ACHEMS - 1995

ABSTRACTS

THE SEVENTEENTH ANNUAL MEETING OF THE ASSOCIATION FOR CHEMORECEPTION SCIENCES

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ACHEMS - 1995

Seventeenth Annual Meeting
of the
Association for Chemoreception Sciences

ABSTRACTS

This book contains abstracts of the volunteer papers and posters of ACHEMS 1995. Abstracts are listed in order of presentation at the meeting. The abstracts for slide presentations precede the abstracts for poster presentations which are scheduled concurrently. An author index is included.

1

Taste and Solution Properties of D-Glucono 1,5-Lactone
GORDON G BIRCH[1], DAVID KILCAST[2], MARIE-ODILE PORTMANN[2], MIRJANA GRANOV[1], and NICOLAS BUTU[1] ([1] Department of Food Science and Technology, University of Reading RG6 2AP, UK and [2] Leatherhead Food RA, Surrey, UK).

D- Glucono-1,5-lactone is an interesting food molecule possessing sweet-eliciting features and which changes over time to a molecule (D-gluconic acid) with sour characteristics. The autohydrolysis of this lactone is thoroughly analysed in both water and 40% sucrose solution in relation to taste changes which accompany the hydrolysis over the course of time. The sweet taste of the 1,5-lactone confirms that the anomeric centre of hexopyranoses is not a pre-requirement for the sweet response. The pure sour taste of the generated D-gluconic acid is confirmed by its apparent specific volume ($0.515 cm^3 g^{-1}$). The rate and extent of hydrolysis of D-glucono 1,5-lactone in water, and hence the accompanying taste changes, are quite different from those in 40% sucrose solution and can be explained in terms of the solute-water interactions in each system. These results are relevant to the tastes of cheese and honey in which the glucono-lactone equilibrium may play a part.

Supported by European Commission
(AIR-PL 94-2107)

2

Taste and Touch in Aging: Assessment by Repeated Threshold Measures. JOSEPH C. STEVENS and L. ALBERTO CRUZ (John B. Pierce Laboratory, New Haven, CT)

As reported earlier for *olfaction* (ACHEMS XV; Perception & Psychophysics, 1993, 54, 296-302), sensitivity to *taste* and *touch* declines ubiquitously with age. Contrary to the impression given by typical brief threshold tests, decline marks nearly everybody. Extensive testing with sucrose (average of, and correlations among, six 15-20 min threshold tests, spread over three days) suggests two principles: (1) That young adults are more uniform in sensitivity than are older adults, and (2) That with respect to sensory loss individuals "age" at reliably different rates. Decline of touch (acuity) thresholds also characterized nearly everybody, also at differential rates. Furthermore, among persons over 65 yrs, one who tested high (low) on taste tended to test high (low) on touch, even after the variance attributable to chronological age was removed by partial correlation. A common aging rate factor appears to underlie these two modalities.

Supported by NIA Grants AG-04287 and AG-10295.

3

Analysis of Taste Mixtures by Children and Adults
M H FREEMAN, N ORAM, D G LAING and I HUTCHINSON (University of Western Sydney, Sydney, Australia)

It is well established that the sense of taste is functional at birth and is advanced in maturity within several years. However, there have been no reports on the ability of children to analyse taste mixtures into the component tastes. Such a task may be beyond their physiological and/or cognitive capabilities. The present study aimed to establish whether 8 yr old children are able to discriminate, identify and estimate the amount of sweetness, sourness and saltiness in binary mixtures of sucrose, citric acid and sodium chloride, and to determine whether their ability was the same as that of adults. Children (26 males, 27 females) and adults (13 males, 12 females), were trained to identify the three single tastants and to rate the perceived intensities of four concentrations of each of the tastants using a simple 5 point category scale. The same scales were then used to estimate the intensity of each tastant in binary mixtures. The results indicated that (1) children and adults produced similar intensity - concentration functions with each of the tastants, indicating that by 8 yr of age, children perceive different concentrations of tastants similarly to adults, and (2) similar levels of a tastant were reported by children and adults with each mixture, indicating not only that children have the cognitive ability to analyse complex taste stimuli, but that their sense of taste has reached a similar functional state as that of adults.

Supported by grants from the Australian Research Council, Meat Research Corporation and Pig R & D Corporation.

4

Cognitive Factors Influence Odor-induced Enhancement of Sweetness: Rating Versus Attending to Attributes. NICOLETTE J. VAN DER KLAAUW & ROBERT A. FRANK (University of Cincinnati, Cincinnati, Ohio)

Frank, van der Klaauw and Schifferstein (1993) have demonstrated that odor-induced enhancement of sweetness is instruction-dependent. When subjects judge only the sweetness of a sucrose-strawberry odor mixture, sweetness ratings are enhanced by the odorant, but this enhancement disappears when several, appropriate stimulus attributes are rated. The goal of the present experiment was to determine whether attending to multiple stimulus attributes of a taste-odor mixture would produce the same pattern of results as making multiple ratings. Three groups of subjects made judgments of seven stimuli (0.3 M sucrose, 50 μM Quinine HCl, 1% strawberry odorant, sucrose-strawberry, sucrose-quinine, quinine-strawberry, distilled water). Group 1 (n=20) rated only the sweetness of the stimuli, Group 2 (n=20) rated the sweetness, bitterness and fruitiness of each stimulus on each trial. Group 3 (n=40) was instructed to judge the sweetness, bitterness OR fruitiness of each stimulus, but was not told which attribute would be rated until after the stimulus had been expectorated. Thus, the subjects in this group made one rating, but attended to at three stimulus attributes. Odor-induced sweetness enhancement was observed for Group 1, but not for the other two groups. Thus, attending to multiple stimulus attributes eliminates odor-induced sweetness enhancement just as effectively as making multiple ratings on each trial. This finding supports the idea that cognitive processes such as attention play an important role in the decision-making behavior of people faced with the task of the rating complex, chemosensory stimuli.

This research was supported by a Sensory Science Scholarship in the memory of R. M. Pangborn to N. J. van der Klaauw.

5

Perceived Odor Character of Concord Grape Juice Components Determined by Gas Chromatography - Olfactometry (GCO) with Eight Subjects RHONDA L. SMITH[1], ANNA B. MARIN[2], JOHN BARNARD[1] and TERRY E. ACREE[1] (Cornell University-Geneva[1], NYSAES, Geneva, NY 14456, International Flavors and Fragrances[2], Union Beach, NJ, 07735)

During the gas chromatography-olfactometry (GCO) of grape juice extracts, subjects were asked to associate labels with odorants after they were detected eluting from a gas chromatograph. The labels were chosen from a personal lexicon developed by each subject. This presentation reports the differences observed between the subjects' use of labels and their differences in sensitivity. Eight people (five females and three males) ranging in age from 16 to 19, in good health with the ability to smell a standard set of stimulants: ethyl butanoate, ethyl 2-methyl-butanoate, ethyl hexanoate, 1,8-cineole, menthone, l-menthol, and l-carvone were used as subjects. Frozen enzyme-stabilized Concord grape juice from a single press lot was thawed to 2°C, stored in glass and brought to 25°C prior to extraction and sensory testing. Grape juice containing 1,8-cineole as the internal standard was extracted with Freon 113 and ethyl acetate and analyzed by GCO on a fused silica capillary column coated with methyl silicone. Sniffing serial dilutions of the extracts provided data for an estimation of potency in units called charm values. There were significant individual differences between the subjects in both the labels they chose to associate with the stimulants and the relative potencies exhibited by each stimulant for each subject.

6

The Perceived Odor Character of Concord Grape Juice Determined by Quantitative Descriptive Analyses (QDA) with Eight Subjects for Natural and Flavor Enhanced Concord Grape Juice. ANNA B. MARIN[1], TERRY E. ACREE[2], and JOHN BARNARD[1] (International Flavors and Fragrances[1] R&D, 1515 HWY 36, Union Beach, NJ 07735, Cornell University-Geneva[2], NYSAES, Geneva, NY 14456)

Eight subjects produced a personal lexicon of odor descriptors for Concord grape juice components during gas chromatography-olfactometery (GCO) of the juice extracts. Concord grape juice components were identified by gas chromatography/mass spectrometry (GC/MS) and related to each panelist's odor descriptors by component retention index. Each subject's list of descriptors was then used as a personal ballot for QDA testing. Four major odor components of Concord grape juice were selected for the QDA flavor enhancement study. These components were methyl anthrenilate, phenyl ethyl alcohol, β-damscenone, and furaneol. Another flavor material foreign to grape juice, 1,8-cineole, was also used in the QDA enhancement study. Enhancement levels for each component were based on the original concentration of that component in the grape juice as determined by GC. Spike levels were then set at 0, 10, 30 and 90 times the concentration level in the juice. Cineole spike levels were based on an average group threshold of 0.40 μg/ml which was used as the 0 level. QDA testing consisted of each panelist rating the intensity of each odor descriptor on his ballot for each of the four spike concentrations, 0 - 90X, for each of the 5 enhanced flavors. Four coded samples of each concentration level of one component were presented for evaluation at once. Each panelist rated all 5 spike components over four replications. Results for all panelists' responses as a group indicate that the cineole spike significantly decreased the intensity ratings for methyl anthrenilate and damescenone but not for furaneol and phenyl ethanol. The damescenone and furaneol spikes had insignificant effect on ratings for any components. However, the phenyl ethanol and methyl anthrenilate spikes increased ratings for some components while decreasing ratings for others.

7

Adaptation to Capsaicin Within and Across Days

DONALD H. McBURNEY, CAREY D. BALABAN and DALE E. CHRISTOPHER, University of Pittsburgh

Human subjects who infrequently consumed foods containing capsaicin rated the intensity of capsaicin for 34 minutes on Day 1 and Day 5. Twenty five μl of 100 ppm capsaicin was pipetted onto filter paper and allowed to dry (Green, 1989). Before being placed on the tongue, the filter paper was wetted with 50 μl of HOH. Ss held the filter paper on the outstretched tongue with a tongue depressor. Stimuli were replaced every minute. On Days 2-4 Ss tasted hard candy containing 75 ppm capsaicin for 10 minutes. On Day 1 the intensity of burn increased monotonically for the entire session. Intensity was less at all times on Day 5 and either levelled off or declined for most Ss. Results were fit to the adaptation model of McBurney and Balaban (1994), which comprises two main processes: a phasic process that responds to transients, and a tonic process that is sensitive to steady stimulation. Analysis of the data suggested an additional process that increased exponentially over the entire session. This second process was manifested on Day 1 as an abrupt rise in the response at about 16 minutes, after the sensation had appeared to level off, which increased throughout the session. This late rising response was absent on Day 5. No subject showed adaptation within session the first day, as defined by a decline in response. On Day 5 most Ss showed a response decline within session. Across days, however, there was considerable adaptation, defined as reduced gains of the tonic and the exponentially increasing processes. When the exponentially increasing process is subtracted from the response, a time course more typical of other sensory modalities remains. Thus, adaptation occurs both within and across sessions, and can be fit by changes in the gains of the components of a simple mathematical model. We predict that, unlike naive Ss, regular users of capsaicin will demonstrate paradigmatic adaptation, owing to long-term adaptation (reduced gain) of the exponentially increasing process.

8

Description and Prediction of Human Nasal Pungency Thresholds by a Solvation Equation. J. ENRIQUE COMETTO-MUÑIZ[1], WILLIAM S. CAIN[1], MICHAEL H. ABRAHAM[2] and JENIK ANDONIAN-HAFTVAN[2] (Department of Surgery -Otolaryngology- University of California[1], San Diego, San Diego, CA 92103-8895, USA, and Department of Chemistry, University College London[2], London WC1H OAJ, UK).

We have previously gathered thresholds for nasal pungency (measured in anosmics), and eye irritation and odor (both measured in normosmics) using members of four homologous chemical series: alcohols, acetates, ketones, and alkylbenzenes. A uniform procedure was used to obtain the thresholds. Common chemical sensations (nasal pungency and eye irritation) displayed a uniform relationship with simple physicochemical properties (e.g., saturated vapor concentration) across the various chemical families. In the present study we developed a four-parameter equation, capable of describing the nasal pungency potency of 31 nonreactive compounds and accounting for more than 95 % of the variance:

$$\log 1/NPT = -8.670 + 2.396\,\pi_2^H + 3.636\,\Sigma\alpha_2^H + 1.352\,\Sigma\beta_2^H + 0.873\,\log L^{16}$$

where NPT=nasal pungency threshold, π_2^H=the dipolarity/polarizability of the stimulus, $\Sigma\alpha_2^H$=its overall or effective hydrogen-bond acidity, $\Sigma\beta_2^H$=its overall or effective hydrogen-bond basicity, and L^{16}=its gas-liquid partition coefficient on hexadecane at 298K. Unlike previous equations applied to chemoreception thresholds, this contains parameters that fall within realistic boundaries and have readily interpretable meaning. This equation, a linear solvation energy relationship, implies that reception of nasal pungency is governed principally by interactions between the stimulus as a solute and the receptor biophase as a solvent. Ongoing measurements of nasal pungency with series that contain substances of clearly greater chemical reactivity in their lower members than in their higher members now give us the opportunity to explore the limits of the predictive power of our equation. We anticipate that lower members of the aliphatic aldehydes and carboxylic acids will produce pungency at levels below those predicted, and that higher members will produce pungency at the levels predicted.

Research supported by NIH Grant DC00284.

9

Pheromones and Other Semiochemicals: Tools for Olfactory Research

THOMAS A. CHRISTENSEN [1] and PETER W. SORENSEN [2] (ARL Div. of Neurobiology, University of Arizona, Tucson, 85721 [1]; Dept. of Fisheries & Wildlife, University of Minnesota, St. Paul, MN 55108 [2])

We have long been fascinated by the unique ability of odors to stir our emotions and to evoke long-forgotten memories, but certain odors play a much more fundamental role in life - they vastly improve an organism's chances for reproductive success and survival. These odorants are called pheromones, a term applied to any semiochemical that conveys information between members of the same species. Pheromones are known for both the specificity and the potency of their actions, which can be behavioral and/or neuroendocrinological. Pheromones can stimulate individuals to aggregate, to disperse, and to react defensively to the presence of a predator, but they are probably best known for bringing the sexes together. Pheromones have also been found to trigger a dramatic release of pituitary hormones in several vertebrate species. Though first identified in insects, more recent studies show that sex pheromones influence the lives of a wide variety of organisms, from microbes to man. In this symposium, we will focus on two animal models of sex-pheromonal communication, the hormonally-derived sex pheromones in teleost fish, and the airborne pheromones of moths. These two examples will illustrate how scientists have used pheromones as important tools to investigate the morphology, physiology and biochemistry of olfactory-receptor systems, the mechanisms of odor-information processing in the brain, and the diverse range of behaviors and endocrinological changes associated with pheromonal communication.

10

Behavioral Responsiveness to Pheromones Provides Fundamental and Unique Insight into Olfactory Function.

PETER W. SORENSEN (Dept. Fisheries & Wildlife, Univ. of MN., St. Paul. MN 55108)

Behavior may be defined as the expression of the integrated actions of an organism's nervous system. Sensory perception of environmental conditions serves as the proximate driving force for the expression of behavior while natural selection is the ultimate determinant of the entire process. Sensory processes are therefore likely to be molded by an organism's behavioral and ecological needs and vary with species and life stage. This is particularly true for the olfactory sense which appears to have great flexibility to detect many types of compounds. but is typically tuned to only a limited number (dozens to 1,000's) of compounds in any species. Especially intriguing is sensitivity to pheromones, or compounds which serve as communicatory signals, because these compounds are frequently unique, yet play fundamental roles in mediating social phenomena such as mating, and are detected exclusively by the olfactory sense. Indeed. detection of pheromones may be the principal function of the olfactory sense of many invertebrate and vertebrate organisms at particular stages of their lives. However, it is difficult for us to grasp this phenomenon because we ourselves lack a good olfactory sense and studies of the behavior of these organisms must therefore serve as the guide to discerning what this system detects and how it is 'designed' to function. In insects, behavioral investigations have led to the discovery of 'specialist' receptor-cell pathways and neural mechanisms associated with the detection and recognition of odorant mixtures. Similarly, in vertebrates, behavioral investigations are responsible for the elucidation of novel classes of specialized receptors for hormonal pheromones in fish, which now appear to be universal in this group. More recent behavioral investigations of fish now suggest that unconventional EEG responses associated with sensitivity to these compounds reflects specialized neural processes (Bresin *et al.*, this meeting). Other studies in insects now suggest that olfactory glomeruli may be specialized not only for the detection of the chemical composition of pheromones. but also for the processing of spatio-temporal information embedded in the pheromone plume. Simultaneous investigation of behavior and neural functioning has proven to be an invaluable tool and it is likely that many exciting discoveries still await those who use this strategy.

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11

Peripheral Mechanisms of Pheromone Reception

KARL-ERNST KAISSLING (Max-Planck-Institut für Verhaltensphysiologie Seewiesen, 82319, Starnberg, Germany)

Within the last five decades, chemical identification of insect pheromones has helped us study the physiology and biochemistry of olfactory-receptor systems. Insect pheromones, for instance those released by the female to attract conspecific males, often consist of two or a few chemical components in species-specific proportions. Each of the components is perceived by a separate type of narrowly-tuned receptor neuron on the male antennae. Thus, the male is able to recognize the conspecific pheromone blend by comparing the excitation pattern across several types of receptor cells. In some vertebrates, sex-pheromones also appear to be blends of odorants, and there is increasing evidence that pheromone-receptor neurons share functional characteristics with those of insects. Electro-olfactogram and cross-adaptation studies in fish, for example, have shown that different classes of olfactory receptors respond to pheromones, amino acids and bile salts, and that pheromone receptors must be extremely selective and sensitive. Pheromones can be detected by male moths over large distances, in concentrations as low as 1000 molecules per cm^3 of air. This remarkable sensitivity of moths is due to large antennae with numerous pheromone-receptor cells that can fire nerve impulses upon capture of a single stimulus molecule. Such specialized olfactory organs offer advantages for the study of chemo-electrical transduction and of perireceptor events. Olfactory transduction in moth pheromone receptors involves several second-messenger systems (direct activation of ion channels via G-proteins not being excluded) and different types of ion channels within the same cell. This has been also found in crustaceans and might be a general property, especially of primary-sensory cells which not only transduce the stimulus into the receptor potential, but also transform the latter into a nerve impulse response. An important peri-receptor event is the termination of the olfactory stimulus, as studied in the moth *Antheraea polyphemus*. Biochemical and electrophysiological experiments provide strong evidence that the pheromone binding protein found in the extracellular fluid surrounding the receptor cell not only solubilizes and presents the pheromone to the receptor site, but eventually deactivates the pheromone. The pheromone is also enzymatically degraded, however much more slowly. Rapid deactivation of stimulus molecules ought to occur in all chemoreceptor organs, including vertebrate noses, which accumulate stimulus molecules and respond to frequent changes in odor concentration. Pheromone detection is thus a fundamental yet specialized aspect of olfactory receptor function in both vertebrates and invertebrates, and many similarities are likely to exist between these systems.

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Central Mechanisms of Pheromone-Information Processing

HANNA MUSTAPARTA (Dept. of Zoology, University of Trondheim 7055 Dragvoll, Norway)

An important advantage of using chemcially-identified and behaviorally-relevant odorants like pheromones in olfactory studies is that they can be used as specific probes to examine odor-information processing pathways in the brain. In many vertebrate and insect species, the olfactory system is separated into two functionally-distinct subdivisions, a "main" and an "accessory" system, one of which is specialized for pheromone detection. In moths, the accessory system mediates physiological responsiveness to pheromones, and this information is first processed in the brain in a large and sexually-dimorphic structure called the macroglomerular complex (MGC). Similarly, in goldfish, the olfactory pathways that regulate responses to pheromones are different from those that process more general odorants. The medial portion of the olfactory bulb appears to mediate responses to pheromones, while the lateral portion is sensitive to feeding stimuli. Likewise, in mammals, responsiveness to pheromones appears to be closely associated with the accessory olfactory bulb, which some suggest to be analogous to the medial olfactory bulb in fish. Another interesting parallel is found in the rat, where a subpopulation of olfactory-receptor-cell axons project to a histologically distinct "modified glomerular complex" in the dorsomedial olfactory bulb that is important in processing information about suckling pheromone. In each of these olfactory centers, pheromonal information is apparently processed in a restricted number of olfactory glomeruli. One important principle to arise as a direct result of using pheromones as tools to explore odor-information processing pathways in insects is that individual olfactory glomeruli can be considered as functional units in the processing of specific information concerning both the chemical *and* spatiotemporal features of the pheromone plume. Indeed, it has now been confirmed in several moth species that the axons of different pheromone-selective receptor neurons project into different MGC glomeruli. Intracellular recordings from the projection (or output) neurons in several species also show that information about single components of the species-specific pheromone blend is preserved in some output pathways, whereas other outputs respond in a unique fashion to the blend, and many of these neurons also accurately encode changes in the temporal characteristics of the stimulus. Recently, using a specific battery of non-pheromonal odorants, similar chemosensory specificity was obtained from mitral/tufted cells in the rabbit olfactory bulb. Continued exploration of the circuits that process this unique information in the brain will undoubtedly reveal further similarities between vertebrates and invertebrates, along with insights into the mechanisms underlying odor-information processing in general.

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Ultrastructural Localization of Putative Odor Receptors and Cyclic Nucleotide-Gated Channels in Rat Olfactory Epithelia. B. PH. M. MENCO[1], O. MATSUZAKI[2], R. E. BAKIN[2], G. V. RONNETT[2], J. STROTMANN[3], and H. BREER[3] (Department of Neurobiology & Physiology, Hogan Hall, Northwestern University, Evanston, IL 60208[1], Department of Neurosciences, Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205[2] and Institut für Zoophysiologie, Garbenstraße 30, D-7000 Stuttgart 70, Germany[3])

Earlier we showed that, at a fine structural level, major effector proteins of one olfactory signaling system, notably G_{olfa} and type III adenylyl cyclase, localize in modified distal regions of rat olfactory cilia (*Menco et al., 1994, J. Neurocytol., 23, 708*). Here we provide preliminary evidence that proteins responsible for the onset of the sensory signal and the evocation of electrical signals in response to odors, odor receptors and cyclic nucleotide-gated (CNG) channels, respectively, localize in the same regions of the cells as above effectors. Postembedding immunolabeling was performed on freeze-substituted rat olfactory epithelia with polyclonal antibodies, E464, to a consensus region of putative odor receptors (*Krieger et al., 1994, Eur. J. Biochem., 219, 829*) and polyclonal antibodies to the 70 kD subunit of cyclic nucleotide gated channels (*Matsuzaki et al. 1994, Chem. Sens., 19, 514, Abstr. 182*). Both, E464 and antibodies to the CNG channels bound to rat olfactory cilia, the latter especially to the distal part of these cilia. While antibodies to the channels worked best in unfixed tissues, antibodies E464, to the putative receptors, bound only in tissues that were chemically fixed and cryoprotected. For the CNG channels we also showed that labeling was reduced when the antibodies were preabsorbed with the antigenic peptide and that the developmental expression of the channels resembles that of the mediating effectors. The combined results suggest that all signal transduction proteins examined as yet localize in olfactory cilia, a long held supposition, but now visualized ultrastructurally. *Supported by NSF (IBN-9109851), NIH (DC02491), the Smokeless Tobacco Research Council, Inc. (031404) (to BPhMM), NIH (DC1704), W. M. Keck Foundation, Whitehall Foundation, McKnight Scholars Award (to GVR, OM and REB) and the DFG (Br712/16-1) (to HB and JS).*

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GBC-1, a Monoclonal Antibody Against Globose Basal Cells. BRADLEY J. GOLDSTEIN AND JAMES E. SCHWOB (Department of Anatomy and Cell Biology, and Chemosensory Disorders Group, SUNY Health Science Center, Syracuse).

Globose basal cells have been defined as a mitotically active population of NCAM (-)/cytokeratin (-) basal cells in the olfactory epithelium that give rise to new neurons. Although other cell types in the epithelium are immunohistochemically identifiable, these basal cells have previously eluded such characterization, and have only been defined by their absence of staining with other cell markers. We have developed a mouse monoclonal antibody, GBC-1, that reacts with this cell population in rat olfactory epithelium. GBC-1 stained cells are situated above the level of the horizontal basal cells in normal and bulbectomized epithelium, and some of them have also incorporated BrdU, demonstrating that the GBC-1 antigen is present on mitotically active basal cells. At 2 days following MeBr lesion, which destroys all of the neurons and sustentacular cells in over 90 % in the olfactory epithelium, GBC-1 (+) cells are very numerous. Neurons have not yet reappeared at this time, and the GBC-1 (+) cells do not stain with a sustentacular cell/Bowman's gland marker. These data are consistent with the conclusion that GBC-1 labels globose basal cells. In addition, GBC-1 labels some neurons in normal and bulbectomized epithelium and in MeBr-lesioned epithelium at later stages in its recovery. The pattern is consistent with the hypothesis that globose basal cells are direct neuronal precursors, and implies that the GBC-1 antigen is retained during some stage of neuronal differentiation. GBC-1 will be useful in understanding neurogenesis in the olfactory epithelium, and in the manipulation of globose basal cells for other analyses.
Supported by NIH grants K04 DC 00080 and R01 DC 02167.

