25$). The main aims were to investigate (a) whether compensated cirrhosis of the liver is associated with a lower olfactory sensitivity; (b) the relationship between global psychometric measurements and performance in the olfactory tests; and (c) the relationship of laboratory findings and olfactory function. The results implicate that patients suffering from cirrhosis had a poorer olfactory sensitivity and performed worse in the odor identification test as compared with age matched healthy controls. There was a straight correlation of odor identification performance and the scores of psychometric tests. Laboratory tests regarding Zn^{2+} levels also revealed a correlation to the olfactory measurements. The lower the Zn^{2+} levels were, the worse they performed on olfactory testing.

Taken together, these findings suggest that the olfactory impairment observed in compensated cirrhosis of the liver is closely related to their Zn^{2+} level and to the psychometric scores.

312. Monorhinal odor identification and detection thresholds in patients with seasonal affective disorder

T.T. Postolache, R.L. Doty[1], T.A., Wehr, L. Sher, E.H. Turner and N.E.Rosenthal

Section on Biological Rhythms, NIMH, 10 Center Drive, Room 3S-231, Bethesda MD 20892-1390 and [1]Smell and Taste Center, University of Pennsylvania Medical Center, 3400 Spruce Street, PA 19104, USA

Because patients with seasonal affective disorder (SAD) feel better in summer than when light-treated in winter (Postolache *et al.*, 1998, Am. J. Psychiat., 155: 1614), because olfaction plays a modulatory role in seasonal rhythms in several non-human species,and because olfactory and visual pathways are interconnected, we hypothesized that, beside light, olfactory factors may play a role in human seasonal rhythms. Previously, in winter, we did not find any difference in olfactory performance between patients with SAD and controls or in patients before and after light treatment, although we did find a lateralized relationship indepressed patients between monorhinal odor identification anddepression scores (Postolache *et al.*, 1999, J. Affect. Disord., in press). This time, we compared olfactory performance in SAD patients and controls in winter and summer. Sixteen patients with SAD and 21 healthy controls were studied during the winter when patients were depressed and again during the summer when patients were in remission. We administered monorhinally phenyl ethyl alcohol (PEA) detection thresholds and UPSIT. Mood was rated on SIGH-SAD and SAM-SAD scales. UPSIT and PEA scores were compared in patients and controls, in both seasons, using ANCOVAs, with post-hoc *t*-tests. In the summer, SAD patients were found to have higher PEA detection thresholds than controls (left, $P < 0.004$; right, $P < 0.001$). Additionally, the lateralized relationship between mood and monorhinal odor identification was partially replicated. Olfaction may be involved in seasonal emotional rhythms in humans.

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313. A mediational model of depression in smell disordered patients

C.A. Ossebaard, W.G. Tayer, P.M. Nicassio and W.S. Cain

University of California—San Diego, San Diego, CA 92037, USA

Many patients with olfactory disorders show elevated levels of depression. A model to illustrate the relationship between olfactory impairment and psychological symptoms would be a useful tool in the development of treatment of depression in this population. The proposed model in the present study consists of disease status/functionality (absence or presence of symptoms related to olfactory impairment) and cognitive factors (perceived helplessness) as predictors of depression. Subjects diagnosed with anosmia, hyposmia and/or dysosmia completed self-administered questionnaires sent through the mail. The questionnaire included an adjusted version of the Multicenter Taste & Smell Questionnaire (an index for olfactory impairment), and widely used measures of helplessness and depression. Results indicate that 1/5 of patients were clinically depressed and 1/3 reported extreme helplessness (perceived inability to control outcomes related to their condition). This degree of helplessness is comparable to that seen in chronic illness populations. The data supported a model in which cognitive factors mediate the relationship between disease status and depression. According to this model, the greater the impairment in olfactory functioning, the greater the helplessness, and in turn the greater the depression. A key finding is that olfactory impairment alone does not predict depression, but that this relationship is mediated by cognitive factors.

314. Failure of physicians to assess olfactory ability in neurologic inpatients

A.R. Hirsch and M.L. Colavincenzo

Smell and Taste Treatment and Research Foundation, Chicago, IL 60611, USA

Due to age, use of medications and presence of neurological disorders, one would expect olfactory dysfunction among hospitalized neurologic patients. Standard texts suggest that assessment of cranial nerve I (CN I) is an essential part of a complete neurological examination. Despite this, our anecdotal experience suggests that it is rarely tested in clinical practice. To assess this, we studied the frequency of CN I evaluation at a teaching hospital. The charts of the 90 most recently admitted

adult patients with neurological diagnoses who were awake, able to follow directions, capable of responding and not intubated, comatose or admitted to an intensive care unit were evaluated for performance of CN I testing by attendings in internal medicine and neurology and consultants in neurology. Ninety-four examinations were performed, 77 by internists, 17 by neurologists. The patients' average age was 69 years (range, 24–100). There were a total of 97 neurological diagnoses of 30 different neurological conditions, including CVA (n = 21), Alzheimer's disease (n = 14), and seizures and convulsions (n = 10). None of the charts describe specific olfactory testing, while 4.2% imply that it may have been performed: 'cranial nerves intact' or 'neuro exam grossly normal'. This lack of testing may have affected the differential diagnosis and was a missed opportunity to detect and appropriately counsel those with chemosensory dysfunction. Given the expected high prevalence of olfactory disorders among neurologic inpatients, the ease of testing (e.g. alcohol sniff test) and the potential benefits of identifying impairment, physicians should routinely test olfactory ability.

315. Solid-state olfactometer for the diagnostic clinic

C.J. Frederickson, D. Taylor, C.J. Frederickson, J.P. Kesslak[1], I. Achiriloaie, M. Stewart[2], N. Comparini and J. Amoore[3]

Laboratory for Neurobiology, MicroFab Technologies, Inc., Plano, TX, [1]University of California—Irvine, [2]Fogelson Neuroscience Center, Presbyterian Hospital, Dallas, TX and [3]deceased; formerly of OlfactoLabs, CA, USA

We are developing a 'clinical olfactometer' (patent pending) that can establish detection thresholds for 1–4 odorants (or trigeminal stimuli), using staircase methods, in ~10 min per test. The sub-briefcase size, 'hands off' convenience and modest projected cost make the system suitable for installation in small clinics or practitioner's offices. Odorant presentation is by direct injection of precisely metered amounts of odoriferous gas into a gentle airstream (filtered, warmed, humidified) that is directed at the subject's nose. PID monitoring of the stimuli shows that the onset and offset are brisk and that intensity (concentration) versus duration can be varied in reliable, predetermined ways. Preliminary data from human subjects using a modified staircase method and the odorant phenyl ethyl alcohol (PE) shows (i) test–retest repeatability within a few decismells; (ii) age-related decline in acuity (ages 10–85 years); and (iii) hypo-anosmia among diagnosed Alzheimer's patients amounting to >30 decismells threshold elevation. Further tests are in progress.

316. Perturbations of the peripheral olfactory system produce distinct patterns of odorant identification at similar performance levels

D.B Kurtz, T.L. White, J.W. Newlon, D.E. Hornung, P.R. Sheehe, P.F. Kent and P. Enko

Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210, USA

Errors in odorant identification can be the result of altered odorant access, altered quality coding and/or altered response bias. Whatever the cause, recent work suggests the possibility that different etiologies of olfactory loss may be reflected in different patterns of odorant identification and misidentification, even at equal levels of olfactory loss. In continuing this work, one normosmic control and two test groups (conductive loss—hyposmic subjects with colds; neural loss—subjects made hyposmic by application of 1 ml 1% cocaine in each nostril) were evaluated with the odorant confusion matrix (OCM) at two perceptual intensities. For each group, the lower intensity level was associated with slightly diminished odorant identification (control: 88%, 93%; colds: 45%, 53%; anesthesia: 53%, 66% average percent correct for the low and high perceptual intensities respectively). Dissimilarities between all pairs of OCMs were determined and submitted to multidimensional scaling to produce a three-dimensional people space. Dimension 1 of this space was primarily correlated to percent correct. The other two dimensions were, by exclusion, related to other aspects of the response pattern. The two test groups segregated from the control group on dimension 1. The test groups segregated from each other's dimensions, suggesting that each perturbation to the peripheral olfactory system produced distinct patterns of odorant identification and misidentification independent of percent correct. These data provide additional evidence that different disease processes result in distinct OCM response patterns.

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317. A clinical test of retronasal olfactory function

B.J. Cowart[1,2], B.P. Halpern[3] and E.K. Varga[1]

[1]Monell Chemical Senses Center, Philadelphia, PA 19104, [2]Department of Otolaryngology—Head & Neck Surgery, Thomas Jefferson University, Philadelphia, PA 19107 and [3]Department of Psychology and Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853, USA

Most patients reporting diminished perception of ambient odors also complain of a 'taste' loss that reflects a concomitant diminution in the perception of food flavors mediated via retronasal olfaction. Indeed, this is the primary complaint in many cases, and the degree of flavor loss reported is often greater than might be expected from orthonasal measures of sensitivity to odors. However, direct measurement of retronasal olfactory function is rare in clinical settings because of the lack of a simple way to present odors to the internal nares with minimal gustatory and oral trigeminal stimulation. We adapted Pierce and Halpern's technique (1996, Chem. Senses, 21: 529–543) to develop a clinical test of retronasal odor identification. Two retronasal presentations of each of five flavor extracts are intermixed with two orthonasal ones in order to compare identification performance in the two presentation modes. Among 30 healthy younger (18–50 years) adults with no chemosensory complaint, only one (3.3%) scored >2 (of 10) points lower on retronasal than on orthonasal presentations. In contrast, 11/40 comparably aged, non-anosmic patients complaining of smell loss (27.5%) scored at least 3 points lower (P = 0.009). Moreover, four of those patients exhibited normal orthonasal olfactory sensitivity on a standard battery of clinical tests, despite their complaints. These data are consistent with patient reports suggesting that retronasal olfactory experience may be more severely impacted than orthonasal experience by olfactory dysfunction, and indicate that retronasal testing may yield unique insights into the nature/degree of dysfunction in some patients.