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Selective Transport of RNA in Sensory Neurons of the Olfactory Neuroepithelium. C. DELLACORTE, L. C. JOHNSON, and D. LYNN KALINOSKI (Monell Chemical Senses Center, Philadelphia, PA 19104)

Principles of cell biology suggest the complex subcellular organization of eukaryotic cells requires selective transport of macromolecules to particular cell compartments. Specificity of sorting is largely achieved by particular signals that target proteins to appropriate destinations after the processing of transcripts at nuclear-associated compartments. Recent studies in neurons, however, suggest some particular mRNAs and associated polyribosomes are translocated to specific cellular positions distant from nuclear locations, near utilization sites.

Signal transduction in olfactory neurons is initiated by stimulation with odorants, resulting in a rapid G-protein-mediated increase in the second messengers adenosine 3'5'-monophosphate (cAMP) and inositol 1,4,5-trisphosphate ($InsP_3$). The proteins that mediate these processes are believed to be localized in cilia that extend from the olfactory knob at the apical end of olfactory neurons. Studies in our laboratory using isolated cilia preparations suggest the presence of selective RNA "trafficking" within these sensory neurons. Electron micrographs reveal polyribosomes localized to apical compartments within the olfactory neuroepithelium. Furthermore, RNA can be isolated from cilia preparations and used to generate cDNA for PCR studies. Examination of the subcellular distribution of specific mRNAs and the role this selection processing plays in olfactory neuronal functions is currently in progress. This work was supported by NIH grant #DC-01228

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OMP-lacZ Transgenic Mice Express lacZ and DBA Binding Sites in Distinct Subpopulations of Olfactory Neurons.
ERIC WALTERS[1], HELEN B. TRELOAR[2], BRIAN KEY[2], and FRANK MARGOLIS[2] (Roche Institute of Molecular Biology[1], Nutley, NJ and Dept. of Anatomy and Cell Biology, Univ. of Melbourne[2], Australia)

Our laboratory has recently generated transgenic mice in which we have targeted expression of lacZ to olfactory neurons under the control of a truncated promoter of the olfactory marker protein (OMP) gene in transgenic mice. In one line, H-OMP-lacZ-6, a subset of olfactory neurons express lacZ in a topographic profile similar to that reported by Key and Akeson (1993) for the *Dolichos biflorus* agglutinin (DBA) lectin. We thought it interesting to compare lacZ expression with (DBA) binding sites in these mice, possibly leading to the identification of a functionally distinct population of neurons. Postnatal day 1.5 mice from the H-OMP-lacZ-6 line were sacrificed, and heads fixed in 4% paraformaldehyde. Serial coronal frozen sections were cut and processed for OMP and lacZ expression, and DBA binding sites. The presence of many lacZ+/OMP+ and DBA+/OMP+ neurons were clearly seen in the neuroepithelium. Though individual, adjacent lacZ+ and DBA+ neurons were localized within the same "zone" of neuroepithelium, we did not detect neurons that were lacZ+/DBA+. Despite the segregated termination of many lacZ+ axons to distinct glomerular sites in the olfactory bulb, DBA+ axons were less convergent to distinct glomeruli. Studies of OMP and lacZ in adult transgenic mice demonstrated the continuance of this pattern throughout life. The topographic lacZ expression within selected populations of olfactory neurons suggests that this may be due to the site of transgene integration, placing it under the control of cis-acting regulatory sequences of an endogenous gene, perhaps an olfactory receptor. If so, one could envision the construction of coding maps that define the topological relationships between receptor neurons and distinct sites in the olfactory bulb. Current studies are aimed at mapping the site of transgene insertion to facilitate the identification of possible genes involved in development and organization of the olfactory receptor sheet.

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Stress-related proteins: Hsp70 and Ubiquitin in the Olfactory Organ of the Channel Catfish (*Ictalurus Punctatus*). ISABELLA ANDREINI,[1,2] CHRISTIAN DELLACORTE,[1] MUZ ZVIMAN,[1] and D. LYNN KALINOSKI[1] ([1]Monell Chemical Senses Center, Philadelphia, PA 19104, [2]School of Veterinary of Pisa, Pisa, Italy 56124).

Heat shock proteins are a group of evolutionarily conserved proteins synthesized in all organisms in response to elevated temperatures and other stress-inducing agents. The intracellular distribution of members of the 70 kilodalton class of heat shock proteins (Hsp70), and the proteolytic pathway-dependent protein ubiquitin, were determined by immunohistochemistry in the olfactory and non-sensory epithelium of the olfactory rosette in the channel catfish under control conditions and those that mimic environmental stress. Changes caused by physical stress (abrupt temperature change) and chemical stress (exposure to Dichlobenil, a pesticide known to be toxic to the olfactory mucosa of mammalians and amphibians) were examined.

Under normal conditions, immunoreactivity to Hsp70 was found to proteins within apical portions of cells in the non-sensory area of the olfactory rosette, while ubiquitin immunoreactivity was localized to the apical surface of cells within the sensory neuroepithelium. Upon heat shock and in the presence of Dichlobenil, a time-dependent emergence, shift and redistribution of Hsp70 and ubiquitin were seen within the non-sensory and sensory portions of the olfactory rosette. Reorganization followed recovery from stress. Western blotting performed with soluble extracts of the olfactory rosette, showed a band corresponding in apparent molecular weight to 70kD when utilizing anti-Hsp70 antibodies, and 8.6 kD when using anti-ubiquitin antiserum. These data demonstrate the presence of cellular stress proteins in the olfactory organ of this vertebrate and suggest a role in maintaining the integrity of olfactory tissues exposed to environmental stress.

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Ultrastructural Localization of NADPH-diaphorase Activity in the Olfactory Mucosa of Larval Sea Lamprey (*Petromyzon marinus*). JASBIR K. OSAHAN, MANSOUR HOSSEINI, ELLA WONG, BARBARA S. ZIELINSKI (University of Windsor, Windsor, ON Canada N9B 3P4)

L-arginine, which is reduced by nitric oxide synthase (NOS) in the presence of NADPH to form the signaling molecule, nitric oxide (NO), elicits strong electro-olfactogram responses from larval sea lampreys, extant fish of ancient vertebrate origin. We have observed that at the light microscope level, brain NOS immunoreactivity (antibody from Transduction Laboratories, Lexington, KY) co-localized with NADPH-diaphorase activity. Both reactions predominated in the supranuclear and basal regions of the olfactory epithelium (OE), the olfactory nerve and olfactory bulb glomeruli. We used NADPH-diaphorase electron microscopic histochemistry to investigate the subcellular sites of NOS distribution. In the supranuclear region of the OE, intense labeling was seen within secretory vesicles of sustentacular cells (SC) and in proximity to mitochondria within dendrites of olfactory receptor neurons and SC. In the OE basal region, staining was intense in the perinuclear cytoplasm of a subpopulation of basal cells and moderate in SC foot processes and in axons. Within these axons and those in nonmyelinated fascicles in the lamina propria, labeling predominated in profiles with mitochondria. These distinct subcellular sites of NOS activity suggest that in larval sea lampreys, NO exerts modulatory effects on processes associated with perireceptor events, axonal activity and the differentiation of basal cells.

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Immunohistochemical localization of two enzymes associated with glutathione metabolism: glutathione S-transferase pi and γ-glutamyl transpeptidase in the olfactory epithelium of rainbow trout. SUSAN L. STARCEVIC and BARBARA S. ZIELINSKI (University of Windsor, Windsor, Ontario, Canada, N9B 3P4)

Glutathione (GSH) is a tripeptide that functions in the cellular defense against free radicals, and endogenous and exogenous oxidants. In rainbow trout, GSH is elevated in dendrites of olfactory receptor neurons (Starcevic *et al.*, 1993, *Chem. Senses* 8:57-65) and may be active in the biotransformation of odorants and xenobiotics. In the present study, we observed elevated activity (477.6± 218 nmol/min/mg protein) of the phase two detoxifying enzyme glutathione S-transferase (GST), which catalyzes the conjugation of GSH onto hydrophobic and electrophilic compounds rendering them more water soluble. The GST pi class was demonstrated by Western immunoblot analysis (Crystal Chemical) and localized by immunofluorescence to the dendritic and perinuclear regions of olfactory receptor neurons. We investigated the expression of γ-glutamyl transpeptidase (GGT, antibody courtesy of Dr. A.R. Beaudoin, Univ. of Sherbrooke, Que.), to determine the cellular origin of the GSH. GGT expression, which indicates the site of cysteine liberation, was localized in the plasma membranes of basal cells, suggesting that cysteine, a rate limiting component for the synthesis of GSH originates from basal cells. These results support the hypothesis that GSH in olfactory epithelium is important for neuroprotection and/or odorant inactivation.

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Solitary Chemosensory Cells in the Olfactory Organs of Fish

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Solitary chemosensory cells (SCC) are epithelial sensory cells which occur in lower aquatic vertebrates. Little is known about their physiology, neuronal connections and even distribution. The aim of this examination was to find out whether SCC also occur in the olfactory epithelium of fish. Olfactory organs of two cyprinids, the goldfish, *Carassius auratus*, and the zebrafish, *Danio rerio*, were investigated by scanning and transmission electron microscope. Additional examinations were carried out on a series of axotomized goldfish. Our results show that SCC are also present in the sensory areas of the olfactory epithelium of the cyprinids examined, but their quantity and shape varies. In the zebrafish we found extremely few SCC. The bipolar cell body is slender. Its apical part ends in a single protrusion that extends into the lumen of the olfactory cavity. With a length of about 5 µm and a diameter of about 0.8 µm this apical protrusion is considerably larger than olfactory receptor cilia and nonsensory kinocilia, and they are easily distinguishable from the so-called giant cilia which sometimes occur as artefacts. The nucleus of the SCC is situated in the basal part of the cell. The upper part of the cell body contains numerous mitochondria and vertically set microtubules and intermediate filaments as well as coated vesicles and multivesicular bodies. In the goldfish, SCC are very common in the olfactory epithelium, but their number varies from one animal to the other and even within the lamellae of one organ. Contrary to the findings in zebrafish, the protrusion has a stout base that divides into several extensions. The number of vesicular structures is very high in many of the goldfish SCC and these vesicles often extend into the protrusions. After degeneration of olfactory receptor cells in axotomized fishes, SCC are easily visible. Their distribution seems to be even on one lamella. SCC are located only in the sensory areas of the olfactory epithelium, they do not occur within the islets of nonsensory kinocilia cells. Studies on frequency and distribution of SCC in the surface epithelia of the two fish species are in progress.

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Different types of olfactory receptor cells project to different areas of the olfactory bulb in catfish. Y. MORITA and T.E. FINGER, Rocky Mountain Taste & Smell Ctr., Denver CO 80262

Olfactory receptors generally are elongate bipolar neurons with an apical dendrite and basally directed axon. Careful examination of the epithelium shows, however, that the receptor cells have a variety of morphologies ranging from elongate, with a deeply-situated nucleus, to rather short and stubby with the nucleus situated relatively high in the olfactory epithelium. These morphological differences often have been attributed in the past to the cell's relative age, but may also indicate different morphological classes of receptors. In order to test whether the receptor cell morphology was related to bulbar projection pattern, DiI was applied to small regions of paraformaldehyde-fixed olfactory bulbs in catfish, *Ictalurus punctatus*. Following 1-2 weeks to permit diffusion of the dye, the bulb and attached epithelium were sectioned on a vibratome and examined with a fluorescence microscope. Following dye applications to the ventral olfactory bulb, the large majority of labeled receptor cells were tall, elongate cells. In contrast, following dye application to the medial or lateral faces of the bulb, relatively few tall cells were labeled. Instead the predominant type of labeled cell was short with a nucleus situated high in the epithelium. Whether these short receptor cells are microvillar receptors or include a class of ciliated receptor cell could not be determined. Future experiments entailing photoconversion of the DiI should resolve this issue. The results so far indicate that different populations of receptor cells connect to different parts of the olfactory bulb in these fish.

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DNA *in situ* hybridization localization of herpes simplex virus type I in the rat olfactory mucosa and the early immune effector cell response. MARILYN L. GETCHELL[1,2] & ANJALI KULKARNI-NARLA[3] ([1]Div. Otolaryngol., Dept. Surg.; [2]Sanders-Brown Ctr. on Aging; [3]Dept. Physiol.; University of Kentucky College of Medicine, Lexington, KY 40536)

Immune barrier function of the olfactory mucosa was investigated by intranasal inoculation of purified herpes simplex virus (HSV) type I (MacIntyre strain) or a control carrier solution (Tris-buffered NaCl) in 6-week old rats. After 1-5 days of incubation, rats were perfused with 2% paraformaldehyde, and the nasal mucosae were frozen and sectioned. DNA *in situ* hybridization was performed with a digoxigenin-labeled HSV probe to identify infected cells. One day after inoculation, virus was localized in clusters of mature olfactory receptor neurons (ORNs) primarily on the turbinates, in several immunocytes in the olfactory mucosa (OM), and in cells in the nasal-associated lymphoid tissue (NALT). At later times, virus was also detected in olfactory nerve fascicles and bundles. Immunohistochemistry with antibodies to markers for natural killer (NK) cells (clone 3.2.2), macrophages (ED2), and neutrophils (IgE, lactoferrin) demonstrated that the numbers and distribution of these cells differed in HSV^+ and control rats. At 1 day, increased numbers of these cells were present in the OM of HSV^+ rats and were observed intraepithelially rather than only the lamina propria as in controls. Marked changes occurred in NALT, where, in contrast to the peripheral localization of a few NK cells and macrophages in controls, these cells were distributed throughout the follicles. In particular, the follicle-associated epithelium and follicles immediately beneath it contained a very large number of macrophages. Increased numbers of neutrophils occurred at the periphery of NALT in HSV^+ rats. By 3-5 days, immunocyte numbers in the OM decreased but were still elevated compared to controls. In NALT, large numbers of these immunocytes were localized near peripheral blood vessels. Thus NK cells, macrophages, and neutrophils are among the earliest immune effector cells to respond to HSV infection of olfactory receptor neurons.

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A Hypothesis about Delayed Recovery of Inflamed Olfactory Epithelium in Cases of Sinusitis

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Sinusitis is reportedly the major cause of olfactory disorders. According to data we collected in the last 3 years, 47.5% of the cases of olfactory disorders could be attributed to sinusitis. During the clinical therapeutic course of sinusitis, olfactory dysfunction often remains after the recovery from other nasal symptoms even in curable cases. To elucidate the cause of this delayed recovery of olfaction morphologically, an influence of sinusitis on-the cell proliferation in the olfactory epithelium was experimentally studies; sinusitis was induced in 4 rabbits, and 2 normal rabbits were prepared as the control group. The labeling reagent 5-bromo- 2'-deoxyuridine (BrdU) was administrated, and 24 hours later the rabbits were sacrificed under intravenous anesthesia and the olfactory and respiratory mucosa were removed. The amount of cell proliferation in these tissues was estimated by immunohistochemical staining with an antibody to BrdU. During inflammation, the uptake of BrdU was significantly increased ($p<0.01$) in respiratory but not olfactory mucosa. Thus, the turnover of epithelial cells in the olfactory mucosa. this delay of the epithelial turnover seems to show one of the important causes of delayed recovery of olfaction in cases of sinusitis.

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Insulin-like growth factor I receptor immunoreactivity in rat olfactory epithelium: effect of diabetes. NANCY E. RAWSON[1], CHRISTIAN DELLACORTE[1], ISABELLA ANDREINI[3], MENEKHEM ZVIMAN[1], AND DIEGO RESTREPO[1,2]. [1]Monell Chemical Senses Center, Philadelphia PA 19104; [2]Dept. of Physiology, Univ. of Pennsylvania, Philadelphia PA 19107 and [3]Dept. of Veterinary, Univ. of Pisa, Italy.

The factors necessary for the ongoing neurogenesis and regenerative capacity exhibited by the olfactory system are not known. Insulin-like growth factor I (IGF-I) is involved in development, regeneration and repair of a variety of neuronal tissues (Ishii et al., Ann. NY Acad. Sci. 692:172-85). In the olfactory bulb, unlike most other brain areas, levels of IGF-I and its receptor remain high throughout life (Bohannon et al., Brain Res. 444:205-213, 1988). The role of this growth factor in the olfactory bulb has not been determined, nor has its presence in the olfactory epithelium been investigated. Using molecular and immunohistochemical methods, we have obtained evidence that the IGF-I receptor is synthesized within the olfactory epithelium of adult rats. A PCR product of the appropriate size was obtained from olfactory tissue cDNA using primers designed to specifically amplify a region of the IGF-I receptor cDNA sequence. To localize receptor protein, olfactory tissue sections were immunostained with a polyclonal IGF-I receptor antibody. Sparse but distinct immuno-reactivity was observed in olfactory epithelium of normal, adult rats localized to the nerve bundles, a population of basal cells and in a narrow layer above the nuclei of the supporting cells. Levels of IGF-I and its receptor in other tissues are altered by diabetes (Werner et al., Diabetes 39:1490-1497). One week after inducing diabetes with streptozotocin, immunoreactivity in olfactory epithelium was more robust and more extensive throughout the supporting cell layer. Immuno-labelling was also found in respiratory regions of the epithelium, with more extensive and intense reaction product in diabetic vs. normal rat tissue. These data suggest a role for IGF-I in the olfactory epithelium, and are consistent with findings of diabetes-induced increases in IGF-I receptor levels in other tissues.

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Quantitative Evaluation of Mitochondrial Size and Distribution in Olfactory Biopsies from Alzheimer's and Control Subjects. PAMELA M. ELLER, EDWARD W. JOHNSON, MIRIAM R. LINSCHOTEN, BRUCE W. JAFEK (Rocky Mountain Taste and Smell Center, UCHSC, Denver, CO)

Initial observations of the ultrastructural characteristics of olfactory epithelium biopsied from subjects with probable Alzheimer's Disease (AD) led to the hypothesis that the overall size and number of mitochondria in support cells increases in the disease state. We have undertaken a detailed quantitative study of mitochondria in support and receptor cells from probable AD and age-matched control volunteers. Tissue sections for electron microscopy were collected according to a strict protocol to insure that no cell was sampled twice. Measures of mitochondrial cross-sectional area and major and minor axes were taken from electron micrographs. Strict criteria were established for inclusion of cells in the quantitative sample. Results indicate that in control and AD subjects a significant difference exists between the size of mitochondria in receptor cells and support cells. However, no significant difference exists between mitochondria of receptor cells from probable AD subjects when compared to those from control subjects. In contrast, mitochondria in support cells from AD subjects showed significant increases in size over those of controls. In age-matched control subjects, mitochondria in support cells averaged $0.052\mu^2$ (±0.003) in cross-sectional area compared to $0.077\mu^2$ (±0.009) for AD subjects (mean ±sem). These differences in mitochondrial size may reflect alterations in mitochondrial cytochrome oxidase function in AD as postulated by other investigators.

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Olfactory epithelium slice cultures: A useful method for investigating olfactory neuronal differentiation, axon outgrowth and target interaction. WEI-LIN LIU, QI-ZHI GONG*, MONICA SRODON, and MICHAEL T. SHIPLEY (University of Maryland and Rockefeller University*)

Early olfactory axons follow a unique path to reach their CNS target tissue and may play a determinate role in olfactory bulb morphogenesis. Several extracellular matrix and cell surface molecules are present along the developing olfactory pathway. However, as it is technically difficult to manipulate embryonic mammals *in vivo*, the functional roles of these molecules in olfactory axon outgrowth, pathfinding and targeting are unknown. The goals of this study were to: (1) develop an olfactory epithelium slice culture from young rat embryos; (2) establish "baseline" conditions that produce consistent patterns of axon outgrowth in defined medium; and (3) investigate the functions of molecules present along the developing olfactory pathway, *in vivo*.

E13 embryos were removed from timed pregnant rats. The heads were embedded in 3% low melting point agarose in L15 medium at 37°C; 400 µm parasagittal slices were cut with a vibratome. The olfactory epithelium was dissected from the slice and placed in Millicell-CM culture plate membrane inserts coated with either type I rat tail collagen, calf skin collagen, laminin, fibronectin or matrigel. Slices were incubated in serum-free modified Waymouth's medium [100% humidity in air/5% CO2 at 37°C] for 5 days; media was changed every 2 days.

Nearly all slices were healthy (vital dye staining) and extended abundant processes identified as axons with either neuronal specific tubulin (NST), which is present in all neurons and axons, or growth associate protein (GAP 43), which is present in growing neurons and axons. Axons extended on all substrates tested, but grew best on membranes coated with matrigel, which contains several growth factors and extracellular matrix molecules. Axons grew least well on membranes coated with fibronectin only. The majority of the neurons in the slice and the growing axons expressed OMP. This demonstrates that olfactory neurons do not require contact with target tissues to express OMP. Numerous cells migrated out from the slices. Some migrating cells were positive for NST indicating that they were neurons; many more cells were identified as glia based on their expression of S-100, GFAP and the low affinity NGF receptor. Neurites always extended in advance of the migrating glial cells.

The present slice culture system should be useful for investigating: (1) growth factors and substrates that regulate olfactory axons outgrowth and targeting; and (2) the influence of olfactory axons on the induction and differentiation of the olfactory bulb. [Supported by: NIH DC00347 & NS29218]

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Expression and Induction of Cyclin D1 and PCNA During Cell Cycle Progression and Apoptosis in the Rat Olfactory Epithelium

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The expression of cell cycle-related proteins, cyclin D1 and proliferating cell nuclear antigen (PCNA) and p53 was investigated in the olfactory epithelium (OE) during embryogenesis and in 3 week-old rats following bilateral olfactory bulb ablation (OBX), two conditions in which cell division and apoptosis are up-regulated. Cyclin D1, a protein involved in the progression of G1 → S phase transition, and PCNA, an antigen primarily induced during the DNA replication phase of the cell cycle, and p53, a tumor suppressor gene product, were localized using monoclonal antibodies in standard immunocytochemical protocols. Apoptotic cells were identified by the end labeling of fragmented DNA with digoxigenin-dUTP using terminal deoxynucleotidyl transferase and the anti-digoxigenin-peroxidase detection system. During development, at embryonic days 14, 16, and 19, and postnatal day 2, cyclin D1 and PCNA immunoreactivities were localized in cells located close to the basement membrane and also in the apical region of the OE; p53 was expressed in the cells of olfactory receptor neuronal layer. Apoptotic cells were observed in the apical region of the epithelium. In the OE of the 3 week-old rat, few cells located only in the basal region, expressed cyclin D1 and PCNA. Apoptotic cells, located apically, were rarely observed. At 3, 6, and 9 day post-OBX, the number of cells expressing cyclin D1 and PCNA in the basal and apical regions of the OE, and the apically located cells undergoing apoptosis were greatly increased when compared with sham-operated rats. The results of this study demonstrate that cyclin D1 and PCNA are induced during periods of active cell cycle progression and apoptosis in the OE.

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Luteinizing Hormone-Releasing Hormone (LHRH) Neurons Migrate Normally In Neural Cell Adhesion Molecule (N-CAM)-Deficient Mice. M. SCHWANZEL-FUKUDA and D.W. PFAFF (Rockefeller University, New York, USA); K.L. CROSSIN (Scripps Research Institute, La Jolla, CA. USA); H. CREMER (Developmental Biology Institute of Marseilles, Marseilles, France); J.-P. HARDELIN and C. PETIT (Pasteur Institute, Paris, France).

LHRH neurons originate in the epithelium of the medial olfactory pit and migrate into the brain along a migration route formed by N-CAM-immunoreactive central fibers of the vomeronasal and terminal nerves. This study examines LHRH cell migration in mice in which the N-CAM gene had been inactivated. Previous studies of these mice showed a complete deletion of the gene for N-CAM and a 36% reduction in the size of the olfactory bulbs (Cremer et al., Nature vol. 367, 1994). Antibodies to LHRH and N-CAM were used to examine the brains and nasal regions of paraffin-embedded embryos 12, 13 and 14 days old. Homozygous embryos showed a complete absence of N-CAM in the brains and nasal regions and a normal pattern of LHRH cell migration. The presence of N-CAM in heterozygous littermates provided good positive controls. The fact that LHRH neurons migrate normally in these mice indicates that N-CAM is not essential for their migration, and suggests that another cell adhesion molecule or protein may compensate for this molecule. Supported by NIH grant DC 00880 (M. S.-F.)