318. Anesthesia of chorda tympani nerve and effect on oral pain

K. Tie, K. Fast, J. Kveton, Z. Cohen, V.B. Duffy[1], B. Green, J. Prutkin and L. Bartoshuk

Department of Surgery, Yale University School of Medicine, New Haven, CT 06520 and [1]School of Allied Health, University of Connecticut, Stores, CT, USA

Two nerves innervate the fungiform papillae on the anterior (mobile) tongue: the chorda tympani (CN VII, taste) and the trigeminal nerves (CN V, pain) with CN V accounting for 75% ofthe innervation. There is genetic variation in the density of fungiform papillae and the ability to taste the compound, 6-*n*-propylthiouracil (PROP). Supertasters have the greatest density of fungiform papillae, perceive the greatest intensity from oral irritants and the greatest bitterness from PROP. Previous research has shown inhibitory interactions within taste; CN VII inhibits CN IX (glossopharyngeal nerve; taste, posterior tongue), resulting in increased intensity of taste stimuli as well as taste phantoms at CN IX when CN VII is anesthetized or damaged. The data in the present study suggest inhibitory interactions between taste and oral pain. Seventeen subjects underwent unilateral anesthesia of CN VII (in separate experiments, CN VII was anesthetized on each side). Anesthesia of the chorda tympani resulted in contralateral intensification of burn with 10 ppm and 100 ppm capsaicin. The magnitudes of these effects depended on genetic taste status (i.e. density of fungiform papillae): supertasters experienced the greatest changes. We conclude from the contralateral intensification that CN VII normally inhibits CN V. These data suggest that supertasters may be at special risk for oral pain as the result of damage to CN VII because of the loss of the inhibition of CN VII on CN V.

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319. Burning mouth syndrome: damage to CN VII and pain phantoms in CN V

L.M. Bartoshuk[1], M. Grushka[2], V.B. Duffy[1,3], K. Fast[1], L. Lucchina[4], J. Prutkin[1] and D. Synder[5]

[1]Yale University School of Medicine, [2]Case Western Reserve University, [3]University of Connecticut, [4]Unilever Research U.S. and [5] State University of Florida, FL, USA

Burning mouth syndrome (BMS) consists of severe oral pain, commonly on the anterior tongue, that occurs in the absence of visible pathology. BMS afflicts predominantly postmenopausal women and is often accompanied by taste phantoms. We have argued previously that CN VII (taste, anterior tongue) normally inhibits CN IX (taste, posterior tongue) such that anesthesia of or damage to CN VII releases inhibition at CN IX, producing taste phantoms. We now suggest a similar interaction between CN VII and CN V that may explain BMS; if CN VII normally inhibits CN V, then damage to CN VII might release that inhibition leading topain phantoms. Since taste buds are surrounded by pain neurons, genetic variation in the density of fungiform papillae (the structures that house taste buds on the anterior tongue) affects the perception of both taste and oral pain. 'Supertasters' taste PROP (6-*n*-propylthiouracil) as intensely bitter and have the highest density of fungiform papillae. We hypothesized that BMS patients would show damage to CN VII and be supertasters. For BMS patients (postmenopausal = 17, premenopausal = 5, male = 9), the peak intensity of burning correlated with the number of fungiform papillae ($r = 0.8$, $P < 0.0001$). That is, the most intense burn was experienced by supertasters. BMS patients showed severe damage to CN VII, particularly for bitter (ANOVA, planned comparison between patients and 72 young adult controls for quinine on the anterior tongue, $P < 0.0000001$). These results suggest that in BMSdamage to CN VII releases inhibition at some point in the projection path of CN V leading to pain phantoms.

320. Radiation induced changes in taste sensitivity

M.R. Linschoten and B.W. Jafek

Rocky Mountain Taste and Smell Center, UCHSC Box B-205, 4200 East 9th Ave, Denver, CO 80262, USA

We carried out a longitudinal study of the changes in taste sensitivity in patients with squamous cell carcinoma of the head and neck receiving radiation treatment. Whole mouth detection thresholds were repeatedly measured before, during and after radiation treatment for six patients. Each patient was evaluated with two out of three tastants: sodium chloride, sucrose and citric acid. Thresholds were determined with a 2-AFC paradigm using an adaptive maximum-likelihood procedure. Thresholds for sucrose and sodium chloride were also measured in five control subjects.

The pattern of loss and recovery is different for different tastants: the rate of loss in this clinical sample appears to be faster for NaCl than for both sucrose and citric acid. Recovery starts as soon as radiation ends for all three tastants. In addition, there is a large variation across subjects in the way their sensitivities are affected by the radiation treatment. Each subject shows a characteristic individual pattern of change that is evident in both tastants. Control subjects demonstrated stable thresholds over the same period of time

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321. The effect of space flight and microgravity on the stimulation of the chemical senses

A. Olabi[1], J.B. Hunter[2], H.T. Lawless[1,3] and D.A. Levitsky[3,4]

Departments of [1]Food Science, [2]Agricultural and Biological Engineering, [3]Psychology and [4]Nutritional Sciences Cornell University, Ithaca, NY14853, USA

Available literature on effect of space flight and associated microgravity environment on stimulation of the chemical senses is reviewed. Soviet and American astronauts have reported attenuation of taste acuity and have complained about Shuttle foods being bland. Microgravity induces physiological changes, including altered body composition, altered fluid homeostasis and an upward shift of body fluids from the lower body to the head and thorax, resulting in facial edema which leads to nasal congestion, decreased airflow and attenuation of the olfactory component in the flavor of foods. Taste in orbit: highly individualized shifts in taste detection were found in one study (Skylab-4), but were not confirmed in a subsequent Canadian investigation (41-G). Soviet electrogustometry measures (Soyuz 30–31) revealed highly significant alterations in taste threshold (220%) and a similar, though smaller effect (7.24%) was obtained with a Polish cosmonaut.

Microgravity simulation: two studies found no effect of simulated microgravity on taste or smell sensitivity; three found

effects on taste perception. Possible mechanisms: include reduced atmospheric pressure rendering movement of volatile compounds from foods to olfactory cells more difficult; space sickness and consequent conditioned taste aversion; fluid shift in the body, resulting in altered saliva electrolytes and resulting taste changes; effect of psychological stress on taste perception; and effect of microgravity on process of chewing and swallowing. Adequacy of methods in studies mentioned above will be discussed. Microgravity provides a unique environment for chemosensory research which could enlighten us about the workings of the chemical senses.

322. Oral stimulation with dietary fat raises postprandial serum triacylglycerol levels in humans

R.D. Mattes and L. Bormann

Purdue University, Department of Foods and Nutrition, W. Lafayette, IN47906, USA

Accumulating evidence indicates there is a non-tactile orosensory component to dietary fat perception. One line of supportive evidence stems from findings of a single study that oral stimulation with dietary fat elicits a rise of plasma triacylglycerol (TG). This within-subject design study sought to replicate and extend this earlier work by comparing TG levels of individuals exposed to mashed potatoes containing butter, Olean (fat-based fat replacer), Simplesse (protein-based fat replacer), Passelli (carbohydrate-based fat replacer), no fat source or no oral stimulation. After an overnight fast, 22 healthy adults ingested 50 g of fat (in capsules to avoid oral fat stimulation) with 300 ml of water in 10 min. This was followed by mastication and expectoration of a given treatment sample every 3 min for 1 h and every 15 min for the second hour. Blood samples were drawn by venipuncture prior to capsule ingestion as well as 0, 2, 4, 6 and 8 h following it. Treatments were presented in random order. Sensory ratings of the samples were obtained following exposure to the first and last sample of each day. The area under the curve for TG was determined by the trapezoidal rule. Increments in TG for the fat replacers and controls were only 16–88% of values following exposure to butter. Sensory ratings for the samples could not account for the findings. These observations provide additional support for the view that there is a non-tactile orosensory detection system for dietary fat.

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323. The effect of sodium gluconate on the sweetness of selected intense sweeteners and their synergistic binary mixtures

S.A. Parke, G.G. Birch and R. Place

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

This experiment investigates the effect of adding sodium gluconate on the sweetness of 0.2% sodium cyclamate, 0.03% sodium saccharin, 0.04% acesulfame K, sodium saccharin:sodium cyclamate (0.005%:0.05%) and acesulfame K:sodium cyclamate (0.02%: 0.08%) in water. These concentrations were chosen as they are the closest in sweetness to a 5% aqueous sucrose solution. The proportions used in the binary mixtures are known to be synergistic. Solution properties such as the apparent specific volumes (ASV) provide information about the packing efficiency of the molecules in water, and isentropic compressibilities [$K_{(S)}$] show the extent towhich the hydration layer around the molecules can be compressed. These parameters therefore give an indication of the state of hydration of the molecules and their interaction with the surrounding water structure. Sensory analyses by the SMURF (Sensory Unit for Measuring Flux) show the temporal sweetness profiles of the molecules. Comparisons of the maximum intensities and persistence times of the sweetness of the solutions show any effect that the sodium gluconate may have on the solutions.

The sensory results (n = 14) show a significant increase in the maximum sweetness intensity observed for mixtures of sodium gluconate with sodium cyclamate (12.6%), sodium saccharin: sodium cyclamate (10%) and acesulfame K:sodium cyclamate (9.6%). The persistence of sweet taste and the overall gustatory response are also significantly increased when sodium gluconate is added to sodium cyclamate. Solution properties show a fall in ASV when sodium gluconate is present in the solutions. The salt therefore promotes solute–solvent interaction, which leads to better packing efficiency of the solutes in water. Sodium gluconate generally decreases isentropic compressibilities so that the solvation layer around the molecules seems to be more compact in the presence of the salt. The increase in sweetness observed can be explained in terms of the accession or positioning of the sapid molecule onto the receptor.

324. Human taste sensitivity to glucose is greater after repeated exposure to fructose rather than to glucose in lemonade

K.D. Sullivan, B. Adamiak and L.M. Kennedy

Neuroscience Laboratory, Biology Department, Clark University, Worcester, MA 01610, USA

Psychophysical response functions for fructose and glucose solutions are different and suggest separate taste mechanisms for these monosaccharides (L.M.Kennedy *et al.*, 1997, Food Chem., 60: 311). Repeated exposure to the sugar solutions increases sensitivity to the sugar for which subjects were previously hypogeusic, but not for the other sugar, and not in normogeusic subjects. An experience-inducible taste mechanism was hypothesized (S. Eylam and L.M. Kennedy, 1998, Olfaction and Taste XII, Ann. NY Acad. Sci., 855: 170; 1998, Chem. Senses, 23: 588). However, regression to the mean was not ruled out, and the specificity of the sugar in the process remained unclear. Here weinvestigated the induction hypothesis further, with attention tothese factors, by repeatedly exposing subjects to beverage sweetened with either fructose or glucose and then testing their sensitivities to aqueous glucose solutions. Forty-two subjects bathed their tongues with lemonade containing fructose 504 mM or glucose 721 mM for 10 s each day for 10 days at home, with instructions to not consider the taste of the beverage. Then in the laboratory, they tasted glucose 17.5, 27, 42, 65 and 100 mM, each paired with water, and indicated the sweeter of each pair. The sensitivities of the fructose-exposed group were greater than those of the glucose-exposed group. These results support the hypothesis of experience-induced increases in sugar sensitivity and show that the increased sensitivity is not specific to the inducing sugar.

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325. Thermal induction of taste

B.G. Green[1,2] and A. Cruz[1]

[1]The John B. Pierce Laboratory, 290 Congress Ave, New Haven, CT 06519 and [2]Department of Surgery (Otolaryngology), Yale School of Medicine, New Haven, CT, USA

The gustatory systems of mammals, including humans, contain afferent fibers that respond to both chemical and thermal stimulation. It has been assumed that the only perceptual consequence of these bimodal fibers is to cause taste sensitivity to vary with temperature. However, we recently discovered that thermal stimulation can induce sensations of taste. A 0.64 cm^2 Peltier thermode was used to heat or cool lingual sites while 20 Ss rated either thermal intensity or taste intensity using the Labeled Magnitude Scale (LMS). Rapidly warming the tongue from 20 to 30–40°C induced sweetness in 18 Ss; cooling the tongue from 35 to 10 or 15°C induced sourness in 15 Ss; and cooling to 5°C induced saltiness in 5 Ss. The best sites for sweetness and saltiness were near the tip of the tongue, whereas sourness was usually more salient on lateral sites. Although 'thermal tastes' were generally rated as less than moderate on the LMS, 0.5 M sucrose, 0.5 M NaCl and 0.1 M citric acid produced comparable taste sensations when they were applied to the same small areas of the tongue. We hypothesize that thermal taste has gone undiscovered because (1) the thermal conditions that produce it are infrequently encountered in daily life and (2) when stimulation affects larger areas of the mouth, input from specific thermoreceptors may nullify the thermal component of taste via cross-modal interactions in the CNS. Experiments are planned to test the latter hypothesis and to explore possible sources of spatial and individual differences.

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326. The nose as a route of administration for therapeutic drugs: nasal metabolism as a possible determinant of efficacy or toxicity

M.B. Genter, V.S. Deshpandee[1] and P.B. Desai[1]

Department of Environmental Health, College of Medicine, University of Cincinnati, Cincinnati, OH 45267-0056 and [1]Division of Pharmaceutical Sciences, College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267-0004, USA

The intranasal route is becoming increasingly common as a route of administration for therapeutic drugs. While nasal sprays are commonly used in treatment of respiratory tract conditions (e.g.seasonal allergies, asthma), newer drugs are being developed for treatment of systemic conditions such as osteoporosis and migraines. Our current work focuses on intranasally administered lidocaine, which is gaining popularity for use as an abortive treatment for migraine headaches. Given the rich complement of metabolic enzymes in the nasal respiratory and olfactory mucosa, we hypothesized that there would be extensive metabolism of lidocaine in these tissues. This may have important implications for drug efficacy, as well as potentially being a significant health issue, as 2,6-dimethylaniline, a metabolite of lidocaine, has been identified as a mutagen and a nasal carcinogen in rodents. To test this hypothesis, lidocaine was incubated with either nasal respiratory or olfactory mucosal microsomes; metabolites were analyzed by HPLC. Incubations with human liver and rat liver microsomes were performed for comparison. Our results demonstrate significant conversion of lidocaine to monoethylglycine xylidide, by both nasal and liver microsomes. The K_m (μM) and V_{max} (μM/min) respectively for the four tissues analyzed are as follows: human liver, 176, 0.2; rat liver, 304, 0.4; rat olfactory, 157, 0.5; and rat nasal respiratory, 608, 0.1. While further work is needed to determine whether nasal respiratory and olfactory tissues produce a mutagenic metabolite, it is clear that nasal tissues are metabolically active toward lidocaine. Nasal metabolism should be considered when predicting the pharmacokinetics and deposition of compounds administered via the nasal route.

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327. A new clinical test for the determination of olfactory recognition thresholds

G. Kobal, S. Roscher and F. Gisbert

Institute of Experimental and Clinical Pharmacology and Toxicology, University of Erlangen-Nuremberg, D-91054 Erlangen, Germany

In Central Europe an olfactory test system has recently been established using the felt-tip pen technique (Kobal *et al.*, 1996; Hummel *et al.*, 1997). Experiences in daily clinical routine revealed that the threshold subtest—a triple forced choice staircase test with 16 dilution steps of *n*-butanol—is very time consuming and stressful for patients and medical personal. Also, it is not well suited for the discrimination of hyposmic and anosmic patients.

In a new test two odorants, phenyl ethyl alcohol (PEA) and citronellal (C), were filled into two sets of 16 pens using dilution steps of 1:2, starting with 100% PEA and 50% C. For stimulation the 32 pens and six blanks were presented in a pseudo-randomized order. The number of correctly identified odorous pens (rose, citrus) was taken as the recognition threshold. For validation, both odorants were also tested in a triple forced choice stair case technique. Mean values of the last four reversals out of seven were taken as thresholds. One hundred subjects participated in the experiments on two separate days. There was a significant correlation between procedures ($r = 0.6$, $P = 0.001$). Test–retest reliability was higher in 'staircase' procedure ($r = 0.6$, $P = 0.001$) than in 'random' procedure ($r = 0.5$, $P = 0.001$). Olfactory performance decreased with increasing age. Preliminary data on 30 patients revealed that the random test discriminated between anosmics and hyposmics. Summarily, the new random test seems to be suitable for clinical settings, because it is simple, fast (10 min) and differentiates between patients with different degrees of olfactory deficits.

328. Etiologies of olfactory loss with similar performance levels produce unique patterns of correct and incorrect odorant identifications

T.L. White, D.B. Kurtz, P.R. Sheehe and J.W. Newlon

Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210, USA

Performance on most tests of odorant identification is computed as a function of the number of smells that have been correctly named without any regard for the pattern of responses (both correct and incorrect). However, previous work with the Odorant Confusion Matrix (OCM) suggests that patterns of response in the analysis of identification performance can provide information regarding the perception of odorants in particular patient groups.

It is likely that similar insights on odorant perception may be obtained through analysis of the response patterns of almost anyforced-choice odorant identification test. The present study examined this possibility through the analysis of UPSITs (University of Pennsylvania Smell Identification Test) from over 200 people tested at either The Smell and Taste Disorders Clinic or the Clinical Olfactory Research Center. Dissimilarities between any two UPSIT patterns were quantified with an information transmitted-based measure, then fit to a four-dimensional space by multidimensional scaling (SYSTAT). The resultant MDS space clustered people with colds both near to each other and isolated from people whose olfactory performance had been reduced to a similar level by the application of 1 ml of 1% topical cocaine. This type of separation provides further evidence that patterns of odorant identification and mis-identification on forced-choice odorant identification tests such as the OCM and the UPSIT reflect changes in odorant quality coding that are unique to different etiologies of olfactory loss.

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329. Long term follow-up on patients with surgically treated phantosmia

T. Loehrl, J. Schwob and D.A. Leopold

Johns Hopkins School of Medicine and SUNY Health Science Center, Syracuse, NY 13210, USA

Since 1988, nine patients with persistent unremitting phantosmia have had excision of olfactory mucosa from the involved nostril(s), and have been followed from 1 to 10 years. Seven of these patients had unlateral symptoms, and two had or developed bilateral disease. Of the 11 nostrils that received surgery, three needed to be done twice since the phantosmia symptoms recurred after the first surgery. The excised olfactory mucosa generally showed a decreased number of neurons and a greater ratio of immature neurons. In addition, disordered growth of axons in olfactory nerve fascicles was noted with some intraepithelial neuromas. Pre-operatively, the olfactory ability was in the hyposmic range in ~2/3 of the nostrils and normal in ~1/3. Post-operatively the olfactory was absent in all but two of the nostrils (where it was in the hyposmia range of 20 and 24 on the UPSIT). In those nostrils where there was an initially absent sense of smell, the olfactory ability improved to the 20–30 range on the UPSIT in six of the nostrils. Once the phantosmia has resolved, it has not recurred in the same nostril. In one patient, however, it did begin in the contralateral nostril and required surgery. This nasal surgical therapy remains the most effective treatment for these unhappy patients.

330. Olfactory recognition in Sjogren's syndrome: a twelve year longitudinal follow-up

J.M. Weiffenbach and M.T. Brennan

National Institute of Dental and Craniofacial Research, Bethesda, MD, USA

Sjogren's syndrome (SS) is an autoimmune exocrinopathy characterized by destruction of salivary and lacrimal glands. It preferentially afflicts peri- and postmenopausal women. Patients commonly complain of dry mouth and may report changes in their experience of foods and declines in their sense smell. Between 1984 and 1988, we assessed the olfactory performance of 60 SS patients (35–72 years old) with the University of Pennsylvania Smell Identification Test (SIT). Although patients' average SIT score was significantly reduced relative to 60 unaffected women (33–73 years old), most of the patients were normosmic (21), a smaller number (eight) displayed moderate deficits while only one demonstrated anosmia. Recently we obtained current SIT scores from 16 of these patients. Their initial scores resembled the initial performance of the total group. Most were normosmic (12), some displayed moderate deficits (one mild, one moderate and one severe microsmic) and one was anosmic. On reassessment after 10.6–15.0 years (mean = 12.9, SD = 1.19), the patients were 51.6–86.3 years of age. Some (six) were still normosmic, but more were impaired to various degrees (four mildly, two moderately and three severely microsmic) and one was anosmic. One had gained three points, four obtained the same score, eight lost from 1 to 4 points and one each lost 8, 14 and 24 points. Interestingly, the only patient whose score improved had the lowest initial score (18) and on re-test was categorized as severely microsmic rather than anosmic. The patient who was anosmic on re-test demonstrated a dramatic decrease from an initial score of 36 (normosmic) to 12 (anosmic). Thus, even long-standing SS is not inconsistent with normosmia. Across more than a decade, some patients, but not all, show some decline in olfaction, and, rarely, the decline may be dramatic.