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Long Term Growth of Rat Olfactory Epithelium Transplants in Host Brain. JOHN C. DENNIS and EDWARD E. MORRISON (Department of Anatomy and Histology, College of Veterinary Medicine, Auburn University, AL 36849-5518)

The olfactory epithelium (OFE) by virtue of life long neuron production offers a convenient system in which to study neurogenesis. The development of in vitro systems has allowed culture of OF neurons up to several weeks. Transplantation offers the advantage, over in vitro systems, of significantly longer survival of all the major OFE cell types and in particular, the sensory neurons. Here, we report some observations of year old OFE transplants made by immunocytochemistry, silver staining and TEM. The transplants contain large vesicles as well as many small epithelium-lined vesicles wherein most of the mitotic activity, indicated by BrdU incorporation, is located. Vigorous axogenesis is revealed by both microscopy and immunocytochemistry. The low epithelium lining the largest vesicles in transplants contain few or no neural cell adhesion molecule (N-CAM) positive cells but prominently staining axon bundles frequently appear in these regions. Conversely, N-CAM(+) cells are numerous in small vesicle profiles and occasionally occur in loosely organized groups at the host-transplant interface. Most or all of these cells and axons are also either growth associated protein (GAP-43) or olfactory marker protein (OMP) positive. The expression of GAP-43 indicates vigorous axogenesis and the existence of OMP(+) cells demonstrates that at least some of the transplant-derived neurons are expressing the olfactory developmental program a year after transplantation. A few cell bodies are GAP-43(+)/OMP(+) suggesting that cells are always being added to the OMP-expressing population. This observation together with the relatively small number of OMP(+) neurons compared to N-CAM(+) cells further suggests that OMP(+) cells are being removed from the transplant neuron population. These results demonstrate that OFE continues to generate neurons that express several OF markers for at least a year following transplantation to cerebral cortex.
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Development of Rat Olfactory Receptor Neuron Subclass Reactive with Monoclonal Antibodies to HSP70. V.McM. CARR AND A.I. FARBMAN (Northwestern University, Evanston, IL 60208-3520)

We have recently described a small subpopulation of olfactory receptor neurons (ORNs) in adult rats that is reactive with a monoclonal antibody (Mab 2A4) directed to the 70 kD heat shock protein (J.Comp.Neur. 348:150-160,'94). Immunoreactivity (IR) is not stress related. These 2A4(+) ORNs are scattered nonuniformly through the olfactory epithelium (OE) and project to just 2-3 glomeruli in consistent locations in each olfactory bulb (OB). We now report results of developmental studies of these 2A4(+)ORNs. Paraffin sections from rats of various stages from embryonic day E14 to postnatal day P42 were examined immunohistochemically as previously described. ORN 2A4 IR first appears between P7 and P10, when a very small number of faintly reactive 2A4(+)ORNs are distinguishable in the OE at widely scattered locations. 2A4(+)ORNs become readily evident by P14 and by P28 occur at densities that appear comparable to those of adults. Axonal IR, pronounced in adults, is first apparent in glomeruli of the OB at P14. Preliminary analysis indicates that the location of these 2A4(+) glomeruli is the same as or only slightly posterior to that seen in adults. This is despite the very large amount of OB growth and development that occurs postnatally. Numbers of reactive glomeruli appear similar as well. Autoradiographic birthdating and quantitative analysis of 2A4(+)ORN densities at various stages are currently in progress.

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Expression of Hu neuronal proteins during early olfactory development MICHAEL T. SHIPLEY[1], WEI-LIN LIU[1] AND HENRY FURNEAUX[2] (University of Maryland and Sloan-Kettering)

There is evidence that pioneer olfactory axons modulate the proliferation and differentiation of telencephalic precursor cells to induce the morphogenesis of the olfactory bulb. If true, the region of the telencephalon containing pioneer axons should have more cells differentiating into neurons than the rest of the telencephalon. The Hu family of mammalian proteins consists of Hu D, Hu C and Hel N-1. These proteins are the human homologues of Elav, which is required for neurogenesis in Drosophila and are the earliest known proteins expressed exclusively by mammalian neurons. Monoclonal antibody 16A11 binds specifically to an epitope present in the products of all known Hu genes. Mab 16A11was used (1) to investigate neuronal development in the olfactory epithelium; (2) to determine if pioneer olfactory axons are accompanied by neurons en route to the olfactory bulb primordium; and (3) to determine if the penetration of pioneer axons into the telencephalic ventricular zone is associated with increased numbers of cells expressing the Hu antigen. Hu expression was examined from E12-20.

At E12-13, Hu+ neurons were located in the basal half of the olfactory epithelium. From E14 to E20 Hu+ cells increased dramatically and were present throughout the basal two-thirds of the epithelium. Numerous globular-shaped cells expressed Hu in the deepest part of the epithelium.

From E12-15, numerous Hu+ cells were present along the trajectory of the developing olfactory nerve. Double staining for Hu and GAP 43 confirmed that these Hu-positive neurons were intimately associated with growing olfactory axons. These cells may be migrating from the olfactory epithelium. From E16-E20, Hu+ cells were not observed along the olfactory pathway.

At E12, Hu+ cells comprised a single layer located just below the surface of the entire telencephalic vesicle. Hu+ cells were not present in the ventricular zone except in a small ventral region corresponding to the site containing pioneer olfactory axons. This part of the telencephalon had more Hu-positive cells. At E15-16 the olfactory bulb appeared. A thick layer of Hu+ cells was present throughout the olfactory bulb but none were present in ventricular zone. From E18 to E20, the Hu-positive cell layer became thinner as the volume of the olfactory bulb increased.

These findings indicate that: (1) Hu is expressed by very immature ORNs and possibly by ORN precursors; (2) neurons are present along the olfactory pathway from its earliest stages of development (3) the arrival of pioneer olfactory axons appears to be associated with the differentiation of neuronal precursors in the telencephalon. [Supported by NIH DC00347, NS29218 (MTS) & NS29682 (HF)]

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The Dynamics of Morphological Transformations in the Olfactory Sensilla of Blue Crabs as a Function of Salinity. RICHARD A. GLEESON[1], LORRAINE M. MCDOWELL[2], HENRY C. ALDRICH[2]. ([1]The Whitney Lab. and [2]Dept. of Microbiology and Cell Science, University of Florida)

The olfactory sensilla (aesthetascs) of the blue crab, Callinectes sapidus, each contain the outer dendritic segments (ODS) of from 40 to 160 chemosensory neurons. For much of there length these processes are separated from the external environment by a thin cuticle that is permeable to odor molecules. It was earlier shown that the length of the ODS is considerably shorter in freshwater-acclimated (FW) crabs relative to seawater-acclimated (SW) animals. In this study we have examined the time course of length changes in the ODS as a function of salinity changes. Using differential interference contrast light microscopy it is possible to measure the lengths of the ODS within aesthetascs. A significant increase in length of the ODS was evident within 24 to 48 hr following transfer of FW crabs to seawater; the lengths of the ODS were comparable to those of SW animals by 96 hr. A similar time course for regrowth was found for SW animals in which the ODS were ablated using distilled water. Conversely, transfer of SW animals to lower salinities results in a concomitant reduction in length of the ODS. In FW crabs transferred to seawater there is an increase in the response of aesthetascs to odor stimulation, as measured physiologically, which corresponds to the increase in length of the ODS. We propose that the increase in length of the ODS, as a function of increased salinity, represents selective growth of the ODS and may possibly reflect a continuous process of turnover such as occurs in the ODS of photoreceptors.

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Localization of superoxide dismutases in rat olfactory and vomeronasal receptor neurons during ontogeny.
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Superoxide dismutases (SODs) are metalloenzymes that scavenge destructive oxygen free radicals produced primarily during oxidative metabolism. Immunohistochemistry with antibodies to mitochondrial MnSOD and cytosolic CuZnSOD as well as PGP 9.5 to confirm neuronal localization was used to investigate the expression of these proteins in olfactory and vomeronasal receptor neurons (ORNs, VRNs) at embryonic (E) days 14, 16, and 19 and postnatal (P) days 2, 6, 11, and 24. At E14, ORNs exhibited immunoreactivity (ir) for both Mn and CuZnSODs. The intensity of MnSOD ir increased gradually from E16 to a maximum at P24. In contrast, a slight decrease in CuZnSOD expression was observed between E14 and E19, followed by a gradual increase through P24. VRNs expressed weak MnSOD ir at E14. Intense MnSOD ir was evident in VRNs from E16 to P24. Weak CuZnSOD ir in VRNs at E16 gradually increased to a maximum at P6 and remained constant through P24. Intense ir was also seen in neurons in the olfactory lamina propria and at the dorsomedial margins around the vomeronasal sensory epithelium at E16. Computer-generated pseudocolor images of the relative intensity of MnSOD ir demonstrated that the most intense ORNs were located superficially and near the basement membrane of the olfactory epithelium at E14 and E16; by E19 intensely stained neurons were located throughout the upper 2/3 of the epithelium. The most intense VRNs were located near the lateral margins and along the basement membrane of the sensory epithelium at E16 and E19. Results suggest that MnSOD protects ORNs and VRNs from oxygen free radicals generated during differentiation and maturation in embryonic and postnatal periods, and that CuZnSOD protects against metabolic oxygen free radicals and those arising from ambient oxygen, xenobiotics and inflammatory reactions postnatally.

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Comparison of Olfactory and Vomeronasal Receptor Cell Division Rate in Breeding and Non-breeding Salamanders: Variation with Natural Life Cycle. ELLEN DAWLEY, CORRIE STANKIEWICZ, STEVEN WAGNER, AND AMANDA FINGERLIN. (Ursinus College, Collegeville, PA, 19426)

Continued mitosis of cells within the olfactory/vomeronasal epithelium to produce new receptors cells is thought to be an adaptation to replace receptors damaged by daily exposure to odorants. We propose that new receptors also may be generated prior to the breeding period of salamanders to enhance chemoreception for courtship activities. Chemoreception may be particularly important for mate recognition and courtship among salamanders, and in previous work we showed that the volume of the vomeronasal epithelium of red-backed salamanders (*Plethodon cinereus*) varies on a yearly cycle. These terrestrial salamanders begin mating in October and the mating season continues until May, as weather permits. Eggs are laid in June and a new gametogenetic cycle begins and continues throughout the summer. Both males and females have significantly larger vomeronasal organs in the summer pre-breeding period than at any other time of the year. We injected freshly collected salamanders with 5-bromo-2'-deoxyuridine (BRDU), a thymidine analog which is incorporated into the DNA of dividing cells. After one week, we sacrificed the animals and used immunocytochemistry to locate cells containing BRDU, and counted labeled cells per mm^2 of epithelium. Salamanders collected in the summer had significantly more labeled cells than salamanders collected in May or October (ANOVA, single classification). We propose that the increased cell division during the summer generates new receptor cells that may be particularly adapted to detecting mates and mediating courtship activities. Hormones (e.g., testosterone) may affect receptor cell division rate, and differences in circulating hormones throughout the year may correlate with differences in receptor cell division rate.
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Distribution of Nestin Positive Cells in the Developing Rat Vomeronasal Organ. TOSHIYA OSADA[1], MASUMI ICHIKAWA[2], and RICHARD M. COSTANZO[1] (Virginia Commonwealth University[1], Richmond Virginia, Tokyo Metropolitan Institute for Neuroscience[2], Tokyo Japan)

The ability to identify neuronal precursor cells is an important element in the study of neurogenesis and development of neurons. Recently nestin has been shown to be a marker for precursor cells in the rat central nervous system (Lendahl, Zimmerman & McKay, Cell 60:585-95,1990). In the present study, we examined the distribution of nestin positive cells at different stages in the development of the vomeronasal organ. At postnatal day 1 (P1), we observed that most cells within the sensory epithelium were nestin positive, except for those occupying the supporting cell layer. By P8, the vomeronasal organ had increased in size although the distribution of labeled cells remained unchanged. At P16, nestin positive cells occupied a more basal region of the epithelium, and by P22 they were restricted to a narrow layer along the basement membrane. By stage P29, the vomeronasal organ had reached structural maturity and only a few nestin positive cells were observed. Vimentin, another marker protein for precursor cells was also examined and its distribution was similar to nestin, with only a few vimentin labeled cells present at maturity. Findings from this study suggest that nestin-positive cells observed in the developing vomeronasal organ represent neuronal precursor cells and that nestin may play an important role in future studies of neurogenesis and development in chemosensory systems.

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Taste Properties of the Quaternary Amine: Benzyltriethylammonium Chloride (BTAC) in Rats. CHARLES N. STEWART and MARCUS W. THOMSEN (Franklin & Marshall College, Lancaster. PA)*

The quaternary amine, BTAC, was selected for study because it may provide a useful model for the study of structure/activity relationships in bitter taste transduction. Two-bottle preference tests revealed that BTAC was rejected when paired with distilled water but preferred over isomolar quinine HCL(Q). In conditioned taste aversion (CTA) tests, the CTA established to BTAC by pairing ingestion with an injection of LiCL (0.15M at 2% of body weight) generalized to Q but not to denatonium benzoate (DB) although a CTA to DB did generalize to Q. Thus BTAC can be regarded as a bitter, in that it has quinine-like properties. Synthesis of the chloro, flouro and nitro (para-substituents) forms of BTAC showed that the bitter properties were retained, in that a CTA to BTAC did generalize to each of these derivatives. Preliminary data suggest that there may also be some rats who are non-tasters of BTAC thus making this compound of possible interest for genetic studies.

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Amylase-Like Enzymes Mediate Polysaccharide Stimulated Feeding Responses in *Uca pugilator*. D. RITTSCHOF, M. JACKSON, J. BASKIN and E. BERGSON. Duke University Marine Laboratory, Beaufort, NC 28516-9721.

Sand fiddler crabs, *Uca pugilator* Bosc, are intertidal deposit feeding crustaceans. Stereotyped feeding is evoked upon stimulation of chemoreceptors on dactyls and minor chelae (Robertson et al., 1982; Rittschof & Buswell, 1989). Natural feeding stimulants are tightly bound to sediment particles. In laboratory feeding studies, dextrose is among the best feeding stimuli. However, crabs respond to dextrose and its polymers on a weight rather than upon a molar basis. We postulated that amylase-like enzymes were involved in chemically stimulated feeding. The hypothesis was that enzymes liberate dextrose residues from polymers and that these residues evoked feeding. Tests of enzymatic activity showed amylase activity in the fluid secretion (spit) that crabs mix with sand brought to the buccal region by the minor chelae. On a per mg of protein basis, *U. pugilator* spit contains about 1/2 the amylase activity of human saliva. Polyacrylamide gel electrophoresis of crab spit showed protein banding patterns with molecular weights similar to amylase in human saliva. Amylase activity was also detected on the dactyls. Dactyl stimulated feeding responses to starch solutions could be eliminated by inhibition of amylase inhibitors. These data support the hypothesis that enzymatic degradation of polysaccharides is involved in detection of, and feeding responses to, polysaccharides. The enzyme in crab spit probably functions in taste and in liberation of food from sediments.

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Synergism Between MSG and IMP in Taste Preference of Rats. E. R. DELAY[1], J. O. HARBAUGH,[1] K. D. CATRON,[1] and S. D. ROPER[2] (Rocky Mtn. Taste & Smell Center, Univ. Colo. Health Sci. Cntr., Denver, CO; [1]Dept. Psychology, Regis Univ., Denver, CO; [2]Dept. Anat. Neurobiol., Colo. State Univ., Ft. Collins, CO)

Monosodium glutamate (MSG) is used widely as a taste enhancer. Taste enhancement may be due to synergism between MSG and other taste stimuli. However, MSG taste synergism has not been well documented for substances other than ribonucleotides. Rifkin and Bartoshuk (1980) described an analytical approach to demonstrate synergism between MSG and disodium 5'-guanylate on taste in humans. We used a variation of this approach to test for synergy between MSG and inosine 5'-monophosphate (IMP) in taste preferences of rats. Non-water-deprived adult rats were tested daily with a Davis MS80 device. Each day, 6 taste stimuli and water were tested in 16 trials. During each trial, a stimulus was presented for up to 5 minutes before the shutter was closed and the next trial began. If the rat responded during the 5 min interval, licks during the next 30 seconds were counted and then the shutter was closed. One to 3 water tests ("washouts") were presented between each taste stimulus. A first series of experiments was conducted to establish concentration preference gradients. Rats preferred MSG up to 200 mM and IMP up to 8 mM. In a second series, 10 naive rats were tested in three phases: Pretest, Test, and Posttest. Pretest and Posttest phases established the baseline lick rates of each rat for MSG (0, 10, 20, 30, 40, 50, and 100 mM) and IMP (0, 1, 2, 3, 4, 5, and 10 mM). During the Test phase, 6 mixtures of MSG and IMP were tested: MSG\IMP, in mM = 0\0, 0\5, 10\4, 20\3, 30\2, 40\1, and 50\0. During the Posttest phase, the Pretest procedures were repeated. Since there were substantial individual differences in lick rates, data for each rat were transformed into a ratio by dividing the lick rates for taste stimuli by the lick rates for water trials. Ratios for the *mixtures* which were significantly higher than the sum of the ratios for MSG and IMP taken *separately* indicates synergy (Rifkin & Bartoshuk, 1980). ANOVA comparison of ratios showed that rats drank at significantly higher rates than predicted from the simple addition of responses to IMP and MSG, alone. These results indicate synergy between IMP and MSG regarding taste preferences of nondeprived rats. The study provides an experimental approach for quantifying synergy between MSG and other taste stimuli.

Rifkin, B., & Bartoshuk, L.M. (1980). Physiology & Behavior *24*, 1169-1172.

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The Role of Taste in Oral Self-Administration of Phencyclidine-like Compounds.
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A procedure has been developed to study the taste qualities of drugs. Rhesus monkeys, trained to discriminate quinine sulfate [0.3 mg/ml] from water, were tested with phencyclidine, dizocilpine, and ketamine. Phencyclidine and ketamine were equipotent in this procedure, producing greater than 80% quinine-appropriate responding at a concentration of 0.56 mg/ml. Dizocilpine was more difficult to assay because, in the range of concentrations where it was active as a taste stimulus, it produced intoxication. A separate group of monkeys was given the opportunity to orally self-administer each of these drugs. Only one of five monkeys reliably self-administered more ketamine [0.0625 - 8 mg/ml] than water. All five monkeys self-administered phencyclidine [0.016-0.5 mg/ml] and three of five monkeys self-administered dizocilpine [0.004-0.125 mg/ml]. These results, taken together, indicate that taste may play a role in whether a compound is self-administered. In the range of concentrations that are pharmacologically active, dizocilpine had little or no taste, phencyclidine had enough taste to indicate that the drug was present, and the taste of ketamine was an obstacle to establishing oral self-administration. The relationship between the "taste" potency and systemic potency may account for the differences that exist among phencyclidine-like drugs in their capacity to function as oral reinforcers in rhesus monkeys.

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Preference for Glucose and L-Amino Acids in Streptozotocin-induced Diabetic Rats
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Amino acid preferences are known to change with alteration of protein and amino acid metabolism under conditions of amino acid imbalance in the diet and metabolic diseases. In diabetes mellitus, impaired insulin secretion is common and is responsible for many of the metabolic abnormalities associated with this disease. The patients with diabetes mellitus generally display a strong preference for sweet-tasting foods containing carbohydrate and sugars. This behavior is reasonable to seek for higher required glucose sources among foods to compensate for disorder of peripheral glucose utilization by failure of insulin secretion. Rats with non-insulin dependent diabetes mellitus had retarded insulin release by glucose, but they were able to release it responding to amino acids i.e., L-leucine and L-arginine, both *in vivo* and *in vitro* studies. In the present study, preference for glucose and amino acids in a choice paradigm of these solutions was examined with the establishment of diabetes mellitus before and after Streptozotocin (STZ) administration to Sprague-Dawley male adult rats. Preference for amino acids was tested to observe the ability to lower plasma glucose level. Preference for glucose in rats was evoked several hours after STZ treatment (50mg/kg, BW) and reached a plateau level for a week. Then rats began to ingest arginine and a mixture of branched chain amino acids (leucine, isoleucine and valine). Thereafter glucose intake declined. In contrast, preference for glucose and amino acids was not altered before and after treatment with STZ (25mg/kg, BW) when insulin secretion and glycemic control were still within normal range, similar to sham-operated controls. Histological findings of the pancreas also supported these behavioral changes. It is well known that amino acids , especially arginine and leucine are potent to release insulin from the pancreas. These amino acids could improve energy metabolism and the abnormal preference for glucose in diabetic animals. The oral administration of arginine or a mixture of branched chain amino acids are therefore possible therapeutic candidates in the status of diabetes mellitus.

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Effect of Selective Vagotomy on Dietary Choice between L-Lysine Deficient and Protein-Free Diets in Rats

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The effect of either total or selective vagotomy on dietary choice between L-lysine (Lys) deficient and protein-free diets with or without i.p. Lys infusion in Sprague-Dawley rats was studied to determine the roles of the vagus nerve to induce adaptive behavioral changes in an amino acid deficiency. Our previous study showed that intact rats developed an aversion to a Lys deficient diet within a few days, choosing a protein-free diet more than the Lys deficient diet and that i.p. infusion of Lys extinguished this aversion to the Lys deficient diet. In the current study rats received either total subdiaphragmatic, hepatic, gastric, or coeliac vagotomy 5 days before the onset of choice paradigm, and subsequent 14 days food intake of each diet was recorded. The sham vagotomized rats gradually chose the protein-free diet more than the Lys deficient diet, whereas this process was delayed in the hepatic vagotomized group. The total vagotomized rats dominantly chose the Lys deficient diet more than the protein-free diet throughout the experiment. When i.p. Lys was infused, both the total vagotomized and the gastric vagotomized rats selected the Lys deficient diet in an amount which was less than sham vagotomized ones. The hepatic vagotomized rats transiently delayed selection of the Lys deficient diet. These results suggest that the vagus nerve is necessary in development or extinction of an aversion to an amino acid deficient diet, and that the cooperativity of each branch of the vagus nerve might play an important role to each other in this adaptive process to Lys deficiency.

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Preference for Glucose and Changes among Activin βA subunit, Follistatin and Insulin in Pancreatic Islet of Diabetic Rats Induced by Cyproheptadine

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It is well known that animals including humans display strong preference for sugars when they fall in the status of diabetes mellitus. Activin A, homodimer of βA subunit, stimulates insulin secretion from the pancreas. Immunoreactive βA subunit of activin was localized in A cells and B cells of Langerhans' islets of pancreas, and follistatin, activin-binding protein, in B cells. The complex of follistatin and activin A has no physiological activity. The interaction between activin/follistatin and insulin was examined immunohistochemically for the βA subunit of activin and for follistatin in the islet of insulin-depressed rats induced by the administration of cyproheptadine (CPH), an inhibitor of insulin synthesis. The administration of CPH (45 mg/kg, BW, p.o.) to Sprague-Dawley rats once a day for two days induced a strong preference for glucose solution along with decreased immunoreactivity of insulin in the B cells. Insulin reactivity was recovered at 48h and 72h after the last administration of CPH. Preference for glucose normalized gradually. Immunoreactivity of follistatin was also decreased by CPH treatment and recovered in a similar manner to insulin in the B cells and the preference for glucose. CPH treatment did not affect the immunoreactive βA subunit in A and B cells. Immunoreactivity of βA-subunit did not change clearly compared with the case of controls. Thus, localization of follistatin in B cells was strongly affected by treatments with CPH, while βA-subunit of activin A remained normal. Therefore, follistatin and its complex with activin A may play more important roles than activin itself in the regulation of pancreatic function, especially insulin synthesis and secretion, by controlling activin activity in the B cell. The degree of glucose preference can be a useful diagnosis for status of diabetes mellitus and whether the function of B cells is normal or not.

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Exposure to an Odor Can Affect Later Ingestive Behavior in Preweanling Rat Pups. Marcy C. Wilson, H. Moore Arnold, W.G. Hall (Duke University)

Behavioral activity, orienting responses, and licking, chewing, and swallowing are all components of ingestive behavior that lead to food intake. Manipulation of the responsiveness of one component may cause changes in the other components. We examined the effect of olfactory experience on later mouthing responses in twelve-day-old rat pups. An anterior oral cannula was inserted into pups' mouths to deliver oral saccharin infusions. A baseline rate of responding was computed from the average of three infusions each two minutes apart. Three groups were tested: the Odor condition, and two controls- the Nothing condition and the Clean air condition. During the Exposure period, the pups in the Odor condition experienced an odor (unsweetened grape Kool-Aid) delivered into their container every two minutes for 40 minutes. Clean air or nothing was delivered into the containers of the Clean air or Nothing condition pups during this period, and activity was measured in each condition. Following the Exposure period, pups in all groups were given three additional oral saccharin infusions and response rates were compared to baseline. Pups in the Odor condition were found to be less responsive to oral infusions relative to their baseline. Pups in the Nothing and Clean air conditions exhibited equivalent and increased responses, respectively. Analysis of activity during the exposure period indicated that pups in the odor condition habituated to the odor over time. This was exhibited by high activity levels early in the exposure period that decreased with time. These results demonstrate that olfaction - an appetitive, non-nutritive component of ingestion - influences consummatory responding.