331. Testing olfactory performance in endocrinological patients

J.E. Steiner and G. Bar-Dayan

Department of Oral Biology, The Hebrew University Hadassah School of Dental Medicine, POB 12272, Jerusalem 91120, Israel

Olfactory functions were assessed in 112 endocrinological patients using a multidisciplinary procedure. Data obtained included: (1) detailed history, focusing on the utilization of olfactory cues in everyday life by patients, as well as on olfactory functions of parents and siblings; (2) psychophysical testing of olfactory functions using 10 common odorants and two blank samples. Odor detection, identification and hedonic ratings were recorded; and (3) direct observation (n = 82) or video-recording (n = 30) of stimulus-triggered expressive facial reactions. Taped behaviors were rated by three independent evaluators in a blind setting. Based on the three sets of data, examinees were classified as severely hyposmic or anosmic, moderately hyposmic or normosmic. Endocrinological profiles obtained from medical records were also classified, rated and correlated with ratings of olfactory performance. All 19 patients with clinical signs typical of Kallmann's syndrome were found to be severely hyposmic or anosmic. Among 41 cases referred with possible other types of hypogonadotropic hypogonadism, one was anosmic, eight hyposmics and 32 normosmics. Among 29 adolescents with delayed onset of puberty, four were hyposmic and 25 normosmic. Testing of olfactory functions can therefore be seen as an efficient, sensitive diagnostic tool, reliably differentiating between Kallmann's syndrome and other endocrine fertility dysfunctions not involving olfactory disorders. Findings also indicate that correct 'reading' of odor induced facial displays is independent of learning of training, since the hedonic ratings ascribed by an expert and a naive evaluator show high correlation.

332. Traumatic brain injury assessed with olfactory event-related brain potentials

M.W. Geisler[1,2], C.R. Schlotfeldt[2], C.B. Middleton[2], M.F. Dulay[2] and C. Murphy[1,2]

[1]University of California Medical Center, San Diego, CA and [2]San Diego State University, Department of Psychology, San Diego, CA, USA

Olfactory event-related potentials (OERPs) were assessed in relation to standard clinical evaluation techniques to help develop an objective, quantitative assessment of sensory and cognitive olfactory loss following traumatic brain injury (TBI). Subjects included 25 TBI patients and 25 age/gender-matched healthy controls. Olfactory functioning was assessed with the following measures. Odor thresholds: *n*-butyl alcohol and amyl acetate; odorsensitivity: Alcohol Sniff Test (AST), odor identification: University of Pennsylvania Smell Identification Test (UPSIT), San Diego Odor Identification Test. TBI patients were divided into three groups: 12 anosmics (loss of smell), six hyposmics (reduced smell) and seven normosmics (normal smell). Cognitive ability was assessed using the Trail Making Test (A and B). OERPs were recorded monopolarly from midline electrode sites using an amyl acetate stimulus with a 60 s inter-stimulus interval; subjects estimated the magnitude of each odor stimulus. Anosmic TBI patients were also tested with ERPs using ammonia to ensure trigeminal nerve function. Amyl acetate OERPs demonstrated that the sensory N1 and P2 amplitudes and the cognitive P3 amplitudes were absent in the anosmic TBI patients and greatly reduced in the hyposmic and normosmic TBI patients compared with healthy controls. The trigeminal ERPs from the anosmic TBI patients were within normal limits indicating that the primary olfactory deficits were objectively measured with OERPs. The present study lends support to the utility of olfactory event-related potentials as anobjective tool for measuring sensory and cognitive loss after traumatic brain injury.

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333. Relationship between structural MRI volumes and olfactory function in non-demented elderly persons

C. Murphy[1,3], C. Fennema-Notestine[2,3], A. Wiser[3] and T.L.Jernigan[2,3]

[1]San Diego State University, San Diego, CA, [2]VA Medical Center, La Jolla, CA and [3]UCSD School of Medicine, San Diego, CA, USA

Olfactory function shows significant impairment in old age, although there appears to be significant variability of function within the elderly population. The current study investigated whether performance on olfactory tasks (odor threshold and odor identification) was related to volumetric measures of areas involved in olfactory information processing, obtained through structural magnetic resonance imaging (MRI) in normal older persons. All subjects were screened for dementia and genetic risk for Alzheimer's disease due to the apolipoprotein e4 allele, at the UCSD Alzheimer's Disease Research Center. Average odor threshold was significantly correlated with amygdala volume in normal elderly persons: those with better threshold performance showed larger amygdala volumes (+0.63). Taste threshold did not show a significant relationship with amygdala volume (+0.07), suggesting that the relationship is unique to the olfactory system and not to threshold measurement *per se*. Amygdala volume was also correlated with odor identification performance (+0.73). The results motivate further investigation of quantitative MRI measures and psychophysical performance in the elderly in order to achieve a better understanding of the neuropathological correlates of olfactory impairment with age.

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334. The aesthetasc–olfactory lobe pathway of spiny lobsters is not necessary for odor-activated searching behavior, odor-associative learning and discrimination of complex odors

P. Steullet, T. Flavus, D. Radman, G. Hamidani, M. Zhou, O. Dudar, R. Hill and C.D. Derby

Department of Biology, Georgia State University, Atlanta, GA 30303, USA

Chemoreceptor neurons that mediate food search and odor discrimination by decapod crustaceans are located in the paired antennules, each of which bifurcates into lateral and medial filaments. Chemosensory neurons densely innervated aesthetasc sensilla, which are located on the lateral filament and project into the glomerular neuropile of the olfactory lobes. Chemosensory neurons associated with non-aesthetasc sensilla are located on both filaments and project together with mechanosensory neurons into the non-glomerular neuropile of the lateral antennular neuropiles. The present study investigates, through ablation of specific sensillar types, the functional role of these two pathways in several odor-driven behaviors of the spiny lobster *Panulirus argus*. Both aesthetasc and non-aesthetasc chemoreceptor neurons are involved in evoking behavioral responses to odors presented in low-flow conditions, and the response magnitude decreases as more antennular chemosensory neurons are removed. However, aesthetasc chemoreceptor neurons are not necessary for odor detection, initiation of search behavior, odor-associative learning, or discrimination between of complex odors. Our results nevertheless indicate a trend that aesthetasc-less lobsters have more difficulty in the discrimination of the most similar complex odors.We are currently investigating the role of aesthetascs in discrimination of different blend ratios of one binary mixture.

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335. Effect of complex odor cues and shelter availability on crayfish foraging behavior

A.M. Tomba, T.A. Keller and P.A. Moore

Laboratory for Sensory Ecology, Bowling Green State University, Bowling Green, OH 43402, USA

Chemical cues play an important role in foraging in crustaceans. Previous studies demonstrate they use food odor to successfully orient to a food source. In nature organisms must forage in the

presence of predators and in structurally complex habitats, yet most studies to date have performed experiments with only a single food source or habitat type. In this study we examined how conflicting chemical cues (e.g. predation event and food) and shelter availability affect the search behavior of *Orconectes virilis*. In order to simulate a predation event, we used crushed chela as an odor source. Clay pots were used to provide shelters. Four treatments were used: shelter versus no shelter; food and crushed conspecific versus food. Crushed conspecific odor significantly increased the time crayfish required to locate the food source. Crayfish also responded to crushed conspecific odor by spending significantly more time in shelters and chose shelters along the sides of the flume, than crayfish in the food only treatment. We also used electrochemical techniques to characterize the spatial and temporal nature of chemical signals and found that the crushed conspecific odor and food odor was absent from the sides of the flumes, where the crayfish spent the most time in shelters. Thus, crayfish are capable of distinguishing the odor of a predation event and orienting around the spatial distribution of the signal. Complex odors can be used to make important ecological decisions regarding search strategies and habitat use.

336. Occupation of chemoreceptors and presence of intracellular cyclic AMP are associated with export of photosynthetically generated carbohydrate by algae living in symbiotic relationships with cnidarian hosts

H. Trapido-Rosenthal[1], J. Austin[1,2], D. Ferrier[1,3] and S. Zielke[1,4]

[1]*Bermuda Biological Station for Research, Ferry Reach GE-01, Bermuda,* [2]*Florida State University, Tallahassee, FL 32306,* [3]*Hood College, Frederick, MD 21701, USA and* [4]*Technical University of Mannheim, D-68305 Mannheim, Germany*

It has been demonstrated by Wang and Douglas (1997, Plant Physiol., 114: 631) that the sulfonic amino acid taurine can stimulate the export of photosynthetically generated carbohydrate from the dinoflagellate algae that live in symbiosis with cnidarians. We have shown that these algae possess cell-surface chemoreceptors that specifically bind taurine (Trapido-Rosenthal and Vallejo, 1998, Chem. Senses, 23: 637). We show here that the phosphonic acid 2-aminoethylphosphonate (AEP), a molecule similar in shape and identical in molecular weight to taurine, andwhich is present in high concentrations in cnidarians both infree form and as a component of membrane phospholipid, canalso bring about the efflux of photosynthetically generated carbohydrate. AEP also competes for the algal binding of radiolabeled taurine. Membrane-permeable analogs of cyclic AMP (cAMP) also induce carbohydrate efflux, whereas externally applied cAMP itself does not. Taken together, these results suggest that efflux of carbohydrate that is stimulated by taurine or AEP may result from the receptor-mediated generation of intracellular cAMP.

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337. Electrophysiological recordings demonstrate that the antennules of the barnacle cyprid larva bear functional chemo- and mechanoreceptors

P.J.H. Harrison[1,2] and D.C. Sandeman[1]

[1]*School Biological Science, University of New South Wales, Sydney, Australia 2052 and* [2]*Department of Biology, Georgia State University, Atlanta, GA 30303, USA*

The life cycle of the barnacle includes seven free-swimming larval stages. The cyprid (final larval stage) must select a site for settlement and metamorphosis. Cyprids are sensitive to various environmental cues and typically settle near conspecifics. This behavior is thought to be mediated by the presence of soluble pheromones, in addition to physical conditions such as flow rate and substrate topography. It has been suggested that sensilla located distally on the antennule function as chemo- and mechanoreceptors. We have previously shown that many of these sensilla are innervated, and that bi-polar neurons located at the base of the antennule are candidates for the receptor cell soma. The purpose of this study was to determine whether the cyprid antennule is sensitive to chemical and mechanical stimuli. Extra-cellular suction electrodes were used to record from the antennular nerve (AnN) and stimuli were focused on distal sensilla. The pattern of electrical activity in the AnN was modulated by both chemical and mechanical stimuli. Effective chemical stimuli included 'barnacle-conditioned seawater', a soluble extract of barnacle shell and 'algal-conditioned seawater'. Both tonic and phasic discharge patterns were recorded in response to mechanical stimuli. The ability to modulate electrical activity in the AnN by extrinsic stimulation demonstrates that the antennule is sensitive to both chemical and mechanical stimuli. This supports an idea that chemo- and mechanoreceptors on the cyprid antennule mediate settlement through the detection of odors, including pheromones, and the discrimination of physical features.

338. The nose of the lobster may also function as a hydrodynamic receptor organ

M. Weaver, C. Guenther and J. Atema

Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543, USA

The lateral antennular flagellum of many crustacea functions as their major olfactory organ. Characteristic aesthetasc sensilla occupy the distal portion of the organ and provide a major chemosensory input. There are several other setal types, mostly of unknown function.

In *Homarus americanus* this flagellum is critical for spatial orientation in odor plumes. Fluid dynamics predicts that important directional information can be provided both by odor dispersal and eddy shedding: the downstream dispersal of flavored eddies should provide the most salient gradient information. Identification of flavored eddies should favor coincident chemo- and hydrodynamic receptors. Specific chemosensory lesion severely impacts the lobster's ability to locate distant odor sources, but does not abolish it. Hydrodynamic receptors (and/or chemoreceptors on non-lesioned organs) may provide the remaining, greatly degraded directional information. For best coincidence

these hydrodynamic receptors should be located on the lateral flagellum.

We described the external morphology of this flagellum, particularly its flexibility and the different setal types with their potential for flow detection. Then we tested the flagellar mechanical response to imposed fluid oscillations. We found a resonance frequency centered around 10 Hz and amplitude maxima shifting along the length of the flagellum with higher frequencies toward the base. This is consistent with the mechanical 'pivot-whip' structure of this organ. It also provides the theoretical foundation for hydrodynamic frequency discrimination in a range consistent with the flicker-fusion frequency of antennular chemoreceptors. The results are consistent with a hydrodynamic receptor function for the lateral flagellum and provide useful direction for further physiological and behavioral studies.

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339. Chemosensory mediation of antennular grooming in the florida spiny lobster requires olfactory input

J. Wroblewska, P.C. Daniel, S. Whalley and M. Fischetti

Department of Biology, Hofstra University, Hempstead, NY 11549-1140, USA

Antennular grooming behavior (AGB) is universally observed in crustaceans. It consists of two repetitive components; antennule wiping followed by autogrooming of the third maxillipeds. In the Florida spiny lobster, *Panulirus argus*, AGB can be induced by only a few chemical stimuli found in prey extracts; very strong responses are observed towards L glutamate (Glu) (Barbato and Daniel, 1997, Biol. Bull., 192: 107). To investigate the source(s) of chemosensory input to AGB, we ablated regions of the two organs involved in AGB and known to contain chemosensory sensilla, the antennules and the third maxillipeds. Six to 12 lobsters were tested with Glu before ablation, ~3 h post-ablation and at least 24 h post-ablation. Distilled water ablation of the distal halves of the lateral filaments of the antennules eliminated AGB response to Glu. Recovery was complete in a little over 24 h. Similar results were obtained when olfactory sensilla (aesthetascs) and non-olfactory setae on the ventral side of the lateral filaments were excised. In contrast, excision of only nonolfactory setae on the ventral side of the lateral filaments did not attenuate chemosensory induction of AGB. Similarly, distilled water ablation of the medial filaments of the antennules and of the third maxillipeds had no effect. These studies strongly indicate that chemosensory induction of AGB occurs exclusively through olfactory receptors tuned narrowly to Glu. Because the olfactory lobe receives input for olfactory receptor neurons tuned to many different chemicals, we suggest that this region acts as a narrowband filter for input to the AGB motor program.

340. Chemosensory mediation of antennular grooming behavior in decapod crustaceans

P. Daniel[1], M. Shineman[1,2] and M. Fischetti[1]

[1]Department of Biology, Hofstra University, Hempstead, NY 11549-1140 and [2]Huntington Senior High School, Huntington, NY, USA

Antennular grooming behavior (AGB) is universally observed in crustaceans. It consists of two repetitive components; antennule wiping followed by autogrooming of the third maxillipeds. In the Florida spiny lobster, *Panulirus argus*, AGB can be induced by onlya few chemical stimuli found in prey extracts; very strong responses are observed towards L glutamate (Glu) and very weak responses towards higher concentrations of glycine (Gly) and adenosine 5′-monophosphate (AMP) (Barbato and Daniel, 1997, Biol. Bull., 192: 107). Many chemicals induce other behaviors incrustaceans, such as foraging. We have begun to examine the possibility of chemosensory mediation in other decapod crustaceans. The Florida spotted lobster, *Panulirus guttatus*, and the American lobster, *Homarus americanus*, were tested with 14 putative chemical stimuli presented at 0.5 mM. As in *P. argus*, onlyGlu elicited strong significant AGB. We propose that chemosensory activation of AGB by Glu may be a fundamental behavior in marine decapod crustaceans.

341. Sensory information used in female assessment of males in *Procambarus clarkii*

L.M. Shauver and P.A. Moore

Laboratory for Sensory Ecology, Bowling Green State University, Bowling Green, OH 43403, USA

Females preferentially associate with males based on their assessment of male quality. Quality is usually assessed through cues that may be perceived through any one or several of the female's sensory systems. Preferable males are identified using visual and chemical cues in related Crustacean species. The use by *Procambarus clarkii* of visual and chemical cues in guiding female preference among males was investigated through two experiments. The first experiment tested the importance of vision and visual cues by altering the visual capabilities of the female. The second experiment tested the influence of male urine cues on female behavior by altering the output of male urine. In both studies, the female was given a choice between two males differing in size and social status identified by fight trials. Three female behaviors—the time spent near each male, the number of chela touches to each male and the total time of chela touches—were used to gauge the females' interest in the males. In the first experiment, a significant difference in the number of chela touches by sighted and blind females was found, indicating the role of vision. Male status also effected female behavior by increasing the time spent near subordinate males. In experiment two, the presence or absence of male urine odors did not effect female choice in any behavioral measure and an overall effect of male status was not seen. The failure of male odors to significantly effected female behavior further supports vision as the primary sensory system guiding female preference.

342. Urine pheromones in the lobster, *Homarus americanus*: both males and females recognize individuals and only use the lateral antennule for this task

J. Atema, T. Breithaupt, A. LeVay, J. Morrison, M. Mallidis and M.Edattukaran

Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543, USA

Both male and female lobsters establish and maintain stable dominance hierarchies. Male lobsters have demonstrated ability

torecognize individuals: they use their antennules to detect and learn urine-based olfactory cues during aggressive encounters. The probability of urine release increases during social interactions and with the level of aggression. Pheromones have not been identified, but the nephropore gland is a good candidate for the production of urine-carried pheromones.

We now report that female lobsters have capabilities similar tomales for the recognition of dominant individuals. They, too, remembered the smell of the animal that defeated them for up to aweek. In the intervening time they did not encounter that individual, but a group of different individuals. Their fighting ability was not impaired, because they fought and could defeat other individuals.

We also identified the specific organ responsible for this individual recognition: after chemosensory lesion lesion of the lateral, but not the medial flagellum losers of a fight re-engaged the dominant of a previous fight as if it were a stranger.

Finally, we began analyzing the urine of males and females before and during a fight using chronic catheters to collect urine. Our initial approach was to look for protein bands with silver stain. Preliminary data showed dozens of different bands in patterns specific for each individual. The protein signal was possibly stronger in urine collected during fights but remained qualitatively similar for each individual. Further work is needed to identify the source, function and structure of these proteins.

343. Pheromonal cues in the goldfish are perceived within the context of the body odor within which they occur

R.L. Kihslinger and P.W. Sorensen

University of Minnesota, St Paul, MN 55108, USA

Pheromones are organismal odors that stimulate stereotypic and innate responses in conspecifics. 15-Keto-prostaglandin F2a (15K-PGF) is a hormonal metabolite that is released to the water by sexually active female goldfish, where it is detected by the male olfactory system and triggers male behavior. 15K-PGF has always been tested on its own. The question of whether and how this cue might function as a species-specific signal has been raised because it is produced by many fish species. One possibility is that 15K-PGF is perceived within the context of the body odor of the fish releasing it. In this study we tested this possibility by exposing male goldfish to 15K-PGF in a background containing the odor of other species of fish. Goldfish behavior was thus observed during three sequential test periods: a pre-test period (blank added); a period when body odor alone was added; and a period when 15K-PGF was added together with fish body odor. The odor of five species of immature fish was used. In no case did body odor influence male behavior. Additionally, and as expected, when 15K-PGF was added together with the odor of either goldfish or carp (which it hybridizes with), dramatic increases in sexual behavior were noted. However, when male goldfish were exposed to 15K-PGF together with the odor of distantly related species, they failed to respond. These results demonstrate that natural pheromone signals in fish are likely specific, multiple-component mixtures which require complex neural mechanisms for their discrimination.

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344. Miniature solid-state pheromone-jet for picoliter dispensing

Ch.J. Frederickson, N. Comparini, A. Romero, E. Wright, I. Frederickson, M. Sinks, A. Knutson[1] and C.J. Frederickson

MicroFab Technologies, Inc., Plano TX 75080 and [1]Texas A&M University, Richardson Extension, Richardson, TX, USA

We are developing a small, solid-state, pager-sized 'PheroJet' (Patent Pending) that dispenses picoliter volumes of pheromone for insect research or insect control. Pheromones are stored in an anaerobic fluid formulation in a microliter reservoir. Dispensing iscontrolled by remote radio-frequency signals (e.g. cellular, satellite) or by a local button press. Dispense commands result in a predetermined number of picoliter-sized droplets being dispensed onto a 'wick' for evaporation into the ambient air or wind tunnelairstream. PID measurement data show that the temporal 'envelope' of pheromone so dispensed can be controlled with great precision.

Data from behavioral testing of microdispensed pheromones will be presented. Testing so far has included (i) attracting house flys with fly-lure; (ii) attracting male coddling moths with codelmone (Bedoukian); and (iii) attracting male boll weevils witha formulation of grandlure (Bedoukian). Data from both wind-tunnel and field (crop and orchard) tests will be presented. Total number of pest insects attracted and trapped by PheroJetted formulations has matched or exceeded the numbers trapped with alternative commercial pheromone traps.

345. Cloning of sodefrin-like peptide cDNA of the sword-tailed newt

S. Kikuyama, T. Iwata and F. Toyoda[1]

Department of Biology, School of Education, Waseda University, Tokyo and [1]Department of Physiology, Nara Medical University, Kashihara, Japan

Sodefrin is a female-attracting decapeptide pheromone isolated from the abdominal gland of the red-bellied newt (*Cynops pyrrhogaster*) (S. Kikuyama *et al.*, 1995, Science, 267: 1643). However, sodefrin did not attract females of a congeneric species *Cynops ensicauda* (sword-tailed newt), suggesting that its action isspecies-specific and that the sword-tailed newt has its own sodefrin-like pheromone. Cloning of cDNA encoding a sodefrin-like molecule from a cDNA library constructed from *C. ensicauda* abdominal gland mRNAs was attempted, using sodefrin cDNA as a probe. A positive clone, 1.4 kbp in length, encoded a precursor protein of 192 amino acid residues, including a predicted signal peptide consisting of a series of hydrophobic residues and a sodefrin-like peptide sequence with the substitutions Leu^3 and Gln^8. The synthetic [Leu^3,Gln^8]-sodefrin attracted *C. ensicauda* females concentration-dependently, but not *C. pyrrhogaster* females. The results raise the possibility that the *C. ensicauda* abdominal gland synthesizes a sodefrin-like pheromone. The sodefrin-like peptide, as does sodefrin, attracts only conspecific females. Minute amino acid sequence differences among species may be important for ensuring reproductive isolation.