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Changes in Licking Microstructure During Sucrose Meals in the Rat. PERRIN A. KLUMPP[1], JOEL M. KAPLAN[2], and ALAN C. SPECTOR[1] (University of Florida[1], Gainesville, FL and University of Pennsylvania[2], Philadelphia, PA)

The quantification of ingestion rate over the course of a liquid meal can provide insight into the manner by which taste and postingestive factors interact to influence amount consumed. Ingestion rate, however, is a derived measure that offers little information about underlying behavior which is expressed as a series of lick bursts and the pauses that separate them. Using a linear regression model, we characterized respectively the sequence of bursts and pauses within sucrose meals. Twenty-two rats were presented with an ascending series of sucrose concentrations (0.03, 0.1, 0.3, & 1.0 M) during consecutive daily 1-h sessions. Rats were tested in both nondeprived and 23-h food-deprived conditions. A line was fit (least squares) to each animal's data for each condition and the parameters of the fits served as scores in an analysis of variance. The y-intercept for burst size increased and for pause duration decreased significantly ($p < .05$) as a function of concentration. This initial behavior presumably reflects the effect of taste. The effects of concentration on the slopes of burst size and pause duration were complementary; the negative slopes for burst size steepened ($p < .05$), and the slopes for pause duration increased positively ($p < .05$). These effects on slope presumably reflect the contribution of postingestive factors. The only effect of deprivation state was to curtail the steepness of the slopes for burst size ($p < .05$). These findings suggest that deprivation state enhances total sucrose intake not by affecting the taste-sensitive initial behavior, but by opposing the satiating influence of the postingestive load on burst size.

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Sucrose/NaCl but not Saccharin/NaCl Mixtures Increase the Intake of NaCl by the Sodium Depleted Rat. S.P. FRANKMANN, N.E. EICHELBERGER, F. KNIGHT and M. ZINGG (U. of Southern Colorado, Pueblo, CO)

Sodium depletion produces a specific appetite for salt and increases the acceptability of normally rejected NaCl solutions. We have previously shown that when the natural sweetener, sucrose, is offered to sodium depleted rats, the rats consume the sucrose and subsequently reduce their intake of NaCl. This experiment was designed to ask if the artificial sweetener, saccharin, produces similar results. Saccharin is a complex taste of both sweet and bitter, thus, quinine (QHCl, a bitter tasting compound) was also used, as a control for the bitter component of saccharin. Male, Sprague Dawley rats (n = 8) were sodium depleted (10 mg furosemide (s.c.) and overnight sodium deficient diet). Twenty-four h later, the rats were given 1 h access to a solution of 0.3M NaCl in choice with: 1) water, 2) 0.3M NaCl/0.3M sucrose mixture, 3) 0.3M NaCl/0.3mM QHCl mixture, or 4) 0.3M NaCl/5mM saccharin. Intakes were measured to the nearest ml at 15 and 60 min. As can be seen in Table 1, the intake of unadulterated 0.3M NaCl was unaffected by the alternate solution. However, the intakes of NaCl in mixture with QHCl (bitter) or sucrose (sweet) were of similar volumes at 15 min (3.45 vs. 4.80 ml), while the intake of the 0.3M NaCl with saccharin was essentially zero; the same as water. At 60 min the intake of the saccharin/NaCl mixture remained essentially zero.

Table 1. Cumulative Fluid Intake (ml)

	15 min		60 min	
Other Solution	NaCl	Other	NaCl	Other
HOH	13.43	0.03	21.64	8.16
5mM Sach+ NaCl	11.74	0.30	20.53	0.33
0.3mM QHCl + NaCl	12.13	3.45	21.84	3.63
0.3M Suc + NaCl	13.61	4.80	22.86	6.80

Surprisingly, the sucrose (sweet) or QHCl (bitter) adulteration produced more intake than saccharin which has both sweet and bitter components. These results suggest that the effect of saccharin adulteration of NaCl in reducing the acceptability of the NaCl is not due to the bitter component alone. Further, something unique to saccharin or general to mixtures of sweet, bitter and salt effectively eliminates intake of the mixture for the sodium depleted rat.

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Amiloride is a Poor Conditioned Stimulus in Taste Aversion Learning. STACY MARKISON and ALAN C. SPECTOR (University of Florida, Gainesville, FL)

Animal studies have revealed changes in responsiveness to NaCl with treatment of the sodium channel blocker, amiloride. Some humans report that the intensity of NaCl is reduced when amiloride is used and that amiloride alone has a bitter taste. Thus, results obtained in animal studies may stem from amiloride's inherent taste and not alterations in NaCl taste properties as a result of blocked sodium channels. We tested the effectiveness of amiloride to serve as a conditioned stimulus (CS) in a conditioned taste aversion (CTA) paradigm. Twenty-two male Sprague-Dawley rats were randomly assigned to four groups (amiloride-LiCl, amiloride-NaCl, water-LiCl, water-NaCl). After they were trained in a computer controlled gustometer, rats were habituated to a restricted water schedule and subsequently given 3 CTA trials on separate days. Each conditioning trial consisted of 15 min access to the CS (100 μM amiloride or water) immediately followed by an injection (ip) of either NaCl or LiCl (2.0 mEq/kg). After the CTA trials, rats were tested in the gustometer for their licking responses to distilled water and 100 μM, 10 μM, and 1 μM amiloride. There was no change in intake across trials for any of the groups. The gustometer test revealed a slight but significant decrease in the number of licks taken to 100 μM as compared to 1 μM and 10 μM amiloride for the amiloride-LiCl group. However, at each concentration there were no significant differences between any of the groups. In conclusion, 100 μM amiloride does not serve as a particularly effective CS in a CTA paradigm, even after 3 conditioning trials with a potent dose of LiCl. Although it is difficult to entirely dismiss the possibility that amiloride has a detectable taste, these results suggest that its intensity is very weak at best. Thus, it appears that the reported behavioral effects of amiloride on NaCl responsiveness in rats is due to its sodium channel blocking properties rather than its inherent taste characteristics.

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Chorda Tympani or Glossopharyngeal Nerve Section Does Not Alter Quinine Detection Thresholds in Rats. STEVEN J. ST. JOHN and ALAN C. SPECTOR, Dept. of Psychology, University of Florida.

Neither glossopharyngeal (GL) nor chorda tympani (CT) nerve section alone alters the rat's unconditioned spout licking behavior to suprathreshold concentrations of quinine hydrochloride. With respect to NaCl, CT section substantially raises the detection threshold but has no effect on unconditioned spout licking behavior, presumably because a) the threshold procedure is more sensitive, especially at low stimulus concentrations, and b) behavioral responses are not guided by affective processes in the threshold procedure. Therefore, the present study examined the effect of gustatory nerve sections on the quinine detection threshold. Fifteen water deprived rats were trained in a computer controlled gustometer to maintain spout licking to water and suppress licking to suprathreshold quinine concentrations (0.047mM - 1.0 mM). Rats that did not lick the spout at least once in the latter 3 seconds (avoidance period) of a 5 second water trial received a 30-second time-out punishment, delaying further fluid reward, and rats that licked the spout at least once in the avoidance period of a quinine trial received a mild footshock. Across days, the stimulus array included lower and lower concentrations of quinine. A sigmoidal curve was fit to the data expressed as a detectability score, quantifying the degree of lick suppression to quinine relative to water. The concentration representing the half-maximum detectability score was arbitrarily defined as threshold. Thresholds were measured before and after sham surgery, CT section, and GL section. Thresholds for sham rats improved 0.954 $\pm$ 0.25 log units after surgery. There was not a significant change in the threshold for the other groups. The fact that thresholds for nerve-sectioned rats did not improve after surgery may suggest some minor impairment. These results, however, compare favorably with our earlier work using suprathreshold concentrations, and indicate that neither GL nor CT section substantially affects quinine detection thresholds in the rat.

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c-Fos Induction in the Nucleus of the Solitary Tract by Sucrose after Conditioned Taste Aversion Acquisition is Not Attenuated by Subdiaphragmatic Vagotomy. T.A. HOUPT, R.A. BERLIN, and G.P. SMITH (E.W. Bourne Behav.Res. Lab., Dept. Psychiatry, Cornell Univ. Med. Coll., White Plains, NY)

After acquisition of a conditioned taste aversion (CTA) against sucrose, intraoral infusions of sucrose induce c-Fos-like immunoreactivity (c-FLI) in the medial intermediate nucleus of the solitary tract (miNTS) of the rat (Houpt et al., Neurosci. Ltrs, 172(1994)1-5). In order to determine if c-FLI expression in the miNTS depends on subdiaphragmatic vagal afferent input to the NTS secondary to gastrointestinal symptoms during CTA expression, we quantified the induction of c-FLI in the miNTS by sucrose infusions after total subdiaphragmatic vagotomy in rats with a previously acquired CTA against sucrose.

Anterior sublingual intraoral catheters were implanted in adult male rats. Rats were conditioned against intraoral infusions of 5% sucrose (6 ml/ 6 min) by pairing sucrose infusions with toxic LiCl injections (0.15M, 12 ml/kg i.p.) 3 times over 1 week. Sucrose intake was measured by weighing rats immediately before and after infusion. The rats consumed all of the infused sucrose during the 1st pairing; by the 3rd pairing, all the sucrose was rejected. After CTA acquisition, 7 rats underwent bilateral subdiaphragmatic vagotomy; 3 rats were sham-vagotomized. One week after surgery, 5 vagotomized rats and all sham-vagotomized rats received a test intraoral infusion of sucrose; 2 vagotomized rats did not receive sucrose infusions. Rats were perfused and processed for c-FLI 1 hr later. The expression of c-FLI in the miNTS was quantified by cell-counting.

Neither vagotomized nor sham-vagotomized rats consumed any of the intraorally-infused sucrose. There was no significant difference in the number of c-FLI-positive cells in the miNTS between vagotomized (77.8 $\pm$ 3.3) and sham-vagotomized rats (84.2 $\pm$ 9.0) after sucrose infusions. Both groups receiving sucrose infusions expressed significantly greater numbers of c-FLI positive cells than did vagotomized rats sacrificed without sucrose infusions (35.4 $\pm$ 11.4, p's < 0.01). We conclude that c-FLI induction correlated with CTA expression is not dependent on subdiaphragmatic vagal afferent input.

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Establishment of Learned Taste Preference for Lysine in Lysine-deficient Rats: Roles of Chorda Tympani and Glossopharyngeal Nerves

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Rats, fed lysine-deficient diet, quantitatively ingest a L-lysine.HCl (Lys) solution in a multi-bottle choice paradigm. Electrophysiological study suggested that the sensitivity of glossopharyngeal nerve (GP) for orally applied Lys was 1,000-fold higher than that of chorda tympani (CT) in mice. In a 8-bottle test, however, the increase in Lys intake in Lys-deficient rats was suppressed by bilateral sections of CT (CTX), but was little affected by those of GP (GPX). In the present study, effects of CTX and/or GPX on taste preference learning for Lys were examined by using a short-term two-bottle preference test. Rats were given free access to a Lys-deficient diet and water. Each rat was tested daily for choice between 0.1 M Lys and water during a 15 min-stay in the test cage. Ingestion of Lys solution in intact rats (controls) increased gradually from day 2 and reached a plateau on days 11 to 15, but that of water was negligibly small. Rats with CTX and/or GPX retarded this learning of Lys ingestion. Either the increase in daily Lys solution ingestion or the maximal amount of Lys intake was comparable to each other as follows: controls > CTX ≧ GPX > CTX+GPX. When Lys concentration varied, the threshold of Lys preference for concentration-dependence was as follows: controls < CTX < GPX < CTX+GPX. These results suggest that both CT and GP nerves are needed either to maintain taste sensitivity for Lys within normal range, or to establish learned Lys preference rather than using other stimuli (odor, etc.) under Lys deficiency.

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Taste Thresholds in Nonhuman Primates.

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The absolute threshold for taste detection is fundamental information that has been reported dozens of times for human subjects since the last century. Many experiments have examined the thresholds for taste preferences and aversions in nonhuman primates, but no experiments have attempted to measure their detection thresholds, even for the 4 basic taste stimuli. In the present experiment detection thresholds were measured in 5 rhesus monkeys (Macaca mulatta) with a conditioned suppression paradigm similar to that used by Thaw and Smith (1992, Chem Senses, 17, 211-223) in rodents. Sucrose, NaCl, HCl, QHCl and distilled water (DW) were presented with a gustometer designed specifically for use with nonhuman primates (Reilly et al., 1994, Physiol Behav., 55, 401-406). Daily test sessions consisted of 50 trials (1 taste trial / block of 10 trials). On DW trials the monkeys were permitted to consume the entire 1.0 ml sample, but on taste trials, the monkeys were punished with a mild 1.0 s shock from an obedience collar if the entire sample was consumed. Each stimulus was tested as part of a descending concentration series in which only 1 stimulus concentration was tested each day. Thresholds were determined at least 2 times for each monkey for each of the tastants. The monkeys received the balance of their daily fluid ration in the home cage and began each test session under approximately 18 hrs of fluid deprivation. The data were analyzed using signal detection theory. The mean detection thresholds for sucrose (56 mM), NaCl (62 mM), HCl (1.9 mM), and QHCl (0.023 mM) were 1.63 - 2.2 log units below the preference and aversions thresholds reported by Pritchard et al. (1994, Physiol Behav., 55, 477-481). The detection thresholds, however, were consistent with the human detection thresholds reported by Pfaffmann (1959, Handbook of Physiology, Neurophysiol. 507-533) and the thresholds derived electrophysiologically from the primary taste cortex of monkeys by Scott et al. (1991, J. Neurophysiol, 65, 76-86).

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Taste Hairs on the Legs of Tsetse Flies and their Function

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Tsetse flies (*Glossina* spp.) are haematophageous insects native to Africa where they are important vectors of trypanosomoses for humans and their livestock. As an alternative to airial spraying of insecticides, insecticide impregnated screens baited with host-derived odors are now used to control tsetse. The flies are exposed to insecticide when they land on the screens. With the aim of prolonging the contact time between insect and screen, we are studying the sense organs which mediate feeding behavior. Man is one of the flies' hosts and at least two tarsal sensilla on each leg of *Glossina fuscipes fuscipes* respond to human sweat. The spike activity from the chemosensory cells in these sensilla is recorded by placing an electrode over the tips of the hairs. Sweat excites 2 or 3 cells in each sensillum as does one of its solutes uric acid. Other components of sweat which are effective stimuli include isoleucine, leucine, phenylalanine, tryptophan, tyrosine, and valine. From the spike trains it is clear that these amino acids stimulate the same cell in each sensillum. Dose-response curves show that the cells are most sensitive to phenylalanine (threshold dose at $\approx 10^{-5}$M). In behavioral studies the flies will not respond to a paper surface treated with the electrophysiologically effective stimuli. Sensory stimuli of other modalities, such as heat, do induce feeding behavior on a paper surface. However, in conjunction with heat, the tastants enhance the feeding activity of the flies to a level far above the response to heat alone (Wilcoxon signed rank test for pairs, $P<0.004$). The effect appears to be maximal after two days of food deprivation (more than 4x the response to heat alone). For the fly species studied so far, including the house flies, the taste quality of the surface is the prime stimulus to trigger extension of the proboscis. In contrast, taste in tsetse flies has a subsidiary -though important- function as a synergistic agent.

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Recognition of Glucose Intake and Utilization in the Brain of Streptozotocin-Treated Adult Rat Using Functional Magnetic Resonance Imaging (MRI) after Insulin Treatment Intraperitoneally

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Animals including humans with diabetes mellitus display strong preference for sugars reflecting the retardation of glucose utilization in their body, especially the brain as well as the muscle, by insufficient insulin function. When glucose utilization has improved, recognition processes in the brain should be operating to sense when plasma glucose level has normalized. Male Sprague-Dawley rats, weighing 150g, treated with or without Streptozotocin (30mg and 60mg/kg, B.W.) intraperitoneally, were employed as a model of diabetes mellitus. Each rat with a catheter into the abdominal cavity was settled at the center of the bore (40cm in diameter) of a super conductance magnet (4.7tesla). The brain O_2 consumption change of each brain area was monitored and visualized chronologically by magnetic resonance imaging using the T2* weighted intensity rapid gradient echo pulse sequence method, following insulin treatment (20 IU/kgBW, i.p.) through the catheter. Fasted glucose in plasma of diabetic rats was above 400 mg / dl and normalized below 100mg/dl at 100min after insulin injection. The intensity changed in the hippocampus at 20-30min, the paraventricular nucleus at 30 min, the thalamus and the dorsomedial hypothalamus at 40min-50min and then the ventromedial hypothalamus at 100 min after insulin injection. These changes were recovered at 120min after normal glucose utilization by insulin treatment. Saline treatment in diabetic rats as a control never caused any intensity change in the brain. These data suggest that STZ-induced diabetic rats with glucose hunger could elicit metabolic changes in the hypothalamus and the thalamus during glucose uptake into the peripheral tissues and that the neuronal activity of nuclei in these areas responded in recognition of sufficient glucose utilization.

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Altered Acceptance of Taste Solutions by Calcium-Deprived Rats.

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Calcium-deprived rats drink large quantities of NaCl. To determine whether this increased salt appetite is specific to the salty modality, young male rats fed diets containing 150 or 25 mmol/kg calcium for 3-5 weeks received two ascending series of 24-hr tests with a choice between d.i. water and one of 29 different taste solutions (1-1000 mM in 0.5-log steps). Relative to controls, those fed low-calcium diet had increased preferences for one or more concentration of NaCl and Na-acetate but not $NaHCO_3$ or Na-gluconate, demonstrating that the increased intake of NaCl was not due to a drive for sodium. Differences in palatability between these sodium salts were unimportant because the rats fed low-calcium diet consumed more NaCl even if this was made less acceptable by adulteration with citric acid. The possibility that calcium-deprived rats have an enhanced general cation or mineral appetite was supported by findings of increased preferences for KCl, $SrCl_2$, $FeSO_4$, $ZnCl_2$, $FeCl_2$, $FeCl_3$, and $AlCl_3$. The difference in preference occurred at lower concentrations and was larger for di- and trivalent ions than for monovalent ones. However, there were no differences in intake or preference at any concentration of CsCl, $MgCl_2$, $MgSO_4$, or Pb-acetate. Moreover, calcium-deprived rats drank more HCl and malic acid than did controls. Thus, the effect of calcium deficiency on intake was not confined to minerals. Acidity or bitterness did not appear important because there was no difference between the groups in intake of H_2SO_4, citric acid, or QHCl. Consistent with their deleterious effects, phosphates ($NaPO_4$, KPO_4) were avoided by calcium-deprived rats, but so were sucrose and saccharin. The only commonality observed in this complex set of results was that differences between calcium-deprived and control rats were expressed only on the descending limb of the inverted U-shaped preference-aversion curve, never on the ascending limb. Perhaps calcium deprivation reduces sensitivity to a common taste quality responsible for the off taste of concentrated solutions. It is clear that calcium deprivation does not induce a general increase in acceptance of all taste solutions but there appears to be no simple explanation for what these animals consume.

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Activated Areas in the Brain of Awake Rats during Ingestion Using Magnetic Resonance Imaging (MRI).

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To understand spatial and temporal dynamics of activated areas in the brain, magnetic resonance imaging (MRI) was undertaken using "awake" rats. Under anesthesia, each rat had a receptacle formed of dental cement put on its head to immobilize the animal's skull to be later fixed painlessly in the correct stereotaxic position. After recovery, they were trained to accept the restraint in a stereotaxic apparatus for more than 2 hrs without struggling, and were permitted to lick a solution from a spout when manually extended close to its mouth, following water deprivation for one day. Each rat was then taken T2* weighted MRI (gradient echo pulse sequence), in which intensity increases in area with high consumption of oxygen (oxy-hemoglobin), before (control), during, and after ingestion of various solutions; water, saline, 5% glucose, 0.2 M lysine, 0.15 M monosodium L-glutamate, 0.05 M arginine, 0.5 M glycine, and 0.05 M histidine. The intensity of T2* weighted MRI increased in the motor area of the frontal cortex (Fr1), the somatosensory area of the parietal cortex (Par1), the amygdala, the piriform cortex, some hypothalamic areas, which are all related to ingestion. The chronological changes in these particularly activated areas by ingestion were quite comparable to those of every solution. The finding directly indicates that these brain regions work parallel to taste sensation during drinking behavior.

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Morphological and Neuroanatomical Properties of a Sex-Specific Chemosensory System: You Can't Judge a Claw by its Cover.

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To understand sex-specific responses to feeding stimulants in the fiddler crab, we assayed neuroanatomy and external morphology of sensilla in the minor claw, the primary feeding appendage. The 1st thoracic ganglion contains all the nerves that project to the claw. This ganglion and attached roots was removed and sectioned where the nerves enter the ganglion. Transmission electron microscopy (TEM) was used to construct a photographic montage of the nerve to determine the size-frequency distribution of the axons. Mature crabs of two species (*Uca pugnax and U. pugilator*) contain 35-55 thousand axons in a bimodal size-frequency distribution. Peaks in the distribution occur at size classes of ca. 0.3 and 1.5 micron dia. Females show axonal populations enriched in small nerves (< 0.5 micron dia.) relative to males. These are putative chemosensory fibers, based on their size. The proportion and absolute number of small axons are greater in females than in males, while the sexes show similar distributions for larger axons (>0.5 micron dia.).Scanning electron microscopy was used to determine if the external morphology of the sensilla on the minor claw reflected sex-specificity of the axonal population. Claws contain ca. 100 uniramous hairs. The largest sensilla (0.5 mm) form a basket-like structure on the claw tips, while smaller sensilla (0.01-0.10 mm) lie in a series of small fissures on the inside and outside margins of the claw, and on the inside of the claw tips. The number of sensilla is not noticeably sex-specific. Deafferentation of basket sensilla does not affect feeding behavior, while feeding time in patches of chemical stimulus mixtures is substantially reduced when the distal tips of the claw are coated with cyanoacrylate. This indicates small sensilla may posses chemosensory cells, although their number appears insufficient to account for the large number of chemosensory fibers based on TEM. Other unidentified and cryptic sensilla may be responsible for the sex-specific size-frequency distributions of axons originiating in the minor claw.

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Differential Roles of Chemosensory Organs in Food Preference by Larvae of the Tobacco Hornworm, Manduca sexta.

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M. sexta larvae possess only five types of bilateral peripheral chemosensory organs. The competence and necessity of each type to mediate food preferences were examined using two-choice feeding tests in which fifth instar larvae were given a choice between leaf discs from a certain plant species and moist filter paper discs. Test larvae had only one type of chemosensory organ remaining after microsurgery. Control larvae had all of these types remaining, showing normal food preferences. Other control larvae had all five organ types removed. The results show that each type of sensory organ is competent to mediate a normal preference for Solanum pseudocapsicum, but no single type suffices for Lycopersicon esculentum. The maxillary palps are competent, but not necessary, to mediate a normal preference for Datura innoxia. The medial maxillary sensilla styloconica and the epipharyngeal sensilla are each solely competent and necessary for rejection of Canna generalis. In contrast, only the lateral maxillary sensilla styloconica play such a role in rejecting Vigna sinensis. Rejection of Pelargonium hortorum can be mediated by the antennae or a combination of the remaining sensory organs. Thus, for some plant species a single type of sensory organ can be both competent and sufficient to mediate normal food preference, but for other plant species only a combination of several types suffices. Often incompetent chemosensory organs mediate an impaired preference and sometimes a normally rejected food becomes preferred. This indicates that various types of chemosensory organs can provide conflicting inputs for feeding decisions. Each plant species seems to be detected by an unique combination of chemosensory organs with some types being more important than others. Dietary experience with V. sinensis results in an increased preference for this plant compared with larvae reared on L. esculentum. This plasticity of food preference appears to be partly due to a change in competence of the antennae and a shift in importance of other chemosensory organs to mediate feeding on V. sinensis.

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Early Dietary Sodium Restriction Prevents the Development of Normal Innervation Patterns in the Rat Peripheral Taste System.
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Neural rearrangement in the pre- and postnatal sheep peripheral gustatory system occurs concomitantly with developmental increases in functional sodium responses. Normal functional development of the postnatal rat taste system mirror that of the sheep. Furthermore, the sodium taste system can be prevented from developing in rats if they are fed a sodium-restricted diet throughout pre- and postnatal development. Thus, the postnatal rat provides an excellent model to examine relationships between taste bud structure and function. In order to determine whether patterns of taste bud innervation in rat change during postnatal development and whether sodium restriction prevents the development of normal peripheral innervation patterns, we determined the number of chorda tympani neurons that innervate single fungiform taste buds. This was accomplished by iontophoretically applying fluorescent tracers into individual papillae and counting the number of labeled geniculate ganglion cells. Data were obtained from normal rats aged 10 and 20 days and adults, and from adult rats that were sodium restricted throughout pre- and postnatal development. The number of labeled geniculate ganglion cells innervating a taste bud was positively correlated with the size of the taste bud ($r=0.91$, $p<0.0003$). By comparison, there was no correlation between taste bud size and the number of geniculate ganglion cells innervating a taste bud in either 10-day-old or 20-day-old rats. Thus, a match between taste bud size and the number of innervating fibers develops after day 20. Additionally, there was no correlation between taste bud size and the number of neurons innervating a taste bud in sodium-restricted rats. In fact, this relationship in sodium-restricted rats is indistinguishable from that in 20-day-old rats. Thus, like functional sodium responses, innervation patterns in sodium-restricted rats remain immature. These findings indicate that 1) taste bud size relates to the number of innervating neurons, 2) this relationship arises over a prolonged postnatal period, and 3) like sheep, the development of sodium taste responses relates to the innervation pattern of taste buds.
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Fungiform Papillae Develop in Organ Cultures of the Anterior Half of Embryonic Rat Tongue Without Intact Neural Ganglia.
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Fungiform papillae first appear in the rat tongue on embryonic day 14 and are well developed by embryonic day 15 (E0: dam is sperm positive). From time of initial formation the papillae are organized in rows on either side of the midline of the anterior tongue. We have reported previously on an organ culture system of the E13 and E14 embryonic rat tongue in which the tongue itself can be maintained and undergo morphogenesis in vitro, including development of fungiform papillae (Mbiene et al, AChemS, 1994). In this system the entire embryonic tongue is dissected from the mandible and cultured, in a preparation that excludes intact sensory ganglia. Therefore, the tongue cultures demonstrate that fungiform papillae can develop without sensory neurons. To exclude the possibility that autonomic ganglion cells in the 'circumvallate ganglion' of the posterior rat tongue have a role in development of fungiform papillae, we have cultured the anterior half of E14 rat tongues. E14 embryos were obtained from anesthetized, pregnant Sprague-Dawley rats. Anterior half (distal to the intermolar eminence) and entire (control) tongues were dissected and maintained in standard organ culture conditions, as described previously, in DMEM/F12 plus 1% fetal bovine serum and B-27 supplement (Gibco). Cultures were fixed after 1, 2 and 3 days and processed for scanning electron microscopy. General tongue morphology progresses through comparable developmental stages and the tongue increases in size, in both half and whole tongue cultures. Fungiform papillae develop in patterned rows on both sides of the midline of the anterior tongue after one day of culture, and are maintained through three days, in both half and whole tongues. These results indicate that: the embryonic half tongue can be maintained and will develop under culture conditions; and, the embryonic half tongue in culture exhibits spatial patterns and morphogenesis of fungiform papillae similar to those of whole tongue cultures. Therefore, fungiform papillae develop in organ culture in the absence of intact sensory or autonomic ganglia.

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A Comparative Ultrastructural Analysis of Circumvallate and Fungiform Taste Buds in the Rat.
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We have previously provided evidence for differences in synaptic structure in circumvallate and fungiform taste buds of rodents. Currently, we are attempting to identify the determinants of synaptic structure in rat taste buds. As part of this study, it is necessary to obtain control data for both circumvallate and fungiform taste buds. In the present study we are comparing and contrasting ultrastructural features of both types of papillae. This information will be compared with data from rats in which the VIIth and the IXth cranial nerves have been cut and then cross–reinnervated. We used light and transmission electron microscopy to elucidate the morphological features of circumvallate and fungiform taste buds in normal adult rats. Using standard ultrastructural criteria, we identified electron-lucent and electron-dense cells in circumvallate and fungiform taste buds. Although a continuum of cellular morphologies is present in both types of taste buds, examples of intensely electron-lucent and intensely electron-dense cells were observed in circumvallate taste buds only. Long chains of rough endoplasmic reticulum (RER) with dense ribosomes and narrow, compact cisternae are often present in the nuclear region of electron-lucent cells from circumvallate taste buds. This is in contrast to the RER in the electron-dense cells. In these cells the cisternae of the RER are typically shorter and the cisternal membranes are often separated by an electron-lucent space. The electron-lucent cells contain elongate nuclei and apical dense-cored granules. Microvilli of circumvallate papillae protrude from the taste pore into the trench. In fungiform papillae, however, the microvilli extend only a short distance into the deeply invaginated taste pore. Unlike circumvallate taste buds, fungiform taste cells have a more homogeneous appearance, particularly with respect to the density of the ground substance. Two major cell types are present in fungiform taste buds. In one cell type, the heterochromatin is evenly distributed and the nucleus has deep invaginations. The other cell type is characterized by a nucleus with patchy heterochromatin and fewer nuclear invaginations. The electron-dense fungiform taste cells also apparently possess RER with long chains and narrow cisternal spaces. No apical dense-cored granules were observed in either cell type in fungiform taste buds.

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Immunocytochemical Studies of PGP 9.5 in Fetal and Adult rat Circumvallate Taste Buds.
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Protein gene product 9.5 (PGP) is a marker for neurons and neuroendocrine cells. In the present study we have studied immunoreactivity of both fetal and adult rat circumvallate taste buds to PGP antisera as a means of obtaining more knowledge concerning the differences between taste cell types. Adult and fetal rats of ages E16, E18 and E20 were used for this study. Animals were perfused with a mixture of glutaraldehyde and paraformaldehyde. Cryo-protected embryonic specimens were sliced at a section thickness of 50 µm using a cryo-microtome. Adult tissues were sliced at a section thickness ranging from 50-100 µm using a vibratome. All sections were treated with 1% sodium borohydride. Before reaction with antisera, sections were blocked with 1% BSA and 1% horse serum. Sections were then reacted with rabbit anti-PGP 9.5 (*Accurate Chemical & Scientific Co.*). After washing, the sections were processed using the ABC protocol. Following incubation in DAB solution and postfixation with 1% OsO_4, the specimens were embedded in epon for electron microscopical analysis. Apical epithelial cells of circumvallate papillae were immunoreactive to PGP 9.5 antisera beginning at day E18. The nuclei of the immunoreactive cells typically were ovoid in shape and the cells possessed prominent cytoplasmic processes which gave the cells a stellate appearance. Some of these immunoreactive cells were in close contact with nerve fibers. Immunoreactive nerve fibers were observed beneath the basal lamina surrounding the circumvallate papillae at days E16, E18 and E20, as well as in adult animals. In addition, immunoreactive taste bud cells were observed which had microvillar processes extending into the taste pore of the circumvallate papilla. Characteristic features of immunoreactive taste cells include a moderately electron-dense cytoplasm and a lack of apical dense granules. On the other hand, the electron-lucent cells possessing apical dense granules were not immunoreactive. Currently we are using immunogold techniques in order to determine which organelles are immunoreactive in the taste cells.

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Synaptic Connections in Adult Hamster Circumvallate Taste Buds.

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It is well known that hamsters have a pronounced behavioral and electrophysiological response to sweet gustatory stimuli. We are currently attempting to correlate structure with function in hamster taste buds. In the present study we are obtaining normative data from hamster circumvallate taste buds at the ultrastructural level to serve as a control for subsequent experiments. In addition to characterizing the ultrastructural features of the taste bud cells, we are particularly interested in the numbers, types and distribution of synaptic foci present in hamster taste buds. Although previous investigators have described "synapse-like structures" in hamster taste cells, detailed descriptions of synapses in hamster taste cells are almost nonexistent. This study presents ultrastructural evidence for both afferent and neuro-neuronal synapses in hamster circumvallate taste buds. Adult female Syrian hamsters were prepared for electron microscopy and examined with a Hitachi H-7000 transmission electron microscope at 100 kV. Afferent synapses are associated with both dark cells (type I) and light cells (type II). These afferent synapses are characterized by parallel, apposed membranes with both clear and dense-cored vesicles in the vicinity of the pre-synaptic thickening with a synaptic cleft of approximately 16-30 nm. Surprisingly, several neuro-neuronal synapses were observed in circumvallate taste buds. These neuro-neuronal synapses are symmetrical or asymmetrical in structure, suggesting that some of these synapses are one-way, while others may be bidirectional. The cytoplasm adjacent to the neuro-neuronal synapses contains abundant mitochondria, clear and dense-cored vesicles, similar to what is observed in the afferent synapses. The significance of these putative neuro-neuronal synapses is unknown, but they may provide an ultrastructural basis for complex neuronal integration that has not been previously observed in adult mammalian taste buds.

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Effects of Chorda/Lingual Denervation on NSE, NCAM and CGRP Immunoreactivity Associated with Fungiform Taste Buds in the Hamster.

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Neuron specific enolase (NSE), neural cell adhesion molecule (NCAM), and calcitonin gene-related peptide (CGRP) are associated with taste buds (e.g. Nelson and Finger, JCN 336:507 '93, Montavon and Lindstrand Reg. Pep. 36:235 '91). We studied expression of these neural markers in denervated fungiform taste buds. Chorda/lingual nerves of 4 hamsters were severed and devitalized unilaterally. 3-15 weeks later the animals were sacrificed and tongue sections evaluated bilaterally with immunocytochemistry. All three markers, on the control side, stained fibers in papillary cores and clusters of cells in taste buds. In experimental buds the staining was reduced overall, but often appeared as a single, brightly NSE, NCAM or CGRP immunoreactive cell. These striking receptor-like cells were spindle-shaped and centrally located among non-immunoreactive cells. On the control side, CGRP stained many nerve fibers in fungiform cores, in perigemmal areas, and in the chorda/lingual nerve trunk deep in the tongue. On the denervated side, CGRP fibers were few and confined to perivascular areas except for a few CGRP fibers near some experimental buds. Thus, sensory denervation of the tongue alters but does not eliminate NSE, NCAM and CGRP expression. Sparse CGRP fibers that survive sensory denervation are likely autonomic (Hino et al., Arch. Histol. Cytol. 56:505 '93) and may be trophically related to fungiform taste buds.

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Apolipoprotein E Immunoreactivity in Human Olfactory Receptor Neurons and Its Increase in Alzheimer's Disease.

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Apolipoprotein E (apo E) is a lipophilic protein of 299 amino acids with a 34,000-*Mr* circulating in the plasma as a constituent of specific lipoproteins, which participate in cholesterol redistribution among peripheral tissues. It is synthesized and secreted primarily by the liver, and to a lesser extent by the central nervous system. Apo E is observed in senile plaques and neurofibrillary tangles in Alzheimer's disease (AD) patients. Furthermore, neurofibrillary tangle-like pathological changes are observed in olfactory receptor neurons (ORNs) of AD patients. The localization of apo E was examined in human olfactory mucosa in which ORNs were identified with protein gene product 9.5 (PGP 9.5) antiserum. Specimens were obtained at autopsy from 6 Alzheimer's disease control (ADC) patients ranging from 76 to 84 years of age, and 9 Alzheimer's disease (AD) patients (2 were early onset and 7 were late onset AD patients) ranging from 52 to 90 years of age. Standard immunofluorescence and streptavidin-biotin-peroxidase complex (SABC) methods were used for immunostaining with antibodies to apo E and PGP 9.5. Several ORNs were immunoreactive to apo E antiserum in AD patients, which were also immunoreactive to PGP 9.5 antiserum by immunofluorescence double staining. Immunoreactivity was observed in the dendrites and perikarya of ORNs. In contrast, only a few apo E-immunoreactive ORNs were observed in ADC patients. The mean number of PGP 9.5-immunoreactive ORNs in ADC, early onset AD and late onset AD patients was 33.7, 30.4 and 33.4 per 200 μm epithelial length, respectively. There was no significant difference among these groups using SABC methods. In contrast, the mean number of apo E-immunoreactive ORNs was 1.3, 3.5 and 4.9 per 200 μm, reapectively. The mean number of apo E-immunoreactive ORNs in both early onset and late onset AD patients was significantly greater when compared with that in ADC patients ($P < 0.01$ and $P < 0.001$). Our results indicate that apo E is selectively increased by accumulation and/or synthesis in ORNs of AD patients. The increased number of apo E-immunoreactive ORNs may be useful marker for AD.

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FGF2 Induces Differentiation In Adult Olfactory Epithelium Cultures.

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The number of mature sensory neurons in adult olfactory epithelium seems to remain stable indicating that the ongoing processes of neurogenesis and differentiation must be highly regulated. The aim of this work is to discover growth factors which control these processes. Olfactory epithelium from adult mouse was isolated, minced and explanted onto fibronectin-coated culture slides in serum-free medium containing basic fibroblast growth factor (FGF2, 50 ng/ml). After a further 5 to 15 days the cultures were fixed and processed for immunocytochemistry. Initially there was an outgrowth of tightly packed epithelial-like cells which formed around the tissue fragment within 2-3 days. These cells were seen in all cultures (FGF2-stimulated and Control). Most of them were immunoreactive for keratin and many were immunoreactive for NCAM. At 3-5 days, FGF2-stimulation promoted a further outgrowth of differentiated cells of several morphologies, including many neuron-like bipolar cells. These neuron-like cells expressed the 150kD neurofilament protein, neuron-specific β-tubulin, and OMP. None of the cells in Control cultures were positive for these antibodies. Pulses of [3H]-thymidine followed by autoradiography and double-labelling with anti-neurofilament antibodies demonstrated that all the neurons were born *in vitro*. FGF2 was effective in stimulating the outgrowth of neurons when it added at Day 5, after the outgrowth of the keratin-positive cells. After 10 days, cultures maintained with or without FGF2 began to degenerate, suggesting that FGF2 does not act as a survival factor. Removal of the explanted tissue prior to the addition of FGF2 led to the death of the established epithelial-like outgrowth, indicating a role for other tissue-derived factors.

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A Light and Electron Microscopic Analysis of Phagocytic Cells in Rat Olfactory Epithelium after Bulbectomy. YUKO SUZUKI, ALBERT I. FARBMAN (Dept. of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208)

It is generally accepted that macrophages play a major role in phagocytosis. However, little is known about the number and distribution pattern of macrophages in degenerating olfactory epithelium (OE). We examined rat olfactory epithelium after unilateral bulbectomy to study phagocytic cells. Immunohistochemistry using a monoclonal anti-macrophage antibody (OX42) and electron microscopy showed that macrophages entered the OE of the operated side 1 day after unilateral bulbectomy. The average number was 6.4 OX42-positive cells/2mm septal epithelium, compared with 2 on the unoperated side. At 3 days the number of macrophages peaked at 17 and subsequently dropped to 2.2/2mm at 7 days when basal cells showed signs of proliferation. In electron micrographs of specimens less than 7 days after bulbectomy most macrophages were round although some had long branched cytoplasmic processes. On the unoperated side, the number of macrophages remained unchanged for two weeks after bulbectomy. Most of the macrophages on the unoperated side and most in 14 day post-bulbectomy specimens on the operated side had branched, ramified cytoplasm. Moreover, we observed large phagosomes in the subnuclear cytoplasm of supporting cells at 6,7 and 14 days, suggesting that they too are phagocytic.

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Relationship between the Spatial Distribution of the Activity in Salamander Olfactory Bulb and Late Responses in Mitral/Tufted (M/T) cells. A.R.CINELLI (Dept.Anat.& Cell Biol., SUNY Brooklyn. NY 11203).

Long lasting excitability changes have been reported in M/T cells either following odor or electric-elicited activity. It is not clear, however, the interrelation between these responses and patterns of activity in the bulb. Olfactory nerve stimulation typically evokes in salamander olfactory bulb a brief period of depolarization often with a single action potential followed by a prolonged period of hyperpolarization. Low stimulation intensities can, however, evoke in some M/T cells a second period of depolarization (Cinelli and Kauer 1993; Cinelli 1994). In the present study, intracellular responses from single M/T cells were analyzed in relation to different spatial patterns of activity. Experiments were conducted "in vitro" in isolated epithelium-bulb preparations. Horizontal slices of the bulb (400-800 um) with the olfactory nerve fibers attached to the epithelium were placed in a split-bath tissue chamber. Stimuli applied to different parts of the epithelium which generated different spatio-temporal patterns of bulbar activity were studied by video imaging voltage-sensitive dye (VSD) signals (RH414 and RH795). Despite some degree of topographic relationship between mucosa and bulb regions, VSD depolarizations were distributed in multiple foci with poorly defined borders. M/T responses recorded in bulbar regions showing the highest levels of depolarization often showed a short latency single action potential, followed by a prolonged period of hyperpolarization. As the stimulus intensity was reduced, the size and duration of the late hyperpolarization decreased, but with this response pattern was conserved. On the other hand, M/T responses recorded in other peripherial regions to the foci of highest activity could instead evoke a second period of late depolarization when moderate stimulation intensities were tested. This late depolarization disappeared, however, when other epithelial sites that evoked larger responses were stimulated. Low bath temperatures (11° C) and GABA blockers enhanced this late depolarization. These observations suggest that late depolarizations in M/T cells depend on either weak activation or reduction of inhibitory inputs. These data support the notion that changes in the spatial pattern of activity that occur during odor or electric-elicited activity might modify the properties of single M/T cells and have implications in the generation of complex response patterns. Supported by NIH Grant ROI-DC01804 and the Dept. of Anat. & Cell Biol., SUNY Brooklyn.

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Odorant Derived Receptive Fields in the Peripheral Olfactory System of the Salamander Observed by Voltage-Sensitive Dye Video Imaging. JOHN S. KAUER[1], ANGEL R. CINELLI[2], (Tufts Medical School, NEMC[1], Boston, MA: SUNY[2], Brooklyn, New York, NY)

We have previously mapped receptive fields of single mitral/tufted olfactory bulb neurons by recording responses after application of odorants to small regions of the olfactory epithelium (Kauer and Moulton, 1974, *J. Physiol.*) using punctate stimulation. These studies showed that when the test odorant elicits suppressive responses, then suppressive responses were generated by stimulating at widely distributed sites across the epithelium (i.e suppressive responses have large receptive fields). On the other hand, when the odorant elicited excitatory responses, then excitatory responses were elicited only by stimulating certain epithelium sites (i.e. excitatory responses have more restricted and sometimes multiple receptive fields). We interpret these experiments as providing evidence for a wide epithelial distribution of receptor cells responsive to the test odorant and for convergence of these distributed receptor neurons (which share similar profiles of responsivity) onto glomeruli connected with a single mitral/tufted cell (see Kauer, 1980, *ISOT-VII*; Kauer, 1987, in *Neurobiology of Taste and Smell*, eds. Finger and Silver, Kauer et al., 1994, *ISOT-XI*). These studies, however, only defined receptive fields for single bulbar neurons. We have now used voltage-sensitive dye video imaging to delineate and decompose the multiple receptive fields associated with the many activated bulbar sites that we observe after odorant stimulation. We call these fields 'odorant derived receptive fields' (ODERFs) and have defined their structure for two odorants - amyl acetate and ethyl-n-butyrate. We hypothesize that each individual ODERF making up the ensemble response to one odorant represents a 'module' consisting of an activated population of receptor and bulbar neurons a number of which we hypothesize are taken together to encode odorant structure (Kauer and Cinelli, 1993, *Micr. Res. Tech.*). The ODERFs for amyl acetate and ethyl-n-butyrate have some constituent receptive fields in similar epithelial locations, as well as some that are different. These findings will be discussed with respect to general principles of odorant coding.

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Neurons Projecting to the Olfactory Bulb in the Salamander Brain. IGOR KRATSKIN and BABAK BINA (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, PA 19104, USA)

Projections to the olfactory bulb from olfactory and nonolfactory brain structures, which provide the central control over the ascending flow and processing of afferent signals, are found in different vertebrate species. In the tiger salamander, central innervation of the olfactory bulb has not been studied, despite the fact that this species has been widely used as an experimental system for investigating and modeling signal processing in the olfactory pathway. In this study, the sources of central projections to the olfactory bulb in the land-phase tiger salamander were examined using horseradish peroxidase (HRP) retrograde axonal tracing. Under tricaine anesthesia, specimens received unilateral iontophoretic application of a HRP solution into the rostral part of the olfactory bulb. Following four days survival, the brains were cut and sections were processed in order to visualize transported HRP. A vast majority of labeled neuronal somata, with different intensity of labeling, were found in the ipsilateral hemisphere. Most of these cells are situated in the lateral, medial, and dorsal pallial areas and in the medial septal nucleus, which are the projection sites of the olfactory bulb. This study suggests that there are reciprocal connections between the primary and secondary olfactory centers in the salamander brain. Additional experiments need to determine whether axons from nonolfactory brain structures also reach the salamander olfactory bulb.

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Dendritic Branching Patterns of Mitral/Tufted Cells in the Salamander Olfactory Bulb: New Observations from Intracellular Staining. K.A. HAMILTON and R.E. MALONEY, JR. (Dept. of Cellular Biology and Anatomy, Louisiana State Univ. Med. Ctr., Shreveport, LA 71130).

Odor discrimination in the salamander olfactory bulb appears to be achieved by subsets of interacting cells that comprise functional modules (J.S. Kauer and A.R. Cinelli, *Microsc. Res. Techn.* 24:157, 1993). We are using intracellular recording and staining methods to determine how cells with glomerular tufts may assist in defining the modules. In the present study, responses to olfactory nerve and tract stimulation were used to identify mitral and/or tufted cells in intact isolated preparations. The response amplitudes and latencies were indistinguishable from those previously studied *in vivo* (K.A. Hamilton and J.S. Kauer, *J. Neurophysiol.* 59:1736, 1988). The recordings were generally more stable, however, permitting detailed analysis of stimulus-response functions and better staining.

The largest cells were mitral-type cells located near the junction of the external plexiform layer with the more caudal (deep) cellular layers. The cells exhibited a variety of dendritic branching patterns, suggesting that several mitral/tufted cell types might occur in this region. The common characteristics included the presence of an axon that projected from the olfactory bulb and thick, smooth dendrites that branched and projected broadly in the general direction of the glomerular layer. There were no well-defined secondary dendrites. In horizontal sections, the dendritic branches were wide-spread and they innervated as few as three and as many as six glomeruli. The glomerular tufts included tight tufts resembling the tufts of mammalian mitral cells and a variety of feathery structures. Many of the largest tufts were each formed by a pair of dendritic branches. In sagittal sections, these tufts appeared to be confined to the same medial/lateral plane of the bulb in which the cell body was located. The results suggest that mitral/tufted cells could define planar modules oriented perpendicularly to the dorsal and ventral surfaces of the salamander olfactory bulb.

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Long-Term Potentiation (LTP) at the Primary Olfactory Synapse

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Mitral cells of the main olfactory bulb (MOB) receive direct excitatory synaptic inputs from the terminals of primary olfactory axons. Recent studies in our laboratory demonstrate that glutamate released from olfactory nerve terminals activates mitral cells (MCs) via non-NMDA and NMDA receptors in rats. Activation of non-NMDA (i.e., AMPA/kainate) receptors evokes rapid, brief excitation of MCs while NMDA receptors mediate a delayed, excitatory response lasting several hundred msec. In the hippocampus and piriform cortex, brief epochs of high frequency stimulation potentiate excitatory responses at NMDA and non-NMDA receptors, i.e., LTP. The goal of the present study, therefore, was to determine if excitatory responses at the ON->MC synapse undergo frequency dependent LTP.

Olfactory bulbs were rapidly removed from chloral hydrate-anesthetized rats (50-150 g), 400 µm-thick horizontal sections were submerged in a recording chamber and a stimulation electrode was placed on the ON layer (ONL). Extracellular recordings were obtained with glass pipettes. A brief, low intensity (4-40 µA) train of electrical impulses (10 sets of 4 pulses at 100 Hz at 200 msec intervals) was applied to the ONL while recording the discharge of MCs. This pattern of stimulation was selected because it mimics the sniff cycle-theta rhythm that rats exhibit in response to novel odors or when exploring the environment.

A single high frequency train produced an immediate, dramatic increase in the delayed, NMDA-like excitatory component in 8 of 9 cells tested. The increase in the delayed response ranged from 138% to 241% of the control response (mean increase = 180.6%). By contrast, the initial, non-NMDA receptor mediated excitatory response was not altered. In all cells tested to date, the potentiation was long-lasting (45 min), comparable to the duration of LTP in hippocampal and piriform cortex slices. In all 5 cells tested, the selective NMDA receptor antagonist AP5 (50-100 uM) prevented the induction of potentiation; potentiation was obtained following washout of AP5.

These results demonstrate that ON—>MC synaptic transmission exhibits use-dependent plasticity *in vitro*. Brief bursts of ON activity induce specific LTP of NMDA receptor-mediated excitation of MCs. LTP at the primary olfactory synapse discloses a novel mechanism for rapid amplification of MC responses to sensory inputs that may, in part, function in the rapid formation of memories to specific odors. Our results *in vitro* suggest that stimuli evoking bursts of ON activity may potently modify the synaptic excitability of MCs *in vivo*. Future experiments will test this prediction. *Support: NIH DC00347 and NS29218.*

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Glutamate receptor subunit localization in the olfactory bulb. ARTIS A. TAGUE and CHARLES A. GREER (Sections of Neurosurgery & Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510).