346. Effects of methimazole on a complex odor discrimination task

L. Hastings and R.L. Doty

Smell and Taste Center, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Whether methimazole, an antithyroid drug, is an olfactotoxin isamatter of debate. In some laboratories, methimazole has beenshown to produce both functional and morphological damage in the olfactory system (Genter *et al.*, 1996, Fundam. Appl. Toxicol., 29: 71; Hastings *et al.*, 1997, Chem. Senses, 22: 698), while in other laboratories such effects have not been observed (Xu and Slotnick, 1997, Chem. Senses, 22: 826). To further investigate this question, rats were trained on a discrete trial, go/no-go complex odor discrimination task using operant techniques and a flow dilution olfactometer. The complex odor discrimination involved training the rats initially on a S+ (ethyl acetate)/S– (air) task and then on a S+ (ethyl acetate)/multiple S– (air, phenethyl alcohol, isoamyl acetate and eugenol) task. The rationale was that depending on severity of insult to the olfactory system, performance on the discrimination task would vary with complexity of the discrimination task. Rats were trained to a criterion of 85% correct responses on the complex discrimination task and then injected with 300 mg/kg i.p. methimazole ($n = 4$) or vehicle control, DMSO ($n = 4$). Preliminary results of this ongoing study suggest that methimazole is an olfactotoxin that alters performance on an olfactory discrimination task, but that complexity of the task is not a factor with regards to recovery of function.

347. Effects of extraneous odors on canine olfactory detection

M. Jones[1,2], T. Boussom[1,2], E. Paletz[1,2], J. Langston[1,2], P.Waggoner[1] and M. Williams[1]

[1]*Department of Psychology and* [2]*Institute for Biological Detection Systems, Auburn University, Auburn University, AL 36849, USA*

Detection dogs are often required to detect target substances under challenging conditions. One of these challenges is to detect contraband in the presence of extraneous odors, whether they are part of the ambient environment or placed there for the purpose of evading detection. This poster presents the results of two studies evaluating the ability of dogs to detect target substances in the presence of varying concentrations of extraneous odors. The studies were conducted under behavioral laboratory conditions, providing good control over vapor sources and a clear basis for evaluation of detection responses. Dogs were trained to sample an airstream consisting of the extraneous odor only or the extraneous odor plus the target odor and then press the appropriate lever toearn food. The results are described in terms of the ability ofdogs to detect target odors in the presence of a wide range of concentrations of the extraneous odors.

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348. Chemistry of the urine and the vaginal secretions of golden hamsters (*Mesocricetus auratus*)

W. Ma, D. Wiesler and M.V. Novotny

Department of Chemistry, Indiana University, Bloomington, IN 47405, USA

The golden hamster has long been used as a model for research in chemical communication. Accumulated evidence from biological and behavioral studies suggests that this species relies heavily on pheromones for chemical communication. However, the chemical nature of the signals has not been fully explored or understood. Inan attempt to isolate and identify active compounds in the excretions or secretions used for pheromonal communication, weinvestigated the volatiles of urine and the vaginal secretions ofgolden hamsters using gas chromatography and mass spectrometry. Forty-six major urinary compounds were tentatively identified and most of them were found to be ketones. Quantitative and qualitative analyses indicated that there were no significant differences in the volatile profiles between males, castrated males or females. In the vaginal secretion, ~41 major peaks were detected. The profile was dominated by sixteen sulfur-containing compounds. The so-called aphrodisin was isolated from the vaginal discharge by size-exclusion chromatography. After lyophilization, this 'purified' protein was screened for possible volatile ligands. We found that nearly all identified components of the vaginal secretions were bound to this 'smelly' protein. It is thus proposed that the aphrodisin may facilitate the transportation of low molecular weight signals rather than being a pheromone itself.

This research was supported by a grant from the National Institute of Deafness and Communicative Disorders (DC02418).

349. Sex and systematic genetic differences in sensitivity to androstenone in inbred mice

V.V. Voznessenskaya[1,2] and C.J. Wysocki[1,3]

[1]*Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA,* [2]*A.N. Severtzov Institute of Ecology & Evolution, Russian Academy of Sciences, Moscow 117071, Russia and* [3]*Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA*

CBA and NZB mice were examined for sexual dimorphism in sensitivity to androstenone (AND). Two tests were used: a buried cookie test and a quinine-aversion test. In the buried cookie test, CBA males showed an average threshold for AND of $0.915 \pm 0.16 \times 10^{-5}\%$ ($n = 12$); the females' threshold was $5.37 \pm 0.49 \times 10^{-5}\%$ ($n = 10$; $P < 0.001$). In the aversion test the threshold for CBA females was $20.5 \pm 3.39 \times 10^{-5}\%$ ($n = 10$); for males it was $2.68 \pm 0.399 \times 10^{-5}\%$ ($n = 10$; $P < 0.001$). AND-insensitive NZB mice showed a similar dimorphism. In the cookie test the average threshold to AND for males was $3.25 \pm 0.383 \times 10^{-2}\%$ ($n = 10$); for females it was $4.75 \pm 0.69 \times 10^{-2}\%$ ($n = 10$; $P < 0.05$). Previous research showed no sex difference in sensitivity to amyl acetate for either strain of mice. Hence, the differences in sensitivity to AND cannot be explained by differences in general olfactory sensitivity. We also investigated sensitivity to AND in F_1 hybrids from matings between CBA females and NZB males and vice versa ($n = 19$ and $n = 21$ respectively) and in the F_2 hybrids ($n = 110$) using the buried cookie test. Data indicate probable involvement of both

X and Y chromosomes. We assume the existence of a gene that suppresses sensitivity to AND on the X chromosome of NZB mice and the existence of a gene promoting sensitivity to AND on the Y chromosome of CBA males.

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350. Fetal MHC odortypes influence behavior toward female mice

K. Yamazaki, M. Curran and G.K. Beauchamp

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA

The major histocompatibility complex (MHC) imparts to each mouse, rat and perhaps human an individual odor called 'odortype', which reflects its MHC genotype. Perception of odortypes in mice affects mate selection, maternal-infant interaction, and embryonic implantation. Using our standard Y maze training paradigm, we have found (Beauchamp *et al.*, 1994, Immunogenetics, 39: 109–113) that odortypes specified by paternal (non-maternal) MHC haplotypes become apparent in maternal urine as early as 9–12 days of gestation. These observations raised the possibility that odortypes expressed *in utero* can be sensed even before birth and may serve in familial identification and communication. As a first test of this surmise, we have investigated stud male and pregnant female spontaneous preferences for genetically identical females carrying fetuses of differing MHC type. Indeed, under some circumstances, test animals show differential preferences. Thus this study provides the first example we are aware of that fetal odortypes are sufficiently salient in maternal secretions and excretions to modulate the behavior of other animals.

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351. Olfactory conditioning and differential immediate early gene expression in mice

C.A. Forestell, H.M. Schellinck, V.M. LoLordo, R.E. Brown and M.Wilkinson[1]

Dalhousie University, Halifax and [1]IWK Grace Health Center, Halifax, NS, Canada

The present experiment investigated which parts of the adult mouse brain mediate acquisition of conditioned responses to an odor paired with a caloric reinforcer. Food-restricted mice were exposed to a differential conditioning procedure in which experimental animals received an odor (CS+) paired with sucrose pellets in a dish covered with bedding and another odor (CS–) presented alone in a dish with bedding. Control animals were either exposed to the odors separately in a dish containing bedding and no reinforcement, or to no odor. In test, experimental animals spent more time digging in the dish containing CS+ than CS– relative to control animals. Fos-like immunoreactivity was assessed in brain structures such as the piriform cortex, the entorhinal cortex and the hippocampus.

352. Olfactory learning-set formation in old Wistar rats

S. Krämer and R. Apfelbach

Department of Zoology, University of Tübingen, D-72076 Tübingen, Germany

Learning to learn occurs when training on a series of discriminations of the same general class results in progressive improvement in solving each subsequent problem. Old animals are often thought of being incapable of performing such complex learning tasks. We therefore investigated whether there are age-dependent abilities in forming olfactory learning-sets. An olfactometer was used (for details see B.M. Slotnick and F.W. Schoonover, 1984, Chem. Senses, 9: 325). After the 28-month-old animals (n = 5) and 3-month-old controls (n = 8) had learned to discriminate between two odors, we presented four different pairs of unknown odorants at a concentration of 0.2% v/v. Each pair was presented for one session (100 trials) only. During the five sessions the rats improved their performance as indicated by the decreasing number of mistakes: in session 1 there were 7.69 mistakes on the average, in session 5 only 3.38 mistakes ($P < 0.05$). Thus, a learning-set was established. No significant differences were found between the two age groups. Based on our results, we conclude that there is no diminished ability to form a learning-set or a decline in cognitive ability in old rats concerning olfactory tasks.

353. Olfaction in rats with depletion of olfactory bulb serotonin

L. Hanford, S. Teldon, B. Slotnick, L. Coolen[1] and M.T. Shipley[1]

American University, Washington, DC 20016 and [1]University of Maryland School of Medicine, Baltimore, MD 21201, USA

Depletion of olfactory bulb serotonin by 5,7-dihydroxytryptamine (5,7-DHT) is said to result in a progressive hyposmia and, within 28 days of treatment, in virtual anosmia (Moriizumi *et al.*, 1994,Neuroscience, 61: 733). To further assess this we tested pre-operatively trained rats on an odor detection task and four progressively more difficult odor mixture tasks. An olfactometer was used and rats were tested 14, 21, 28 and 35 days after bilateral injections of saline or 5,7-DHT into the olfactory peduncle. As determined by immunohistochemistry, 5,7-DHT produced severe depletion of olfactory bulb serotonin. In behavior tests, experimental rats performed as well as controls in the first 28 days after treatment but on day 35 performed more poorly on the most difficult of the odor mixture tasks. We conclude that severe depletion of olfactory bulb serotonin does not result in anosmia but may produce a gradual decrease in odor sensitivity.