In situ hybridization studies have localized mRNA for several glutamate receptors (GluRs) in rat olfactory bulb (OB) (Sato et al., 1993). The subcellular location of the GluR proteins, however, remains to be elucidated. To address this question, we studied the laminar and cellular immunoreactivity (IR) of antibodies (Abs) to specific subgroups of the GluRs. Adult rats, 200-300g, were anesthetized and perfused with 4% paraformaldehyde and 0.1% glutaraldehyde in 0.1M PBS. Tissue sections were incubated for 48 hrs in primary antibody, processed with Vector Labs ABC Kits. IR to GluR1 (Chemicon) appeared localized to periglomerular (PG) and short axon (SA) cells. IR was heaviest in the glomerular layer (GL) and moderate in the external plexiform layer (EPL). In general, the pattern of GluR1 IR appeared consistent with subcellular localization to PG and SA somas and dendrities. The IR to GluR2/3 (Chemicon) appeared localized to granule cells (GCs). The heaviest concentration was in the EPL with moderate staining in the granule cell layer (GRL). This is consistent with the IR of both the somas and apical dendrites of GCs. The IR to GluR4 (Chemicon) did not appear specific to any subpopulation of cell bodies but rather, showed heavy staining of EPL and slightly lighter staining of the olfactory nerve layer (ONL) and GL. This suggests that GluR4 receptors may localize to dendritic processes in the EPL but may also be present on olfactory receptor cell axons. The IR to GluR5/6/7 (Pharmigen) appeared localized to GCs and mitral cells. Very heavy dendritic staining was seen in the EPL, slightly less in the ONL and moderate staining in the GL. This pattern appears consistent with the subcellular distribution of GluR5/6/7 in the dendrites of both GCs and mitral cells in the EPL. NMDA IR (Chemicon/Pharmigen) IR was heaviest in the ONL, slightly less in the EPL and moderate in the GL and GRL. The NMDA Abs did not show the clear localization to subpopulations of cells or subcellular compartments seen with the other Abs. These results support the idea that glutamate is one of the neurotransmitters utilized in the OB (Berkowicz et al., 1994). The data also suggest strongly that different subsets of GluRs localize to different cell populations and subcellular compartments. This may indicate that GluR subunits differentially participate in OB local synaptic circuits.

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D2 Receptor Modulation at the Olfactory Nerve Synapse. D.A. BERKOWICZ[#], P.Q. TROMBLEY and G.M. SHEPHERD. (Section of Neurobiology and [#]Interdepartmental Neuroscience Program, Yale University School of Medicine, New Haven, CT 6510).

Dopamine can produce a variety of effects in central neurons, including changes in membrane potential, regulation of cyclic nucleotides, and modulation of transmitter release. In the olfactory bulb, tyrosine hydroxylase, the synthetic enzyme for dopamine, is largely confined to neurons in the glomerular layer (Baker et al, 1988). D2 dopamine receptors were shown to be present in both the glomerular and olfactory nerve layers by Nickel et al (1991) who proposed that these receptors are located on presynaptic boutons of the olfactory nerve and thereby act to reduce transmitter release. We previously provided evidence that olfactory receptor neurons use glutamate as their transmitter to excite olfactory bulb neurons through activation of both NMDA and non-NMDA receptors. We tested the hypothesis that dopamine modulates neurotransmission at the olfactory nerve synapse using the hemisected turtle olfactory bulb preparation and patch-clamp recording techniques. We found that the magnitude of the excitatory postsynaptic response to olfactory nerve stimulation was reversibly decreased in the presence of dopamine (10-300 µM). The application of 10 µM of a D2 specific agonist, quinpirole, mimicked this effect. The modulatory effects of dopamine (100 µM) could be reversed by the addition of the D2 specific antagonist sulpiride (300 µM). Dopamine appeared to affect both the NMDA and the non-NMDA mediated components equally and, in cultured bulb neurons, had no effect on membrane currents evoked by glutamate, kainate, or NMDA. Our data therefore support the notion that dopamine modulates synaptic transmission at the olfactory receptor neuron synapse through a presynaptic D2 receptor-mediated mechanism.

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The Efficacy of Dendrodendritic Synapses Affected by the Noradrenergic Stimulation in the Rat Olfactory Bulb

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Vaginocervical stimulation at parturition accelerates maternal behavior in the rat with the change of reaction to pup odors. In virgin rats, pup odors are aversive and maternal behavior is not elicited until this aversion is avercome. These findings suggest that vaginocervical stimulation modulates the neural activities in the rat olfactory bulb (OB). Somatosensory stimuli activate centrifugal noradrenergic inputs from the locus coeruleus (LC) to the OB as well as other regions in the central nervous system. We have already shown that the dendrodendritic interaction between mitral and granule cells in the OB is modulated by LC activation. Field potentials were recorded in the granule cell layer evoked by antidromic electrical stimulation of the lateral olfactory tract. Paired-pulse inhibition (PPI) in the evoked field potentials reflects the efficacy of the dendrodendritic synapse between mitral and granule cells. PPI was decreased immediately after glutamate activation of the LC, and then increased for a few minutes. Infusion of a β-blocker, timolol, into the OB blocked this LC activation effect, but an α-blocker, phentolamine, did not. These results suggest that centrifugal noradrenergic inputs from the LC act on the dendrodendritic synapses through β-adrenoceptors. We further examined the effect of infusion of the β-agonist, isoproterenol, into the OB on the dendrodendritic interaction. The results show that the bulbar infusion of isoproterenol modulates PPI in field potentials.

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Rat Olfactory Bulb Neurons Express Components of the Cyclic GMP Second Messenger System. PAUL A. KINGSTON[1], COLIN J. BARNSTABLE[2], and FRANK ZUFALL[2] (Interdepartmental Neuroscience Program[1], Section of Neurobiology[2], Yale University School of Medicine, New Haven, CT 06510)

Increasing interest in the role of cyclic nucleotide second messengers in regulating neuronal activity has led us to investigate the possibility that cyclic GMP (cGMP) modulates neuronal signaling in the olfactory system. Synthesized by guanylyl cyclase enzymes regulable by intercellular messengers like nitric oxide and atrial natriuretic peptide, cGMP in turn regulates both cyclic nucleotide-gated (CNG) cation channels and a protein kinase. The presence of CNG channels and guanylyl cyclase, considered together with recent findings that CNG channels are distributed many areas of the nervous system, led us to test for the presence of cGMP system components in the olfactory bulb. Tissue samples obtained from adult rats were screened by reverse transcriptase-polymerase chain reaction (RT-PCR) to identify the expression of specific molecules. Primers specific for regions of the olfactory receptor neuron and rod photoreceptor CNG channels, both isoforms of a Type I cGMP-dependent protein kinase, and soluble and particulate guanylyl cyclases all amplified cDNA from olfactory bulb total RNA. Southern blot tests of the resulting PCR products with digoxigenin-labelled probes confirmed their identity. The expression patterns of cGMP system components in the bulb examined by *in situ* hybridization experiments with digoxigenin-labelled probes indicate that RNA for both forms of guanylyl cyclase and the protein kinase are expressed strongly in mitral/tufted neurons and cells surrounding the glomeruli of the olfactory bulb. Further studies are underway to determine the localization of the CNG channels and identify cells in the glomerular layer expressing cGMP system components. The expression and heterogeneous distribution of these enzymes demonstrate the presence of a cGMP system regulable by nitric oxide and atrial natriuretic peptide, and suggest a model for how such a system might modulate activity in the bulb.

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Orbital cortex damage impairs individual odor discrimination in Golden Hamsters

ARAS PETRULIS and ROBERT E. JOHNSTON (Department of Psychology, Cornell University NY)

Hamsters, like many mammals, are capable of discriminating individuals on the basis of their odors. Although this ability appears to be common-place, the neural mechanisms underlying individual discrimination have not been well characterized. To investigate this topic further, we have focused on the orbital prefrontal cortex as a possible neuroanatomical substrate for individual odor discrimination. This area is reciprocally connected to olfactory cortical areas and has been shown to be involved in complex, albeit artificial, odor discriminations and learning tasks. We tested the ability of female hamsters to discriminate two individual male's flank scents (using a habituation paradigm) prior to and following lesions of the orbital cortex or sham surgery. We also assessed their ability to discriminate sex-specific odors and to scent mark in response to male flank scent before and after surgery. Damage to the orbital cortex but not sham surgery eliminated the hamsters ability to spontaneously discriminate individual conspecific odors. However, animals with orbital cortex lesions were not impaired in discrimination of sex odors nor did they differ from shams in scent marking frequency. Thus, it appears that orbital cortex is important for complex social discriminations but not for less demanding discriminations such as those of sex. In addition, orbital cortex appears to be unimportant for stereotyped scent marking responses to social odors.

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Altered Levels of NGF & NGF-Receptor in Olfactory Bulb of Developing Hypothyroid & Recovering Rats. TIMOTHY SENDERA & ESMAIL MEISAMI (Physiol.Dept., Univ. Illinois, Urbana, IL 61801)

Effects of early thyroid hormone deficiency and recovery from this condition was investigated on concentration of nerve growth factor(NGF) and its low affinity receptor (NGF-R) in the rat olfactory bulb(OB). Thyroid deficiency was induced by adding propylthiouracil (PTU) in water (0.1% v/w) from birth which reduced body and brain growth; >50% of olfactory receptor neurons (ORNs) fail to develop & 20% of mitral cells degenerate; number of glomeruli is unchanged but their size is reduced. PTU withdrawal at weaning (day 25) partially restores body and brain growth. By 90 days, total ORN number and much of OB volume is restored. The rat OB is one of a few brain regions to express a low level of NGF-R($p75^{NGFR}$) throughout life. Immunohistochemical expression of NGF-R (MAb 192-IgG, Bohringer) in normal OB was patchy and limited to the glomeruli among which expression intensity varied from none to low to moderate. In 25-day hypothyroid OB, NGF-R expression was markedly elevated: most glomeruli showed moderate to high levels of NGF-R. By day 50, NGF-R expression in hypothyroid OB was very intense while OB of recovery rats showed an opposite trend. NGF levels, determined by an ELISA method, indicated a reduction in 25-day hypothyroid OB. Results suggest a link between thyroid hormones and NGF system in OB. Changes in NGF-R and NGF may also be secondary to altered proliferation and maturation of ORNs (Paternostro & Meisami, *Dev Brain Res*, 76:151-161, '93; *ibid* 83:151-162, '94). Long-term reversibility of these hypothyroid-induced changes in NGF and NGF-R is under study in 90-day rats where somatic recovery is stabilized.

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Localization of Neurotrophic Factor mRNA Expression in the Rat Olfactory Bulb

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Several lines of experimental evidence suggest that olfactory receptor neurons receive target-derived trophic signals from the olfactory bulb. When deprived of its normal target by olfactory bulb ablation, the olfactory epithelium becomes repopulated by new receptor neurons but does not fully recover structural and biochemical features characteristic of the mature epithelium. Furthermore, in vitro studies have demonstrated that co-culturing olfactory epithelium with olfactory bulb both increases the total number of mature olfactory receptor neurons and influences their morphology. The bulb signals responsible for these effects have not been identified, but the demonstrated actions of neurotrophic factors on the growth and differentiation of other neuronal populations suggests that such factors play a role in this system. In order to identify neurotrophic factors expressed by the olfactory bulb, previous work from this laboratory examined the cellular localization of mRNAs encoding the NGF-family of neurotrophins. To further characterize the expression of neurotrophic factors in this system, the present work describes the cellular localization of mRNAs encoding insulin-like growth factor-1 (IGF-1), transforming growth factor-α (TGF-α), the acidic and basic fibroblast growth factors (aFGF, bFGF), and ciliary neurotrophic factor (CNTF) in rat olfactory bulb using in situ hybridization of ^{35}S-labeled cRNA probes.

IGF-1 mRNA was expressed at high levels by mitral and tufted cells, and at lower levels by glial cells in the olfactory nerve layer. Dense hybridization of TGF-α cRNA occurred in the granule cell layer, with much lower levels seen over scattered juxtaglomerular neurons. Expression of aFGF mRNA was restricted to a subpopulation of mitral cells. Basic FGF mRNA was expressed at comparatively high levels by glial cells in the nerve layer, as well at low levels by astrocytes scattered throughout the other bulb laminae. Dense hybridization of the CNTF cRNA exclusively labeled glial cells in the olfactory nerve layer. While most bulb cell types expressed some type of putative trophic factor, the glial cells of the olfactory nerve layer appear to be a particularly rich source of several such factors, suggesting the involvement of these cells in the trophic support of olfactory receptor neurons and their afferents.

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Early Deafferentation & Development of Rat Olfactory Bulb Glomeruli: Histo- & Immunochemical Study of Cytochrome Oxidase, Synaptophysin, NGF & NGF Receptor. NIDA GLEVECKAS & ESMAIL MEISAMI (Dept.Physiol., Univ. Illinois, Urbana, IL 61801)

Early olfactory deafferentation through zinc-sulfate induced chemical destruction of olfactory epithelium results in marked retardation in growth of the olfactory bulbs (OB) [Meisami & Manoochehri *Brain Research* 128: 170-175, 1977]. Here we report on the effects of the same treatment on the size and number of olfactory glomeruli. The nasal cavity of 3-day-old rats were irrigated by a zinc sulfate solution. At days 12 and 25 of age the OBs of the deafferented and control animals were isolated, sectioned and stained histochemically for cytochrome oxidase (CO) and immunohisto-chemically for synaptophysin. Deafferented OBs were consistently smaller than controls at both days 12 and 25. Quantification of glomeruli showed a marked reduction in size but a moderate reduction in number of glomeruli. Although, these changes were evident throughout OB, reduction in glomerular size varied proportionally with extent of reduction in the thickness of olfactory nerve layer. Interestingly, early olfactory denervation did not have a marked effect on intensity of CO or synaptophysin staining; this may be secondary to reduced glomerular size. Preliminary study of deafferented OBs for concentration of nerve growth factor (NGF) (determined immunochemically by an ELISA method) and expression of NGF low affinity receptor(NGF-R, $p75^{NGFR}$) determined immunohisto-chemically using MAb192-IgG (Bohringer) indicate reduced NGF concentration in the denervated OB together with elevated NGF-R expression in glomeruli. Results indicate critical influence of sensory afferent neurons on OB development.

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Glial Cell Response to Deafferentation of the Rat Olfactory Bulb.

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It is well established that astrocytic glia respond to injury of the CNS either directly at or removed from the site of injury. In the olfactory bulb, several subtypes of astrocytes are present (Bailey and Shipley, 1993). Olfactory sensory deprivation causes increased gliofibrillary acid protein (GFAP) expression in the bulb by immunocytochemistry (Martinez Garcia et al. 1991). We hypothesize that the subtypes of astroglia and elements of glia respond differentially to deafferentation of the bulb. In the present study, zinc sulfate injection into the olfactory epithelium of PND10 rat pups was used to deafferentate. Following 3 days survival the pups were sacrificed and the olfactory bulbs processed by immunocytochemistry to demonstrate expression of different glial intermediate filament markers including vimentin and GFAP. In addition an antibody to the 5-HT_{1A} receptor, reported to be predominantly on glial cells (Whitaker-Azmitia, 1993), was examined. The contralateral bulb and bulbs of unoperated littermates were used as controls. Relative to controls, GFAP immunoreactive glia in deafferentated bulbs exhibited marked hypertrophy in all layers, especially the external plexiform layer. Vimentin immunoreactive cells also appeared to react to the deafferentation. The location and extent of cell processes immunoreactive to the 5-HT_{1A} receptor did not differ between deafferentated and control bulbs; the 5-HT_{1A} receptor was located on radially oriented processes especially in the mitral cell layer. We are presently analyzing the response of astrocyte subtypes to the deafferentation. In conclusion, glial markers expressed differential responses to deafferentation of the bulb.

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Use of Magnetic Resonance Imaging in Neural Degeneration and Regeneration in Rat Olfactory System SAIED AGAHI, ESMAIL MEISAMI & PAUL LAUTERBUR (Dept. Physiol. & Biophys. & Biomedical Magnetic Resonance Lab., Univ Illinois, Urbana, IL 61801).

To determine whether olfactory bulb (OB) layers can be visualized by magnetic resonance imaging (MRI) and to utilize this tool for *in vivo* visualization of olfactory degeneration and regeneration, we conducted several experiments: Normal rats were anesthetized with a cocktail of ketamine, xylazine and acepromazine and placed in specially constructed plastic cylindrical chambers wound with an Alderman-Grant rf coil 6.3 cm in diameter. Using a 4.7T/33cm bore magnet and SISCO imaging spectrometer, the OBs were imaged using moderately T_2 weighed images. The imaging data sets were processed and displayed using the NCSA supercomputers and *Viewit* visualization software. Coronal MRI images were compared with homologous histological sections of OB. The MRI images of OB revealed 4 zones corresponding to 1)olfactory nerve-glomerular layers, 2) external plexiform layer, 3) internal granular layer and 4) ventricular-ependymal layers. Indeed it was possible to measure the thickness of these layers from MRI images. We are now determining whether these findings can be applied to olfactory degeneration and regeneration. Nasal cavity of newborn or adult rats are irrigated with a zinc sulfate or triton-x solution to destroy olfactory receptor neurons; this results in reduced thickness of OB and some of its layers. At weekly intervals after treatment, the OBs are imaged and width of various layers determined from the MRI images and compared with the age-matched controls.

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The Effects of Unilateral Naris Occlusion on Spontaneous and Odor-Driven Unit Activity in the Rat Olfactory Bulb. BENJAMIN D. PHILPOT, THOMAS C. FOSTER, and PETER C. BRUNJES (University of Virginia, Charlottesville)

Early unilateral naris closure has profound effects on the morphological development of the ipsilateral olfactory bulb. Although increasing evidence has shown long-term naris closure affects bulb physiology (e.g. Guthrie et al., *J. Neurosci.* 10, 1990; Wilson and Wood, *Neurosci.* 49, 1992), little attention has been given to the immediate effects of occlusion on spontaneous and odor-driven unit responses in the bulb. The present study examined the possibilities that 1) spontaneous activity changes following naris occlusion and 2) the bulb ipsilateral to occlusion remains competent to respond to odorants. Extracellular unit activity was examined in postnatal day 25-30 rats using conventional electrophysiological techniques. Subjects were anesthetized with chloral hydrate. A recording electrode was placed in the vicinity of the mitral cell layer by observing characteristic field potentials evoked by lateral olfactory tract stimulation. Baseline spontaneous activity levels were recorded prior to stimulation with amyl acetate Only units with excitatory responses to the stimulus were further investigated. After activity returned to baseline, the ipsilateral naris was occluded by applying warmed Vaseline. Unit activity was recorded over the next 10 minutes, at which time amyl acetate was delivered once again. Our results suggest that spontaneous unit activity decreases after 10 minutes of naris occlusion. Although stimulation with amyl acetate evoked a response in occluded bulbs, the increase was attenuated. Occluded bulbs may remain minimally responsive to odorants via information passage through, for example, the septal window or anterior commissure. In summary, naris occlusion affects both spontaneous and odor-evoked activity. Either of these factors may contribute to the subsequent observed morphological changes within the bulb.

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Subcutaneous Odorant Administration Increases Tyrosine Hydroxylase Expression in Olfactory Bulbs of Naris-Occluded Adult Mice HARRIET BAKER[1], LINDA FRANZEN[1] and JOEL MARUNIAK[2]. ([1]Cornell Univ. Med. Coll. at Burke Med. Res. Inst., White Plains, NY and [2]Univ. Missouri, Columbia, MO.

Unilateral naris closure in adult mice produces a profound reduction in the expression of the dopamine phenotype in juxtaglomerular neurons of the olfactory bulb. It is presumed that prevention of odorant access to the olfactory epithelium and thus stimulation of receptor neurons results in the down regulation in dopaminergic expression since either chemical or surgical deafferentation produce similar alterations. The previously reported occurrence of odor perception following either intravenous or subcutaneous application of odorous compounds was exploited to further assess the role of receptor stimulation in dopamine expression. At least two months after naris occlusion performed on adult CF-1 mice, either a mixture of four odorants, pentyl actetate, butyric acid, allyl sulphide and limonene (each at 0.5%), in corn oil or corn oil alone (control) was applied by single, daily, subcutaneous injection. After 10 days, dopamine expression was analyzed by tyrosine hydroxylase immunocytochemistry. Mice receiving odorant injections showed a patchy increase in the number of tyrosine hydroxylase immunoreactive neurons as compared to control animals in the olfactory bulb ipsilateral to the closure (115 ± 8.0 versus 41.7 ± 5.5 cells per section, respectively, $P < .05$). Although increased, the number of TH-containing neurons was small in the ipsilateral compared to the large number in the contralateral olfactory bulb. The treatment resulted in no obvious changes in the number of neurons in the contralateral olfactory bulbs. These data suggest that odorants gain access to the olfactory epithelium following parenteral application and that stimulation by this route may result in normal activation patterns. (Supported by AG09686).

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Metabolism and Transport of Inhaled Xylene to Olfactory Bulb Glomeruli: Nasal Metabolism as a Component of the Nose-Brain Barrier. J. L. LEWIS, A. R. DAHL, and D. A. KRACKO (Inhalation Toxicology Research Institute, Albuquerque, NM).

Our studies on olfactory transport of inhalants postulated a "nose-brain barrier" that served to protect the central nervous system from toxicant insult during inhalation much as the blood-brain barrier protects the brain from systemic toxicants. This nose-brain barrier is proposed to be multifaceted, with two likely components being 1) tight junctions between epithelial cells of the olfactory epithelium and 2) high xenobiotic-metabolizing capacity in the olfactory epithelium. F344 rats inhaled a human threshold limit value concentration of ^{14}C-xylene for 1 hr subsequent to inhalation of 1) methyl bromide to damage the olfactory epithelium; 2) cold xylene to inhibit xylene metabolism; or 3) clean-filtered air. At 30 min post exposure, nearly all (93%) of the radioactivity in the olfactory bulbs was associated with less-volatile metabolites of xylene rather than the parent compound. We found no xylene metabolism in olfactory bulb microsomes, but a high capacity for xylene metabolism in the olfactory mucosa. Therefore, xylene appeared to be metabolized within the olfactory mucosa and the metabolites subsequently transported to the olfactory bulbs. Metabolites were still detectable by 4 hr post exposure, and 10% of the radioactivity remained at 18 hr. Autoradiographs showed that, at 30 min post exposure, radioactivity was localized in brain only in the glomeruli of olfactory bulbs. At 4 hr post exposure, radioactivity was still detectable in the autoradiograms, but not at 18 hr. Results from other exposure conditions are not yet complete, but suggest epithelial lesions result in increased transport of parent xylene into the olfactory bulbs.

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Identification of a Discrete Subset Of Rat Olfactory Glomeruli and their Reinnervation Following Methyl Bromide Lesion. RING, G., YOUNGENTOB, S.L. AND SCHWOB, J.E. (Depts. of Anatomy and Cell Biology and of Physiology, SUNY Health Science Center, Syracuse, NY).

The functional organization of the olfactory epithelium and its projection to glomeruli in the olfactory bulb are not fully elucidated. The ability to identify a discrete subset of olfactory neurons and glomeruli can be useful in understanding the pattern of projections of olfactory neurons onto the olfactory bulb, and the mechanisms of reinnervation. Using the monoclonal antibody KH10 (Developmental Studies Hybridoma Bank) which recognizes a lactoseries carbohydrate antigen (Dodd and Jessell, 1985, J. Neurosci. 5:3278), we determined the projection pattern of a subset of olfactory epithelium neurons onto specific glomeruli in the main olfactory bulb (MOB) in normal rats and in rats whose olfactory epithelium (OE) was either partially or completely destroyed by exposure to the gas methyl bromide (MeBr). A single exposure of MeBr (330 ppm) for 6h selectively destroys >90% of the olfactory epithelium, leaving respiratory epithelium and the vomeronasal organ intact. MeBr in combination with skatol destroys 100% of the olfactory epithelium including the nasal septal organ. In normal rats olfactory neurons whose cell bodies and dendrites are labeled by KH10 are concentrated in the caudalmost extent of the dorsal recess and in the cul-de-sacs of ectoturbinates 1 and 2. In the main olfactory bulb KH10 intensely labels a small population of glomeruli at the caudalmost extent of the bulb, which topographically correspond to the "necklace olfactory glomeruli" described by Shinoda, et al, (1989, J. Comp. Neurol. 284:362), and "atypical glomeruli" described by Zheng et al., (1987, Neuroscience 23:1083). In contrast to the vast majority of glomeruli of the MOB, these glomeruli stain very weakly, or not at all with anti-OMP (olfactory marker protein). In addition, KH10 variably labels glomeruli in the dorsal and dorsomedial edge of the MOB and strongly labels all of the axons of the vomeronasal organ to their terminations in the glomeruli of the accessory olfactory bulb. We determined the staining pattern of KH10 and OMP of 6 identifiable "necklace/atypical" glomeruli in rats 1-21 weeks after their olfactory epithelium was totally or partially destroyed by exposure to MeBr. One week post lesion, KH10 staining was extremely weak, indicating that the afferent innervation from the OE was largely destroyed. In some cases 6-21 weeks following exposure to MeBr, the staining pattern of KH10 was undistinguishable from normal, indicating that the original "necklace/atypical" glomeruli had been successfully reinnervated by KH10(+) olfactory neurons. In other long-term cases, especially those in which the OE was completely destroyed, the original KH10(+) glomeruli stained very weakly or not at all with KH10, and some became OMP(+). In contrast, many glomeruli which are not normally labeled by KH10, became spuriously labeled by the antibody and lost their staining for OMP. These results suggest the hypothesis that regenerating olfctory neurons may require intact axons as guideposts to successfully reinnervate their normal targets. (supported by DC 00080, 00220, 00467)

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A theoretical investigation of the neural mechanisms underlying odor processing in the honey bee antennal lobe.