354. Odor quality recognition in rats with reduced connections to the olfactory bulb

B. Slotnick and N. Bodyak

American University, Washington, DC 20016, USA

The object of this study was to determine whether selective deafferentation of the olfactory bulb (OB) would disrupt recognition of odors. To this end, rats were trained to discriminate among four homologous fatty acid and four homologous aldehyde odors and a control set of eight unrelated odors prior to aspiration of the dorsal and dorsomedial segments of the OB or i.p. injection

of 3-methyl indole (3MI). Anterograde transport of HRP from epithelium to the OB provided an index of deafferentation in 3MI-treated rats. Memory tests given 10 days (operated rats) or 3 days (3MI rats) after treatment provided no feedback for correct or incorrect responses. Aspiration lesions removed the bulbar area identified electrophysiologically as responsive to a homologous series of fatty acids but left intact a region responsive to aldehydes (Imamura *et al.*, 1992, J. Neurophysiol., 68: 1986). 3MI treatment eliminated input to both bulbar areas but left intact input to a restricted group of glomeruli on the mid-lateral wall of the bulb. Each control and all but two experimental rats had good or essentially perfect retention for each of the 16 test odors. The two exceptional rats, both treated with 3MI, had only barely detectable HRP reaction transport in their bulbar glomeruli and performed largely at chance on both sets of odors.

355. Does nasal irrigation with zinc sulfate produce anosmia in the rat?

N. Bodyak, P. Glover and B. Slotnick

American University, Washington, DC 20016, USA

Anterograde transport of horseradish peroxidase (HRP) from olfactory sensory neurons to the olfactory bulb and odor detection and discrimination were examined in rats in which each nasal epithelium was irrigated with 0.1–0.5 ml of 0.17 M zinc sulfate. In each case there was at least light HRP reaction product in olfactory bulb glomeruli. In most cases, moderate-to-dense reaction product, similar to that obtained in saline treated controls, filled >50% of olfactory bulb glomeruli 2–4 days after treatment with $ZnSO_4$. The 0.5 ml application produced a greater deficit in axonal transport than did 0.1 ml. Rats trained in an olfactometer to detection 1–0.0001% ethyl acetate had little or no deficits in detecting high concentrations of the odor and variable deficits in detecting lower concentrations 1 or 3 days after treatment. Rats trained to discriminate between eight different odors were able to perform at or near their pretreatment level when tested 1–2 days after $ZnSO_4$ treatment. The histological results are in agreement with prior anatomical studies indicating that areas of olfactory epithelium remain intact after intranasal application of $ZnSO_4$. The behavioral outcomes are in agreement with recent reports of considerable savings in odor detection and discrimination, even after severe reduction of afferent projections to the olfactory bulb. We conclude that, in the rat, intranasal application of $ZnSO_4$, as generally practiced, does not produce anosmia.

356. Olfactory testing in rats without deprivation

N.E. Rawson, O. Crenshaw and S. Hyman

Monell Chemical Senses Center, 3500 Market St, Philadelphia, PA 19104, USA

A variety of techniques have been used to evaluate the olfactory function of rodents. Traditionally, these involve overnight food or water deprivation in order to 'motivate' the animal to learn and perform the task to obtain the appropriate reward. This type of deprivation has a variety of physiological consequences, including changes in brain neurochemistry, blood metabolite levels and hepatic fuel status. Under some conditions, deprivation is inadvisable to the health of the animal, and may even be modifying the function of the olfactory system directly. We aimed to determine whether rats could be trained to carry out simple olfactory testing tasks in the absence of food or water deprivation, and whether their performance would be sufficiently reliable to permit further studies of olfactory function under these conditions. We began with a simple 'buried stimulus' task and found that by testing the rats at the very beginning of the dark cycle, when rats naturally begin to eat, they quickly learned to find a buried capsule containing an odor source reinforced by a nutritionally inconsequential but flavorful reward. Performance became extremely consistent for each rat within 1 week, although some rats performed better than others. Further studies will be carried out using more sophisticated olfactory testing methods. The ability to learn and carry out these tasks reliably without food or water deprivation opens up the possibility for further studies of rats with altered nutritional status or diseases in which deprivation is not possible.

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357. Quantitative analysis of changes in the rat vomeronasal epithelium during degeneration and regeneration

M. Matsuoka[1,2], R.M. Costanzo[3], J. Yoshida-Matsuoka[3] and M.Ichikawa[1]

[1]Anatomy and Embryology, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Tokyo 183-8526, Japan, [2]JSPS Research Fellowships for Young Scientists and [3]Department of Physiology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA 23298-0551, USA

To investigate the turnover process of vomeronasal receptor cells, we used electron microscopy to make quantitative measurements of changes observed at the surface of the vomeronasal epithelium after sensory nerve transection. Animals were examined at postoperative recovery times of 2, 6, 10, 15, 30 and 60 days. We measured both length of the cell surface membrane and density of receptor and supporting cells. The number of receptor cells decreased to a minimum at 6 days. After 15 days, receptor cell density returned to control levels. The average length of surface membranes for regenerated receptor cells was similar to that of controls, although their morphological appearance was characteristic of immature cells. The density of supporting cells did not change during degeneration and regeneration. However, supporting cell surface membranes increased in length, reaching a maximum at 6 days. These results suggest that during receptor cell degeneration supporting cell membranes make adjustments to maintain the integrity of the epithelium. Their surface membranes expand to compensate for the loss of receptor cells, while the total number of supporting cells remains constant. Thus, an important function of supporting cells may be to maintain the structural integrity of the sensory epithelium during degeneration and regeneration of the receptor cell population.

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358. Layer organization of the vomeronasal epithelium during regeneration

J. Yoshida-Matsuoka, N.J.P. Ryba[1] and R.M. Costanzo

Department of Physiology, Virginia Commonwealth University, MCV Campus, PO Box 980551, Richmond, VA 23298-0551 and [1]Taste and Smell Unit, NIDCR, National Institutes of Health, Bethesda, MD 20892, USA

Recent studies have demonstrated that there is a layered organization to G-protein and G-protein linked receptor expression in the sensory epithelium of the vomeronasal organ (VNO). Gi2α- and Goα-expressing neurons are localized to the apical and basal halves of the receptor cell layer. Moreover, the ordering of Gi2α and Goα expression is preserved in the accessory olfactory bulb (AOB). In rats, the layered organization of the VNO develops over the first few postnatal days and remains constant thereafter. After olfactory bulbectomy or vomeronasal nerve transection, the receptor neurons in the VNO degenerate over a period of ~6 days. At later recovery times considerable neural regeneration has been demonstrated. We were interested in whether the laminar organization of the VNO was an intrinsic property of the VNO or whether it resulted from developmental cues or interaction with the AOB. To examine this question, the distributions of VNO molecular markers were determined at various times after olfactory bulbectomy and neonatal sensory nerve transection. Six days after bulbectomy and nerve transection, there was a marked decline in the number of receptor cells expressing receptor and G-protein molecular markers. However, within 15 days the numbers of cells expressing all markers recovered to ~25%. After recovery, the Gi2 and Go-expressing neurons were intermingled and no apparent order was discernable in the epithelium even at 60 days recovery. Therefore the layering established during normal development is not replicated during recovery from bulbectomy or nerve transection.

359. Culture of rat vomeronasal neurons

T. Osada[1,2], A. Ikai[1], R.M. Costanzo[3], M. Matsuoka[4] and M. Ichikawa[2,4]

[1]Department of Biological Sciences, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 226-0026, Japan, [2]CREST of Japan Science and Technology Corporation, [3]Department of Physiology, MedicalCollege of Virginia Campus, Virginia Commonwealth University, Richmond, VA 23298-0551, USA and [4]Anatomy and Embryology, TokyoMetropolitan Institute for Neuroscience, Fuchu, Tokyo 183-8526, Japan

The main olfactory and vomeronasal systems have been regarded as an ideal model for the study of neurogenesis, regeneration, axonal guidance and development of neurons in the mammalian nervous system. Olfactory and vomeronasal neurons undergo continuous neurogenesis throughout the life span of most animals. Precursor cells have the ability to undergo cell division and differentiate into neurons after they receive signals perhaps from damaged or degenerating cells.

In the present study, we describe vomeronasal cell culture from rat embryos. Partially dissociated VNO cells when cultured on the feeder layer formed vomeronasal epithelium-like structures called pocket-vomero (PV) and continuous degeneration and regeneration of axon bundles were observed. PVs increased their size during the first 2 months of culture and then maintained this size with only slight changes. PVs contained both vomeronasal neurons and supporting cells. They formed a spherical structure with a central cavity where microvilli protruded from supporting cells. Mature vomeronasal neurons with well-developed microvilli were not observed in the PV. The time period between degeneration of axon bundles and the next was ~2 weeks. When PVs were incubated with 5 μg/ml aphidicolin, an inhibitor of cell division, regeneration of axon bundles was not observed after degeneration. These results suggest that vomeronasal neurons in culture undergo continuous regeneration but did not fully mature. In this culture system, PVs survived for over one year. The culture system may provide an important tool for future studies to examine the mechanisms underlying neurogenesis and differentiation of vomeronasal neurons.

360. Evidence for receptor neurons in the thick epithelium of a newborn elephant's vomeronasal organ

E.W. Johnson and L.E.L. Rasmussen[1]

Department of Biological Sciences, Idaho State Univeristy, Pocatello, ID 83209 and [1]Department of Biochemistry and Molecular Biology, Oregon Graduate Institute of Science and Technology, PO Box 91000, Portland, OR 97291, USA

At birth, Asian elephants do not display flehmen, a common mechanism for accessing pheromones, suggesting that at birth the accessory olfactory system (AOS) may not yet be functional. Also, little is known about the structure of the vomeronasal organ (VNO), where chemosignal detecting neurons are located, before adulthood. The present study was conducted on the VNO of a newborn Asian elephant because of inconclusive data from an earlier newborn. Thick sections cut from VNOs embedded in plastic were stained with toluidine blue. The overall organization was similar to the VNO of other mammals, with thick and thin pseudostratified, columnar epithelia on either side of an elliptical lumen. In the thick epithelium, cells with superficial, elongate nuclei had unique mucus globules and surface cilia. The nuclei of cells in the deeper layer were ovoid, with prominent nucleoli, reminiscent of receptor neurons. Unmyelinated nerve bundles were seen only under this epithelium, and a large, unmyelinated nerve was observed adjacent to this mucosa. These observations suggest a structurally mature neuroepithelium and that axons project from receptor neurons into the vomeronasal nerve.

The thin epithelium had mucus and ciliated cells. Acinar glands with ducts to the lumen were abundant in the lamina propria. These observations suggest that this is the respiratory mucosa and that the numerous glands and mucus cells are evidence of a copious secretory activity. Additional analysis is being conducted to characterize cell types. The existence of receptor neurons and their degree of structural maturity will be confirmed with ultrastructural studies.

361. Passage of the Harderian gland secretions to the vomeronasal organ of the snake, *Thamnophis sirtalis*

S.J. Rehorek, W.J. Hillenius[1], W. Quan[2] and M. Halpern[2]

Department of Anatomy, NYCOM, Long Island, NY 11568-8000, [1]Department of Biology, College of Charleston, Charleston, SC29424-0001 and [2]Department of Anatomy and Cell Biology, SUNY, Brooklyn, NY 11203-2098, USA

The Harderian gland is a poorly understood structure, found in the anterior orbit of most terrestrial vertebrates. In the snake *Thamnophis sirtalis* this is a serous–mucous secretory glandular structure, with a large post-orbital section. Numerous functions have been ascribed to this gland, including orbital lubrication and in the vomeronasal lubrication. Anatomically, the Harderian gland is connected to the vomeronasal organ (VNO) via the nasolacrimal duct. In this study, we traced the serious secretions of the Harderian gland using autoradiographic techniques at the light microscopic level. We had two treatment groups (unilateral and bilateral). Snake Harderian glands were injected either unilaterally (right side) or bilaterally with [^{3}H]proline. The right Harderian glands of both treatment groups were then injected with a potassium rich solution. On the right side labeling was observed in the Harderian gland, Harderian gland ducts, nasolacrimal duct, and the lumen and duct of the VNO. On the left side labeling was observed only in the Harderian gland of the bilaterally injected animals, but not in the other structures listed above. Additionally, since no labeling was observed in the orbital space, the serous secretions of the Harderian gland in *Thamnophis* do not appear tofunction in orbital lubrication. Thus, the serous secretions of the Harderian gland in snakes flow to the VNO, and may be considered part of the vomeronasal system. Whether this is the sole source of the fluid on the VNO remains to be determined.

362. Selective activation of G-protein β subtypes in the vomeronasal organ

I. Boekhoff, J. Krieger, A. Schmitt, D. Löbel and H. Breer

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

Chemosensory neurons in the vomeronasal organ (VNO) detect pheromones related to social and reproductive behavior in most terrestrial vertebrates. Current evidence indicate that the chemo-electrical transduction process is mediated by G-protein-coupled second messenger cascades. the present study, attempts were made to identify the G-protein subtypes which are activated upon stimulation with urine-derived components. G-protein-specific antibodies were employed to interfere specifically with IP_3 formation induced by urinary stimuli; furthermore, in photoaffinity labelling experiments, the identity of receptor-activated G-proteins was analyzed using [α-^{32}P]GTP azidoanilide followed by immunoprecipitation of the labelled G-protein α-subunits.

The results of both experimental approaches indicate that stimulation of female VNO membrane preparations with male urine samples induces activation of G_i as well as G_o subtypes. Experiments using different fractions of urine revealed that upon stimulation with lipophilic volatile odorants only G_i proteins were activated, whereas G_o activation was elicited by α2u-globulin, a major urinary protein, which is a member of the lipocalin superfamily. Since each G-protein subtype is stereotypically co-expressed with one of the two structurally different candidate pheromone receptors (V1R and V2R), the results provide first experimental evidence that V1Rs co-expressed with G_i may be activated by small, lipophilic odorants, whereas V2Rs co-expressed with G_o seem to be spezialized to interact with pheromonal components of proteinaceous nature.

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363. Electrophysiological properties and GTP-binding proteins putatively involved in vomeronasal signal transduction

F. Murphy, K. Tucker, E.E. Morrison, J.C. Dennis[1], V. Voydanoy[1], D.Srikumar[1], J.H. Kehrl[2] and D.A. Fadool

Zoology Department and [1]Department of Anatomy, Physiology, and Pharmacology, Auburn University, Auburn, AL 36849 and [2]Laboratory of Immunoregulation, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD 29892, USA

Cell signaling in the vomeronasal organ (VNO) of vertebrates isnot fully understood. Towards a better comprehension, we haveimmunocytochemically screened turtle VNO cryosections for various G-protein subunits. $G_{\alpha i(1-3)}$ and G_α were localized to the microvillar layer and $G_{\alpha o}$ was localized primarily to the VN axon bundles. In the rat VNO, $G_{\alpha i(1-3)}$ immunoreactivity occurred apical to the supporting cell somata and in a nonuniform pattern among the sensory cell somata. Additionally, one family member of the regulators of G-protein signaling (RGS proteins), RGS3, was expressed in rat VNO and olfactory bulb by RT-PCR. Western analysis of purified VNO membranes has revealed the presence of $G_{\alpha i(1-3)}$, G_α, and $G_{\alpha o}$ in both rat and turtle. Further studies with turtle VNO have demonstrated via quantitative densitometry that $G_{\alpha i(1-3)}$ expression is 2- to 3-fold greater in females. This gender difference is not observed in juvenile turtles and juveniles have a lesser total G-protein concentration than that of adults. We have isolated single turtle VRNs by a combination of enzyme dissociation and trituration; an amendable acute preparation for voltage-clamp studies. Voltage-evoked outward transient currents are tetraethylamonium (TEA) sensitive, whereas 25% of sustained outward currents are not blocked by 10 mM TEA. The voltage-evoked inward current is completely blocked by 1 nM tetrodotoxin (TTX). We are presently exploring turtle musk, urine, skin extracts and holding tank water as potential compounds to elicit chemical-activated electrophysiological responses.

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364. Initial molecular studies of (*Z*)-7-dodecenyl acetate as a mammalian pheromone

L.E.L. Rasmussen, J. Lazar[1] and G. Prestwich[1]

Department of Chemistry, Oregon Graduate Institute, Beaverton, OR97006 and [1]Department of Medicinal Chemistry, University of Utah, Salt Lake City, UT, USA

We are conducting an in-depth investigation of the proteins involved in transport, recognition, and signaling by the female-produced sex pheromone (Z)-7-dodecenyl acetate, (Z7-12:Ac) in the Asian elephant, *Elephas maximus*. Utilizing a radiolabeled photoactivatable analog, [^{3}H](Z)-7-dodecenyl diazoacetate, we have established that the primary urinary transporter protein

binds the pheromone or its photoactivable analog loosely and unspecifically; this 66 kDa protein has an 80% sequence homology to bovine serum albumin. Bioassays demonstrated that a semi-purified albumin-rich protein fraction enhanced the bioresponses elicited from males to Z7-12:Ac.

The male response to the urinary pheromone involves the process of pheromone transport toward the vomeronasal organ (VNO). The urinary pheromone mixes with truncal mucus; this mixture is placed onto the mucous-laden incisive ducts leading to the VNO. At least two truncal mucous proteins (21 and 16 kDa), with a >50% sequence identity to bovine olfactory binding protein, bind the pheromone somewhat tighter than the urinary albumin, but with apparent low discrimination between various lipophilic ligands. Neither nasal/truncal nor vomeronasal mucous proteins that bind the pheromone demonstrate differences between male or female secretions. However, the vomeronasal mucous proteins that bind the pheromone are distinct from the nasal proteins.

We have prepared cDNA libraries from elephant liver, mucosal and vomeronasal tissue, and are in the process of screening these libraries by biotinylated DNA probes. We have prepared cDNA from single sensory cells of female vomeronasal organ. The comparison of two separately evolved solutions to binding and transducing signals from the same chemical entity, Z7-12:Ac, is of special interest during these ongoing studies.

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365. The anatomy of the vomeronasal organ: characterization by means of nasal endoscopy and magnetic resonance imaging

T. Hummel, D. Kühnau, M. Knecht, N. Abolmaali[1] and K.B. Hüttenbrink

Departments of Otorhinolaryngology and [1]Radiology, University of Dresden, Fetscherstraße 74, D-01307 Dresden, Germany

Although the human vomeronasal organ (VNO) receives increasing attention, there are little data regarding its appearance in relation to age or gender. This study aimed (1) to describe the VNO's presence and location in a large sample, and (2) to identify size and course of this tubular organ in selected subjects. We investigated 88 women and 85 men (age range 2–91 years). The nasal cavity was endoscopically inspected by two otorhinolaryngologists. In 39% of the subjects the VNO was found bilaterally; in 21% it was detected on just one (either) side. It had a mean distance of 2.65 cm from the naris (SD 0.47), 0.92 cm above the floor of the nasal cavity (SD 0.42). Although its occurrence did not differ in relation to gender, the VNO appeared more frequently in younger than in older women (<40 years 63%, >40 years 53%). The second part of the study was performed in seven women and four men (age range 18–66 years). T1-weighted MR sequences (1.5 T spin-echo, 1 mm) were performed before and after instillation of hydrophilic contrast agent (Magnetvist™). In three cases contrast agent was found in the organ's spot-like opening. However, in eight cases the VNO appeared to be tubular, with a mean length of 15 mm (5–40 mm); most of these were slightly bent carnially. In two of these eight cases the VNO appeared to be connected to the contralateral side. These data indicate that appearance and course of this organ may be different from what has been reported previously.

366. Immunohistochemical analysis of rat vomeronasal organ transplanted to brain

J.C. Dennis, K.G. Wolfe and E.E. Morrison

College of Veterinary Medicine, Auburn University, AL 36849, USA

Rat vomeronasal organ (VNO) tissue fragments, following transplantation to the brain, develop as a series of epithelial structures, sensory, respiratory and glandular. We examined the immunoreactivity of cytokeratin, neural cell adhesion molecule (NCAM), nerve growth factor receptor (Trk A) and epidermal growth factor receptor (EGFR) in paraffin-embedded brain sections which contained transplanted VNO epithelial structures. Cytokeratin immunoreactivity occurred in respiratory and glandular epithelia but was rare in sensory structures. Many, but not all, cells within sensory structures as well as transplant-derived axon bundles were NCAM(+). Antibody to Trk A labeled a subpopulation of cells *in situ* but gave no definitive label in transplant sensory structures. EGFR antibody labeled some basal cells and the apical portions of cells *in situ*, but labeled almost exclusively the apical portions of cells in sensory transplant structures. The differences of staining pattern in VNO transplants compared with *in situ* VNO may reflect the disruption of normal neuron–target interactions and/or the disruption of the normal epithelium exemplified by the dearth of basal cells in large areas of the transplant.

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