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In the honeybee, a large amount of experimental data have been collected at different levels of observation within the olfactory system, from signal processing to behavior, including cellular and molecular properties. However, no set of data considered by itself can give insight into the mechanisms underlying odor discrimination and pattern recognition. Here, by concentrating on deciphering the neural mechanisms underlying encoding and decoding of the olfactory signal in the two first layers of the neural network, we illustrate how a theoretical approach helps us to integrate the different experimental data and to extract relevant parameters (features) which might be selected and used to store an odor representation in a behavioral context. Our model neural permits to study the computational capacities of the neural circuitry in the antennal lobe and to investigate a number of features concerning odor discrimination and feature detection in the antennal lobe layer. No precise knowledge as to the neural mechanisms which cause the observed increased activities of ONs in response to olfactory stimulation exists up to now. From a theoretical point of view, two basic mechanisms can be implemented: (i) indirect excitation of ONs via excitatory local interneurons; in this case, the lateral inhibition in-between glomeruli should mainly affect these excitatory elements, as they are responsible for the activation of the glomerulus, (ii) release of spontaneous activity of ONs by inhibition of their presynaptic inhibitory local interneurons. In this case, the lateral inhibition affects mainly the inhibitory interneurons (no excitatory interneurons are needed). A comparison between these two mechanisms shows that the resulting across fiber patterns of ON activities are statistically comparable, but the emerging activities of local interneurons are more "realistic" (in comparison with the electrophysiological data) in the second case.

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Phylogenetic Analysis of Main and Accessory Olfactory Bulb Anatomy.

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The main and accessory olfactory bulbs contain many interacting elements that have been modified repeatedly over the course of vertebrate evolution. If we are to understand the roles of these elements and the relative functions of the main and accessory olfactory systems, we must understand the evolutionary relationships among their components. We therefore conducted a cladistic analysis to determine ancestral and derived features of the main and accessory olfactory bulbs in vertebrates. Our analysis included data from hagfish, lampreys, sharks, teleosts, sturgeons, lungfish, amphibians, squamates, crocodilians, and rodents. We postulate that the ancestral condition for the vertebrate olfactory bulb is (1) the presence of glomerular, mitral cell, and granule cell layers; (2) indistinct laminar boundaries and no intervening plexiform layers; (3) somata of some mitral cells located in between glomeruli, with each mitral cell possessing several primary dendrites that can project to different glomeruli; (4) granule cells with centripetally-projecting axons; and (5) absence of an accessory olfactory bulb or any homologue. Some features of the olfactory bulb have been modified independently in teleost fishes and possibly in lungfish. The main olfactory bulb is greatly modified in tetrapods: (1) the laminae are distinct, and include both external and internal plexiform layers; (2) the mitral cells possess "basal dendrites," with the primary dendrites projecting to only one or a small number of glomeruli; (3) the granule cells lack an axon; (4) tufted cells are present; and (5) periglomerular cells are present. The modification or emergence of some these features may be interrelated, and might be linked to the emergence of the accessory olfactory bulb, which appears to be a novel feature of tetrapods. In general, the accessory bulb contains laminae with indistinct boundaries and mitral cells that project to multiple glomeruli, lacks tufted cells, and may lack true periglomerular cells. These observations indicate that the anatomy of the main olfactory bulb has diverged in different tetrapod lineages, whereas the form of the accessory bulb is highly conserved. Function of the accessory bulb may therefore be more highly conserved in tetrapods than is that of the main olfactory bulb.

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Interpretation of Olfactory Stimulation by Lateral Protocerebrum Neurons in the Crayfish.
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In crustaceans, projection neurons (PN) from the olfactory midbrain ascend the olfactory-globular tract and terminate within the lateral protocerebrum (LPC). A large proportion of PN synapse directly upon bursting neurons of the hemi-ellipsoid body. We have studied responses of these and other LPC neurons to application of electrical or odorant stimuli applied to the lateral antennular filaments in perfused, isolated head preparations of the crayfish *Procambarus clarkii*. Stimuli were solutions of individual amino acids, mixtures and tetramin. Records were obtained with sharp micropipette electrodes from the LPC neuron cell bodies. Hemi-ellipsoid cells responded to odorants or single shocks with EPSP's and a modest number of impulses. Underlying subthreshold baseline oscillations of ca. 1 Hz sometimes were enhanced for a few cycles by the stimulus, generating impulses at the peaks of depolarization. Responses to individual amino acids as well as to mixtures were recorded, suggesting that mixture complexity is not a necessary feature of excitation at this level of the nervous system. Hemi-ellipsoid neurons receive highly convergent input from PN axons[1]. Furthermore there is strong evidence for electrical coupling between individual cells. Interpretations of odorant stimuli by these neurons may thus involve widescale changes in the overall activity of the cell population, while the changes in activity of the individual neurons remain subtle. Other LPC neurons resonded to either onset or the cessation of fluid flow over the antennules, providing a possible basis for interactions between mechanical and chemical stimuli by higher order units.

[1] Mellon D, Sandeman D, Sandeman R (1992) J Exp Biol 167:15-38
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Histamine-mediated inhibition in the olfactory lobe of the spiny lobster. M. WACHOWIAK and B.W. ACHE (Whitney Laboratory and Dept. Neuroscience, Univ. Florida, St. Augustine, FL, 32086).

Last year (AChemS XVI, Abstr. #248), we reported that histamine (HA) mediates the long-lasting hyperpolarization that normally follows excitation of projection neurons in the lobster olfactory lobe (OL) in response to electrical stimulation of the olfactory nerve. We also reported that blocking the HA-mediated hyperpolarization with cimetidine reveals a slow, protracted depolarization lasting 6 - 10 seconds which can lead to further spiking. We now show that cimetidine also lowers the threshold, but not the duration of the initial excitation, suggesting that there are both tonic and stimulus-dependent components to HA-mediated inhibition. The effect of cimetidine is reversible and dose-dependent. Ranitidine and *d*-tubocurarine have similar effects on the responses of OL projection neurons, although both appear to be slightly less potent than cimetidine. The pharmacology of the HA-mediated inhibition thus roughly matches that of a HA-gated chloride channel previously characterized in lobster olfactory receptor cells (McClintock and Ache, PNAS 86:8137-8141), suggesting that this or a similar channel mediates HA's action in the OL. A polyclonal antibody known to specifically label HA in carbodiimide-fixed lobster tissue reveals a distinctive pattern of HA-like immunoreactivity (HA-IR) in OL glomeruli, in which the subcap stains much more heavily than the cap or the base regions. HA-IR occurs in many somata and primary neurites in the cell cluster containing the somata of OL interneurons, but not in the cell cluster containing the somata of OL projection neurons, suggesting that HA-mediated inhibition within the OL is mediated primarily by local interneurons, at least some of which preferentially innervate the subcap of glomeruli. We are currently using biocytin and HA-IR double-labelling to identify individual HA-ergic interneurons.

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Olfactory and Accessory Lobe Development in *Homarus americanus* and Effects of Serotonin Depletion. B. BELTZ, J. BENTON, S. HELLUY. Dept. Bio. Sciences, Wellesley College, Wellesley, MA. 02181.

The paired olfactory lobes (OLs, primary chemosensory areas) in the lobster brain are of interest because chemoreception plays a key role in behaviors such as mating, food acquisition and territoriality in mature animals. The paired accessory lobes (ALs, higher order processing centers) develop in close association with the OLs. Both OLs and ALs are composed of glomerular neuropil and receive a dense serotonergic innervation. Histological methods were used to document the enlargement of the OLs and ALs and the accompanying changes in numbers of glomeruli from embryonic through adult life. The developmental effects of serotonin depletion by the toxin 5,7dihydroxytryptamine (5,7DHT) also were examined. The OLs emerge at 10% embryonic development (E10%); ALs appear later at E40-45%. Serotonin is detectable immunocytochemically in the deutocerebrum by E40%, and the ALs receive a dense serotonergic innervation from their first appearance. The columnar OL glomeruli begin to form at E40-45%, coinciding with the ingrowth of antennular sensory axons. During embryonic and larval life, OL glomeruli expand in both size and number until the animal adopts a benthic life at the end of stage 4 when each OL has in the range of 200 glomeruli with an average diameter of 25μm. This number remains constant in juveniles and adults, but glomerular size increases to approximately 100μm in the mature adult. The sperical glomeruli of the ALs appear at larval stage 2, and their numbers are stable at approximately 2000 per lobe after stage 4. When 5,7DHT was injected into embryos weekly for 1 month, serotonin levels were reduced by 90% compared to control embryos receiving injections without toxin (Benton et al.[1994] Soc. Neurosci. Abstr. 20: 775). When embryos were allowed to develop following toxin injections, serotonin depletion persisted for at least 2 months. In embryos that were toxin-treated beginning at E38% and sacrificed at E55%, OL glomeruli formed and ALs emerged as expected during this period, but the sizes of both OLs and ALs were significantly reduced in the experimental group. The growth of the antenna 2 neuropils, which do not receive early embryonic serotonergic innervation, was not affected by toxin treatment. These data indicate that the basic construction of the OLs and ALs is complete by the end of larval life and that after stage 4 ingrowing axons must project to existing glomeruli. The smaller volumes of the OLs and ALs following treatment of embryos with 5,7DHT suggest that serotonin may be involved in the normal development of these regions.

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Neurogenesis in the Olfactory Bulb of the African Clawed Frog, *Xenopus laevis*. ANNE FRITZ, DENNIS L. GORLICK, and GAIL D. BURD. University of Arizona, Tucson, AZ.

Previous studies have shown that olfactory bulb (OB) development is dependent upon innervation from olfactory receptor cell axons (Stout and Graziadei, 1980; Byrd and Burd, 1993). In *Xenopus*, prior to metamorphosis there are two areas of sensory epithelium; at the onset of metamorphosis, a third area of sensory epithelium forms (Key, 1986; Weiss, 1986). In addition, there is plasticity of the sensory projections at metamorphosis. The principal cavity receptor cells change their innervation pattern; initially, axons from this cavity innervate the ventral OB. After metamorphosis, the axons from the principal cavity innervate the dorsal OB (Weiss, 1986). As a continuation of our previous work on the influence of sensory afferents on the development of the OB, the goal of our current study was to determine the birth dates of neurons in the OB throughout development. *Xenopus* embryos and tadpoles (early neurula [stage 11/12] to metamorphic climax [stage 62]) were injected with ^{3}H-thymidine and the tissue was processed for autoradiography when the animals reached stage 62 or two weeks postmetamorphosis. We found that the mitral/tufted cells in the ventral OB are born first, beginning as early as stage 11/12 and continuing through metamorphosis. The early mitral/tufted cells, however, were not distributed uniformly throughout the mitral/tufted cell layer; they were primarily concentrated in the lateral quadrant of the OB. The interneurons, granule cells and periglomerular cells, in the ventral bulb are first born much later, beginning at stage 41. Neurogenesis in the ventral OB followed the normal pattern of CNS development observed in vertebrates, with output neurons born first followed by interneurons. In contrast, the mitral/tufted, periglomerular, and granule cells of the dorsal OB were born at about the same time beginning at stage 54, the onset of metamorphosis. In summary, we observed that the ventral region of the OB formed first. In addition, our results are consistent with the hypothesis that the principal cavity sensory axons, innervating the dorsal OB at stage 54, are responsible for the development of the dorsal region of the OB.
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The Primary Olfactory System of the Salmon exhibits both Punctuated and Sustained Growth Across the Life History

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In the Coho salmon (*Oncorhynchus kisutch*), olfaction plays a major role in successful completion of the life history: juveniles imprint to odor cues in their natal stream, then, when adult, home during the spawning migration to that same stream. In ongoing experiments studying the primary olfactory system across the life history of the Coho, the olfactory bulb (OB), its laminae the glomerular layer (GL) and inner cell layer (ICL), and the olfactory nerve (ON), were observed in fish from several ages. Results, normalized to remove the confounding influence of increasing body length, suggest several major allometric changes: 1) Dramatic, sustained growth of the OB and ON occurs throughout the life history. In spawning adults (45-55 months old; n=4), OB volume is 7.5x that in juvenile parr (14 mos.; n=4), and 35x that in fry (5 mos.; n=3). Similarly, cross-sectional area of the ON increases 6x between parr/adult stages. 2) Rapid, punctuated change in the laminar composition of the OB occurs in juveniles. The contribution of the GL to OB volume increases 1.2x between 5/14 months, while only increasing 1.1x between 14/55 (adults) months. The ICL shows a linear decrease in OB volume across the life history. These anatomical changes may represent changes in the function of the primary olfactory system, such as an increase in olfactory sensitivity. The precise timing of these anatomical changes within the context of the life history, as well as their hormonal basis, is currently being explored.

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Subunit Architecture of the Macroglomerular Complex and the Integrative Function of its Output Neurons in the Moth, *Manduca sexta*

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The sexually-dimorphic macroglomerular complex (MGC) in the antennal lobe of the male moth *M. sexta* receives input from pheromone-responsive antennal neurons that are each tuned to one of the two key components of the female's sex pheromone. The MGC consists of two large and identifiable glomeruli - the cumulus and toroid. In some MGC projection neurons (PNs) that arborize in both MGC glomeruli, mixed inhibitory/excitatory responses are evoked by stimulation with the blend of both components. Mixed responses consist of an initial hyperpolarization (I_1), a subsequent depolarization leading to a burst of action potentials, and a long afterhyperpolarization (I_2) with a different apparent reversal potential than I_1. Further investigation into the role of inhibitory input to MGC-PNs has revealed that mixed responses may sometimes be evoked by stimulation with only one pheromone component, and that the appearance of the three response phases which characterize the mixed response is dependent upon a number of different experimental variables. For example, an observable I_1 is strongly dependent upon resting membrane potential. I_1 is often not visible at rest, but is revealed upon injection of depolarizing current. It also reverses polarity with hyperpolarizing current, typical of a chemically-mediated IPSP. Another variable that can affect the appearance of I_1 is the position of the stimulus along the length of the antenna. These findings indicate that under the proper conditions, stimulation with only one pheromone component can mediate both the inhibitory and excitatory phases of the mixed response in some MGC-PNs, reflecting new features of the underlying circuitry within MGC glomeruli. Some cells appear to receive both inhibitory and excitatory input in the same MGC glomerulus, and these inputs may be differentially stimulated from different parts of the antenna, suggesting that complex integrative processes take place in one glomerulus. Could the integrative properties of different MGC-PNs be correlated with their anatomical specializations? We are exploring the subunit organization of MGC glomeruli by combined intracellular recording, neuronal tracing methods, and laser-scanning-confocal microscopy. Massive fills of primary-afferent axons with Neurobiotin have revealed a lobular structure within the cumulus, and a partitioning of the toroid into two distinct glomeruli. Some MGC-PNs, stained intracellularly with Biocytin, have arborizations restricted to distinct sub-zones in one MGC glomerulus, whereas others have more extensive arborizations throughout one or all MGC glomeruli. The main neuritic branches of PNs often have a columnar pattern of arborization in the MGC, suggesting that PN neurites sample input from a subset of functionally distinct columns of neuropil within the MGC glomerulus. [Supported by NIH grant AI-23253 to J.G.H.]

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Characterization of Inward Currents in Vomeronasal Receptor Cells of Neotenic Salamanders (*Ambystoma*).

HEATHER L. EISTHEN and VINCENT E. DIONNE (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA)

The vomeronasal system is an accessory olfactory system that has been identified only in tetrapods and may have originated in aquatic amphibians. The relative functions of the olfactory and vomeronasal systems have been examined only in a small number of terrestrial reptiles and mammals. To be able to formulate hypotheses concerning the evolutionary origin and adaptive roles of the olfactory and vomeronasal systems, we must understand the functions of these systems in aquatic amphibians. We have therefore begun to investigate the physiological properties of the vomeronasal receptor cells in neotenic members of two subspecies of tiger salamanders, axolotls (*Ambystoma tigrinum mexicanum*) and barred tiger salamanders (*A. t. mavortium*). Using whole-cell patch methods, we have recorded from receptor cells in thin slices of vomeronasal epithelium of adult animals. The inward currents in vomeronasal receptor cells from *Ambystoma* were transient with peak values of -24.1 ± 5.5 pA/pF (n=34). Approximately 80% of the inward current was reversibly blocked by application of 10-100 nM tetrodotoxin (TTX), a specific blocker of sodium channels (n=12). The activation and inactivation kinetics of the sodium current voltage sensitivities were typical of those seen in other neurons. The addition of 1 mM cobalt, a calcium channel blocker, eliminated the remaining inward current (n=3). Inward currents of similar magnitude have been reported in *Ambystoma* olfactory receptor neurons, although in these cells the sodium current was not blocked by TTX and its inactivation kinetics were markedly voltage-insensitive (Firestein and Werblin, *Proc. Natl. Acad. Sci.* 84: 6292-6296). Our data indicate that both sodium and calcium channels contribute to the inward current in vomeronasal receptor cells of ambystomatid salamanders, as has been reported for vomeronasal receptor cells in the frog *Rana esculenta* (Trotier et al., *Eur. J. Neurosci.* 5: 995-1002), and contrast with those from mammalian vomeronasal receptor cells, which appear to lack a calcium component.

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The effects of olfactory mucosal lesion on vomeronasal investigative behavior in the gray short-tailed opossum.

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We examined the effect of temporary olfactory loss on a specific vomeronasal investigative behavior ("nuzzling" behavior) in the gray short-tailed opossum (*Monodelphis domestica*). This behavior is stimulated by the presence of conspecific scent deposits and is often followed by scent marking. Six adult males were injected with 300-400 mg/kg of methimazole or 200 mg/kg 2-pentenenitrile (2pn); two others were injected with the vehicle controls (DMSO and mineral oil, respectively). The animals were then tested for behavioral responses to conspecific odor deposits and were sacrificed 48 hours later. Histopathological results indicated that about 80-90% of the olfactory mucosa was destroyed, with the mucosa lining the dorsal medial airways affected most severely. No histological changes were observed in the epithelium of the vomeronasal organs (VNOs). Methimazole and 2pn injections resulted in a significant drop in nuzzling duration of novel conspecific odor deposits after 24 hr (8.20±5.27 s post-test vs 40.09±15.33 s pretest; paired $t=2.06$, $p< 0.05$). An even more dramatic effect was observed after 48 hr when nuzzling duration of both own (0.16±0.14 s) and novel conspecific odor deposits (2.23±0.62 s) nearly disappeared ($p<0.03$). Scent marking was completely abolished 24 and 48 hr after injection. Vehicle controls had no significant effect on nuzzling or marking behavior. One animal which was monitored and tested daily after injection started to regain nuzzling and scent marking behaviors 4 days post injection. Behavioral recovery was nearly complete after 6 days. The study demonstrates that in spite of seemingly intact VNOs, an intact olfactory mucosa is necessary for vomeronasal investigation and scent marking behaviors in *M. domestica*.

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Differential expression of G proteins in the Vomeronasal System of the Opossum (*Monodelphis domestica*) CHANG-PING JIA and MIMI HALPERN (SUNY Health Science Center at Brooklyn, Program in Neural and Behavioral Sciences, 450 Clarkson Ave., Brooklyn, NY 11203)

Recent work in several laboratories has demonstrated that the accessory olfactory bulb (AOB) of several mammals is not homogeneous. The anterior and posterior portions of the nerve-glomerular layers of the AOB contain different levels of lectin VVA-binding sites, NADPH-diaphorase activity, olfactory marker protein, G proteins and other markers. In order to understand the possible significance of the heterogeneity of the anterior and posterior portions of the AOB, immunocytochemical experiments have been conducted in the AOB and vomeronasal (VN) organ using antibodies to G proteins ($G_{\alpha i2}$ and $G_{\alpha o}$). Adult opossums were perfused with Bouin's fixative and parasaggital sections of the brain were immunocytochemically stained with antibodies to $G_{\alpha i2}$ and $G_{\alpha o}$. In the nerve-glomerular layers of the AOB, the antibody to $G_{\alpha i2}$ stains the anterior half, and the antibody to $G_{\alpha o}$ stains only the posterior half. Since the nerve-glomerular layers contain axons and terminals of VN receptor neurons, sections of the VN organ were processed for staining with the same antibodies. The antibody to $G_{\alpha i2}$ stains the neurons in the middle 1/3 of the VN epithelium and the antibody to $G_{\alpha o}$ stains neurons in the deep 1/3 of the VN epithelium. Staining is located in the cell bodies, axons, dendrites and on the microvillar surface. The neurons that express $G_{\alpha o}$, which are located in deep 1/3 of the VN epithelium, are not basal or undifferentiated cells because (1) they have a morphology typical of mature bipolar receptor neurons; (2) after VN nerve sectioning, the number of neurons expressing $G_{\alpha o}$ decreases; and (3) 48 hours after BrdU injections labeled cells along the basal lamina do not express $G_{\alpha o}$. These results not only show the two subdivisions of the AOB, but also indicate that there are two spatially segregated populations of receptor neurons in the VN epithelium, one expressing $G_{\alpha i2}$ and the other expressing $G_{\alpha o}$. These results strongly suggest a topographic projection from the middle layers of the VN epithelium to the anterior portion of the AOB and from the deep layers of the VN epithelium to the posterior portion of the AOB.

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Histochemical, Immunocytochemical, and Tract Tracing Studies Investigating the Heterogeneity of the Primary Vomeronasal Pathway in the Brazilian Gray Short-tailed Opossum, *Monodelphis domestica*. LENA SHNAYDER SHAPIRO, CHENG-SHU LI, CHANG-PING JIA AND MIMI HALPERN (SUNY Health Science Center at Brooklyn, Program in Neural and Behavioral Sciences, 450 Clarkson Ave., Brooklyn, NY 11203)

We have previously demonstrated that antibodies to OMP (Shnayder et al., 1993), and the VVA lectin (Shapiro et al., in press) and NADPH diaphorase (Shnayder et al., 1994) stain the adult opossum accessory olfactory bulb (AOB) differentially. All three markers stain the rostral half of the AOB much darker than the caudal half, where there is little, if any, staining. The three markers show the same developmental pattern of differential expression: at the end of the first month of life the markers stain the AOB homogeneously, whereas two weeks later the differential pattern begins to emerge. The differential pattern is most striking in the adult opossum AOB. We have also noted that different G proteins are differentially localized in the adult opossum AOB: the rostral half is $G_{i2\alpha}$-positive, whereas $G_{o\alpha}$ is expressed in the posterior half (Jia and Halpern, 1995). Where along the primary vomeronasal pathway does this heterogeneity emerge?

After injections of WGA-HRP into the anterior half of the AOB, retrogradely labelled vomeronasal (VN) receptor cells are found only in a layer occupying the middle 1/3 of the sensory epithelium; when the injections encompass the entire extent of the AOB, retrogradely labelled cells are found in both the middle and basal layers of the VN epithelium. Repeated attempts to inject WGA-HRP into the posterior AOB exclusively were unsuccessful. Following injections of WGA-HRP into the VNO for anterograde labelling of the AOB, the label was restricted to the anterior half of the AOB, and almost no label was observed in the posterior AOB. These results suggest that there may be few binding sites for the WGA lectin in the posterior AOB, and could explain our difficulty in producing "clean" injections of the posterior AOB in the retrograde tracing study. We know from parallel studies (Jia and Halpern, 1995) that the middle 1/3 of the VN epithelium is $G_{i2\alpha}$-positive and that the basal 1/3 is $G_{o\alpha}$-positive.

These results indicate that the vomeronasal receptor cells located in the middle layer of the VN epithelium project to the anterior, darkly stained portion of the adult opossum AOB. We suspect that the basally situated cells project their axons to the posterior, lightly stained part of the AOB. We are in the process of characterizing, histochemically and immunocytochemically, the vomeronasal receptor cell population in order to understand more fully the phenotypic heterogeneity of the adult opossum primary vomeronasal pathway.

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FOS-ir After Exposure to Chemical Cues that Stimulate or Block Reproduction in Female Prairie Voles. MAUREEN L. TUBBIOLA, and CHARLES J. WYSOCKI (Monell Chemical Senses Center, Philadelphia, PA)*

Female prairie voles (*Microtus ochrogaster*) do not have estrous cycles. Exposure to chemical cues from male voles initiates uterine growth and exposure to female chemical cues can block this activation of reproduction. We are investigating the central pathways mediating this phenomenon using FOS-immunocytochemistry. Naive female prairie voles were exposed to two stimuli (urine from male voles, urine from females voles, or water) with 10 minutes between exposures. Subjects were exposed to male/male, male/female, water/female, water/water, or female/female stimuli and sacrificed 48 hours later. The only stimulus combination to induce uterine growth was male/male and exposure to female urine successfully blocked activation of reproduction (similar to Getz et al., 1983). In another experiment subjects were exposed to the same stimuli as above and sacrificed 1 hour later for FOS-immunocytochemistry. FOS-immunoreactivity (FOS-ir) was observed in the accessory olfactory bulbs of all animals except those exposed to water/water. FOS-ir is currently being examined in other regions along vomeronasal pathways including the bed nucleus of the stria terminalis, medial amygdala, and premammillary nuclei. Vomeronasal pathways may be differentially excited following exposure to chemical cues that stimulate or block reproduction, and sequential exposure to such cues may further alter neuronal activation.

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Fos expression during mating behavior in inexperienced male hamsters: Contributions of main olfactory input. GWEN FERNANDEZ-FEWELL and MICHAEL MEREDITH. Florida State University, Tallahassee, FL.

An intact vomeronasal (VN) system is important for mating behavior in inexperienced male hamsters and removal of vomeronasal organs (VNX) causes deficits in mating. Lesions of olfactory receptors (OLFX) on the other hand do not affect mating behavior and inexperienced OLFX males mate normally with a receptive female. After sexual experience main olfactory input may sustain mating behavior in the absence of a functional VN system since subsequent removal of the VNO produces no behavioral deficits but removal of both the VNO and olfactory receptors abolishes mating in sexually experienced males. Central vomeronasal pathways of inexperienced male hamsters had increased Fos expression after mating or pheromonal exposure compared to controls while main olfactory pathways: the main olfactory bulb (MOB), anterior cortical and posterior lateral cortical nuclei of the amygdala (ACN & PLCN) showed equivalent Fos expression in mating and control animals (Fernandez-Fewell & Meredith 1994). This study was designed to determine the contribution, if any, of main olfactory input to Fos patterns seen in intact inexperienced male hamsters after mating behavior. Sexually inexperienced male hamsters were either made anosmic (n=8) by intranasal infusion of zinc sulfate or were left intact (n=11). All OLFX animals were given food finding tests and only those that failed were included in the study. Animals from both groups were presented with a receptive female and allowed to mate for 45 minutes. After an additional 45 minutes they were perfused with 4% paraformaldehyde. Control animals from each group were put into clean boxes with fresh bedding and perfused 90 minutes later. Fifty μm sections were processed for immunocytochemistry using a polyclonal Fos antibody (Cambridge Research). Fos expression was analyzed in main olfactory and vomeronasal pathways of intact and OLFX animals. Fos expression in central vomeronasal pathways: the accessory olfactory bulb (AOB), medial amygdala (Me), and posterior medial cortical nucleus (PMCN), and in the medial preoptic area (MPOA) and bed nucleus of the stria terminalis (BNST) were not significantly different in OLFX and intact animals. As expected, main olfactory pathways (MOB, ACN, PLCN) had significantly less Fos expression compared to the low levels seen in intact stimulated and in unstimulated animals. Thus main olfactory input does not contribute much to the Fos expression in central areas known to be critical for mating behavior in intact inexperienced male hamsters.

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Induction of Fos expression in the accessory olfactory bulb (AOB) of female rats following stimulation of the vomeronasal organ (VNO).

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Previous studies from our lab have shown that application of pheromones to VN receptor cells produces an outward current which hyperpolarizes the membrane and inhibits neuronal firing. Yet, electrical stimulation of the VNO has been demonstrated to elicit orthodromic excitation in mitral cells of the AOB. To resolve this discrepancy, the present study was designed to compare the effects of electrical and pheromonal stimulation of the VNO on cellular activity in the AOB. Urethane anesthetized female rats were placed in a stereotaxic instrument and the right and left VNOs were exposed. A stimulating electrode was used to deliver trains of electrical pulses to the right VNO while the left VNO was unstimulated. In other animals, dehydro-*exo*-brevicomin, a pheromone isolated from male mouse urine, was infused into the right VNO while PBS was delivered to the left VNO. After delivering VNO stimulation periodically for two hours, the animals were perfused and the AOBs were processed for Fos immunoreactivity. In most animals, Fos expression was higher in mitral cell layer than in the granule cell layer. Electrical stimulation was associated with more Fos expression in the right AOB than in the left AOB. Also, high levels of electrical stimulation induced Fos expression in periglomerular cells. To date, the results of pheromone infusion are inconclusive, perhaps as a result of spread of the infusion and/or the small number of animals. Fos induction in the periglomerular cells has not been observed in previous studies where VNO dependent Fos expression was induced by repetitive mating. Also, repetitive mating induced Fos expression in the AOB is higher in the granule cells than in the mitral cells. The present results coupled with previous findings suggest that electrical and pheromonal stimulation induce Fos expression in different populations of AOB cells.

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Induction of c-fos Gene Product in the Male Hamster Accessory Olfactory Bulbs by Natural and Bacterially Cloned Aphrodisin

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Immunohistochemical analyses of c-fos gene product induction were performed in sexually experience male hamsters exposed either to the pheromonal protein aphrodisin purified from hamster vaginal discharge, the aphrodisin protein backbone cloned in E. coli, or the homologous lipocalin β-lactoglobulin. All three proteins were subjected to chromatographic deodorizing procedures. In previous behavioral bioassays, the hamster aphrodisin had been shown to account for most of the ability of vaginal discharge to elicit copulatory attempts by male hamsters via sensory stimulation in the accessory olfactory system, and that the E. coli aphrodisin, like the control protein β-lactoglobulin, lacks this aphrodisiac activity. The males were housed in isolation chambers like those used in our behavioral bioassays to control their exposure to odors. The proteins were presented as 50 µl aqueous solutions on a watch glass placed in a corner of the male's home cage within the chamber. The watch glass served as a novel stimulus which reliably induced contact investigation and vomeronasal sampling of the solution. Each male was sacrificed 45 minutes after introduction of the stimulus. Free-floating sagittal sections of the accessory olfactory bulbs (AOB) were incubated with sheep c-fos antiserum (Cambridge Research), and immunoreactive c-fos product was visualized with the ABC technique (Vectstain). Blind procedures where used to count labeled mitral/tufted cells in the AOB. Both the native hamster and E. coli aphrodisin produced greater labelling of mitral/tufted cells in comparison to the β-lactoglobulin control protein. This implies that the aphrodisin protein backbone does have stimulatory activity in the hamster accessory olfactory system, even though a ligand and/or conformational feature of the native protein is necessary for the pheromonal activity. The labeling induced by the E. coli aphrodisin was evenly distributed in the external plexiform and mitral body layer of the AOB, whereas the native aphrodisin produced considerably more labelling of mitral/tufted cells in the posterior region of AOB as compared to its anterior region, suggesting that the neurons which mediate the pheromonal response may be concentated in the posterior region.

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Vomeronasal Receptor Neurons: Species Specific Pheromones Decrease Membrane Conductance.

ROBERT L. MOSS, ROBERT E. FLYNN (University of Texas Southwestern Medical Center, Dallas, Tx).

Experiments were conducted on dissociated rodent bipolar neurons from the vomeronasal (VN) organ under tight-seal whole-cell voltage-clamp and current-clamp recording conditions. Puffer electrode contained dehydro-*exo*-brevicomin and a bath solution. The chemical agents were puffed (2.0 - 5.0 psi) onto the dendrites of the bipolar neurons while varying the holding potential. Previously, we observed that the general odor induced an inward current, while each pheromone induced an outward current. To address the question of whether pheromone-induced current involves a decrease or increase in conductance, the effect of pheromone (dehydro-*exo*-brevicomin at 6.5 µM) or bath application to a single bipolar VN neuron was compared. Under voltage-clamp the bath current was linear and behaved in the expected passive manner. Under current-clamp, the spontaneous firing of action potentials was observed and the application of pheromone suppressed the frequency of action potential firing. Pheromone-induced current was nonlinear and outward at negative potentials under voltage-clamp conditions. Current differences were obtained by subtracting the voltage-induced currents in the presence of bath from the pheromone, while change in conductance was obtained by dividing the current difference by the membrane potential minus the reversal potential. The associated conductance change in bipolar neurons in the presence of pheromone is a decrease. Coupled with previous findings from our lab, present results suggest that pheromones decrease the conductance of a nonspecific cation channel leading to a decrease in the spontaneous firing of action potentials. These findings suggest that the pheromone signal evokes an outward current in bipolar VN neurons. Supported by NIH grant DC02120.

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Chemosignal Transduction in the Vomeronasal Organ of Garter Snakes: Ca^{2+}-regulation of cAMP Generation.

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Earthworm shock secretion (ES) contains a 20 kD vomeronasally mediated chemoattractant for garter snakes (ES20). When ES20 binds to its G-protein coupled receptors IP_3 levels are increased, but forskolin-stimulated high levels of cAMP are reduced. We hypothesized earlier that the reduction by ES20-receptor binding of stimulated levels of cAMP was the result of activation of a Ca^{2+}-dependent cAMP-phosphodiesterase (cAMP-PDE). The following result provide evidence that Ca^{2+} regulates the synthesis of cAMP by inactivating adenylate cyclase rather than by activating a Ca^{2+}-dependent cAMP-PDE: (1) Although ES20-receptor binding requires Ca^{2+} or Mg^{2+}, Ca^{2+} alone but not Mg^{2+} can cause reduction in GTPγS- or forskolin-stimulated cAMP levels; (2) PDE inhibitors, IBMX and RO 20-1724, do not reverse the Ca^{2+} effect on the reduction of stimulated levels of cAMP; (3) no difference is observed in the hydrolysis of exogenously supplied [^{3}H]-cAMP in the absence or presence of Ca^{2+}; (4) following forskolin induction of cAMP, addition of Ca^{2+} does not result in a change in cAMP levels; (5) addition of [^{3}H]-cAMP during cAMP generation does not result in any significant reduction of the specific radioactivity of cAMP in the pool; (6) ADP-ribosylation by PTX does not alter the action of Ca^{2+} on GTPγS-stimulated high levels of cAMP; (7) ADP-ribosylation does not alter the stimulatory action of forskolin on cAMP accumulation; and (8) using intact vomeronasal sensory epithelium, binding of ES20 to its receptors in the presence of Ca^{2+} does not alter forskolin-stimulated cAMP levels; however, when a Ca2+-ionophore is included, the cAMP levels were reduced considerably.

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GENDER-SPECIFIC ACTIVITY IN NERVES FROM THE ADULT RAT VOMERONASAL ORGAN. H. Mack Brown, Zhian Wang and Larry Stensaas*. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT.

Reports of gender-specific behavioral responses to putative pheromonal substances are common. It is uncertain if this function lies in the sensory components of the VNO or is due to interpretation of VNO output by the CNS. We have explored this problem in terms of coding of chemical stimuli by nerve recordings from the isolated VNO of male and female rats in response to representatives of 3 classes of stimuli: (1) heptanone, (2) n-pentylacetate, and (3) dimethyldisulfide (10^{-3} M to 10^{-5} M). The discharge frequency from individual nerves from rostral, middle and caudal regions of the VNO was analyzed for a 50 sec interval by integrating consecutive 10 sec bins with an electronic frequency counter. Data from 34 male and 33 female VNO preparations indicated a demonstrable preference for certain sequences of the test compounds, and it was possible to distinguish the sex of the VNO donor based on these sequences. Male VNOs had a preferred sequence of 3>1>2 ($P<.05$), whereas female response to this sequence was not different from chance. VNO nerve discharge from female rats had a preferred sequence 1>3>2, whereas the male response was at the chance level for this sequence. VNOs from both sexes had a preferred sequence 3>2>1 ($P<.05$). There was also some evidence that the VNO has spatial specificity as well as sequence coding since the majority (70-80%) of the sexually specific sequences were obtained from the rostral and middle VNO nerves with little response from the posterior VNO nerve. Thus, while there is no sexual distinction based on individual compounds, there appears to be information coding in the preferred sequence of response to different classes of compounds which indicates that the rat VNO encodes meaningful gender-specific information at the sensory level.

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Olfactory transduction is intrinsically noisy.
GRAEME LOWE & GEOFFREY H. GOLD. (Monell Chemical Senses Center, Philadelphia, PA.)

The high sensitivity of olfaction suggests that olfactory receptor cells may reliably detect single odorant molecules. Discrete events, presumably triggered by single odorant molecules, have been observed in insect pheromone receptor cells. Are vertebrate olfactory receptor cells also capable of resolving single molecular events? To address this question, we recorded near threshold responses from dissociated olfactory receptor cells from rat. Whole cell currents evoked by: menthone, 2-isobutyl-3-methoxypyrazine, isoamyl acetate or 2-hexylpyridine, exhibited pronounced low frequency fluctuations. Such fluctuations might represent summated single molecular (quantal) responses. However, they might also represent noise intrinsic to the transduction mechanism. To determine the relative contribution of quantal vs. intrinsic noise we compared fluctuations in odorant-evoked currents with fluctuations in currents evoked by cyclic AMP in the absence of odorants. Intracellular cyclic AMP was elevated by inhibiting basal phosphodiesterase activity with IBMX, or by photolysis of caged cyclic AMP. Surprisingly, fluctuations in cyclic AMP-evoked currents exhibited similar amplitude and power spectral density as fluctuations seen in odorant-evoked currents. Thus, the odorant-induced fluctuations do not reflect the quantal nature of the odorant-receptor interaction. Rather, they constitute the basal, or intrinsic, noise of olfactory transduction. How is a high instrinsic noise to be reconciled with the quiet baseline currents of whole cell recordings from vertebrate olfactory receptor cells? We have proposed that, in the unstimulated cell, spontaneous fluctuations in the cyclic AMP signal are relatively large, but are attenuated by the threshold for the generation of current by cyclic AMP (Lowe & Gold, 1993 *Nature* **366**, 283 (1993)). High intrinsic noise may preclude the reliable detection of single odorant molecules by vertebrate olfactory receptor cells.

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Properties of Odorants Producing Maximal Responses in Different Parts of the Rat Olfactory Epithelium. JOHN W. SCOTT, LISA M. DAVIS, and DONNA E. SHANNON. Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia 30322.

We have reported that some odorants evoke larger electro-olfactogram (EOG) responses in the dorsal recess of the olfactory epithelium, near the nasal septum. Other odors evoke larger responses in the recesses between the turbinate bones. These two regions are anatomically different in projections to the olfactory bulb, in antigen expression and olfactory receptor gene expresssion. We studied EOG responses to homologous series of odorants: (1) acids, esters, ketones, aldehydes, alcohols, alkanes and cyclic alkanes with 6, 7, or 8 carbon atoms; (2) acids, alcohols, esters, and ketones related to benzene and toluene; (3) terpene compounds related to limonene and carvone. Tests were run on intact animals anesthetized with ketamine. Odors were tested over a two log unit concentration range. Iso amyl acetate was used as a standard for response normalization. All odorants without functional groups containing oxygen (eg. hexane, benzene, or limonene) evoked larger EOG responses in the lateral recesses. Esters evoked larger responses in the dorsal recess. Responses to acids and alcohols were small in both regions. Aldehydes and ketones related to benzene or terpinene evoked larger responses dorsomedially, but aldehydes and ketones based on the alkanes evoked very large responses at both sites. No odorants evoked responses exclusively in one region, but some (benzaldehyde or methyl benzoate) were often five times as large dorsomedially, while hexane and heptane were usually four times as large ventrolaterally. These results suggest that the receptors expressed in the two regions have systematically different properties.

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Evidence for Imposed and Inherent Olfactory Mucosal Activity Patterns in a Mammalian Species. MAXWELL M. MOZELL, PAUL F. KENT and STEPHEN J. MURPHY (Olfactory Research Center, Departments of Physiology and Neurology, SUNY Health Science Ctr, Syracuse NY)

Based upon earlier data from amphibians, two mechanisms which could possibly underlie the encoding of different odorants by the different activity patterns they engender across the olfactory mucosa are: 1) receptors with similar odorant selectivities could be aggregated into the same mucosal regions and those with different selectivities could be aggregated into different regions (the basis of inherent patterns); 2) in analogy to gas chromatography, as odorants are drawn along the surface of the mucosa, the strongly sorbed ones could be preferentially deposited upstream whereas the weakly sorbed ones could be more evenly distributed (the basis of imposed patterns). Do either or both of these possible coding mechanisms operate in mammals, and, if so, how do they interact? These questions were investigated by using the voltage sensitive dye technique on the olfactory mucosas of rats decapitated following CO_2 anesthetization. Fluorescence changes in di-4 ANEPPS applied to the mucosa were monitored by a 10 X 10 pixel photodiode array. Three odorants (propyl acetate, ethyl acetoacetate and l-carvone) of varying sorbabilities were first puffed in a uniform manner over the entire mucosa to observe the inherent patterns. These same odorants were then drawn at three different flow rates along the flow path of the olfactory mucosa which was mounted in a chamber designed to anatomically replicate the rat's nasal cavity. This gave composite patterns of the inherent and imposed activities. After subtracting the inherent from the composite patterns (which revealed the imposed patterns),the results clearly demonstrated, for the first time, the existence of both imposed and inherent patterns in a mammalian species. The strongly sorbed odorants, in contrast to the weakly sorbed one, showed marked imposed patterns. These imposed patterns were two to five fold more pronounced than the inherent patterns. Within physiologic limits, increasing the flow rate decreased the magnitude of the imposed patterns. This ability to alter the composite patterns (imposed + inherent) with stimulus conditions raises the question as to how directly the mucosal activity patterns can encode different odorants.

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Carbon Monoxide Controls Excitable Properties of Olfactory Receptor Cells through Activation of Cyclic Nucleotide-Gated Channels. TRESE LEINDERS-ZUFALL, GORDON M. SHEPHERD, FRANK ZUFALL (Section of Neurobiology, Yale Medical School, New Haven, CT 06510

Recent interest has focused on the possible role of carbon monoxide (CO) as a diffusible messenger in the central nervous system. In the vertebrate brain the highest concentrations of heme oxygenase-2 (HO2), the enzyme producing CO, occur in the neurons of the olfactory epithelium (ORNs) and the olfactory bulb. Therefore, the olfactory system provides an ideal model to study a putative functional role of CO.

We now report that CO is a highly potent regulator of excitable properties of freshly isolated ORNs from salamander. If voltage clamped to their resting potential ORNs respond with the activation of a sustained inward current to the application of CO. A series of pharmacological and ionic tests has shown that this inward current is due to the activation of cyclic nucleotide-gated (CNG) channels that were previously described to mediate odor transduction in these neurons. Activation of CNG channels by CO is highly consistent and can be measured in nearly every cell. CO does not exert its effect directly on the olfactory CNG channels; rather channel activation has an absolute requirement of GTP in the intracellular environment. This result is consistent with the idea that CO leads to cGMP production through the activity of a guanylyl cyclase. Full dose-response curves for CO-mediated cGMP-production (monitored through CNG-channel activity) have been obtained. CO is surprisingly potent, generating significant CNG currents in the submicromolar range. In a second set of experiments we applied the perforated patch technique to study quantitative aspects of CO-mediated depolarization of ORNs and the consequences for action potential generation. These experiments have shown that micromolar application of CO can lead to tonic shifts in membrane potential due to CO-mediated CNG channel activity. This effect has a strong modulatory action on responses to other incoming stimuli.

These results demonstrate that two second messenger pathways, the odor stimulated G-protein coupled cAMP system and the guanylyl cyclase/cGMP pathway, converge onto the same effector molecules, CNG channels, to control the output of ORNs.

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Involvement of a Ca^{2+}-activated K^+ Channel in Odorant-triggered Inhibitory Responses of Toad Olfactory Neurons. BACIGALUPO, J.[1], MORALES, B., [1], LABARCA, P.[1,2], MADRID, R.[1]. (Dept. Biologia, Fac. Ciencias, Universidad de Chile[1] and Centro de Estudios Cientificos de Santiago[1], Santiago, Chile).

Toad olfactory neurons can be either excited or inhibited by volatile odorants (Morales et al., Proc. R. Soc. Lond B. 357:235, 1994). Inhibition, reflected as a transient decrement in action potential firing, is due to the activation of an outward K^+ current that hyperpolarizes the cell. We have investigated the nature of the K^+ conductance associated to the inhibitory responses in olfactory neurons isolated from *Caudiverbera caudiverbera*. Electrical recording was attained using the cell-attached and whole-cell configurations of the patch-clamp method. Chemical stimuli, a mixture of putrid odorants, were applied onto the cilia from a close distance (20 μm), with a multibarreled pipette (2 μm diam. each) connected to a picospritzer. The I-V curve of the odorant-dependent current departed from 0 at around -50 mV, reached a maximum near +30 mV and decremented at more positive voltages. Its shape closely ressembles that of Ca^{2+}-activated K^+ currents. We further examined the possibility that the K^+ conductance involved in inhibitory responses is Ca^{2+} activated. Charibdotoxin (10 nM), which blocks large conductance Ca^{2+}-activated K^+ channels, also blocked the odorant-triggered K^+ conductance, having no effect on the voltage-activated conductances. The odorant-activated outward current was reversibly abolished upon removal of extracellular Ca^{2+}. Activation of the outward current was much more pronounced when odorant stimuli were addressed to the cilia than to the soma of the neuron. Confocal microscopy measurements using fluo 3,AM show that the same odorant mixture caused an elevation of Ca^{2+} in the apical end of the neuron. These results are in agreement with the notion that a ciliary Ca^{2+}-activated K^+ channel is involved in the inhibitory responses to odorants, and that its activation is mediated by an increase in apical $[Ca^{2+}]$ mainly due to an influx of this cation from the extracellular saline.

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Expression and Purification of Rat and Human Olfactory Receptor Proteins URI GAT, MICHAEL NATOCHIN, ELINA NEKRASOVA & DORON LANCET (Dept. of Membrane Research & Biophysics, The Weizmann Institute of Science, Rehovot 76100, Israel).

The multigene Olfactory Receptor (OR) family stands at the basis of the sense of smell. Olfactory Receptors belong to the superfamily of seven transmembrane domains G-protein coupled receptors (7RG) and are thought to be activated by odorant ligands. Our goal is to study in detail the properties of OR proteins, including their odorant binding characteristics and G-protein coupling. To this end we have expressed several OR genes both *in vitro* and *in vivo*. The ORs studied are the rat olp4 and F5 genes (Gat et al. Eur. J. Biochem. 225:1157, 1994)) as well as human OR17-2 and OR17-4 (Human Molec. Genet. 3:229, 1994)). To monitor expression, we have attached a Flag octapeptide epitope tag, which allows immunodetection of the fusion proteins by a monoclonal antibody, as done for other 7RG receptors. To purify the OR proteins we have also added a 6 histidine (6xHis) tag, that tightly binds to a Ni^{2+} chelate affinity column. Both in *vitro* translation and *in vivo* baculovirus expression showed a glycosylated ~30 kDa band, identified as the OR protein monomer, as well as a ~55 kDa band, potentially an OR dimer. Flow cytometry and immuno-histochemistry showed a correct localization and orientation of the Flag-OR product in the Sf9 cell membrane, with the amino terminus facing outside. We are currently developing methods for detergent solubilization of the extremely hydrophobic OR proteins from the cell membrane, and for purifying the 6xHis-tagged OR proteins to homogeneity. These purified OR proteins may be biochemically and physically characterized, reconstituted in lipid vesicles with transduction components such as G_{olf} and screened for specific odorant ligand binding and activation.

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Spatial and Temporal Patterns of Gene Expression in Olfactory Epithelia of Insect (*Manduca sexta*) and Fish (zebrafish *Danio rerio*). RICHARD G. VOGT[1], CHRISTINE A. BYRD[2], MATTHEW E. ROGERS[3] ([1,2]Univ. of South Carolina, Dept. of Biological Sciences; Univ. Virginia, Dept. of Psychology).

Spatially and temporally regulated gene expression contributes strongly to the functional assembly of the olfactory system. Such patterns are common elements of diverse olfactory systems including arthropods and vertebrates. We present histological *in situ* hybridization studies (whole mounts and sections) of the differential expression patterns of three homologous odorant binding proteins (OBPs) of the moth *Manduca sexta*: Pheromone Binding Protein (PBP) and the General Odorant Binding Proteins GOBP1 and GOBP2. PBP and the GOBPs are expressed in different spatial domains known to be functionally distinct. GOBP1 and GOBP2 are differentially expressed among two classes of olfactory sensilla in a single overlapping domain, suggesting that functionally distinct domains can physically occupy the same space. The three *M. sexta* OBPs are a simple model for examining spatially regulated gene expression in the olfactory epithelium.

We further present histological *in situ* hybridization studies (whole mounts and sections) of olfactory receptor expression during embryogenesis in the zebrafish *Danio rerio*. At 26°C, receptor expression is first observed in cells of the olfactory placode by 36 hrs post fertilization, around the approximate time sensory neurons are reported to make first brain contact (Hansen & Zeiske, 1993). The number of cells showing positive receptor hybridization increases in a roughly linear fashion past hatching (72 hrs post fertilization). This pattern becomes increasingly complex during juvenile life as the olfactory organ proceeds through morphogenesis to its adult form. This pattern of expression, coupled with behavioral studies presented elsewhere (see presentation by Lindsay *et al.*) suggest the zebrafish is a suitable model for evaluating the ontogeny of chemosensory behavior at the level of gene regulation.

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A Mutant with Reduced Nitric Oxide Synthase (NOS) Levels in the Olfactory System.

DEBASISH RAHA (Dept. of Biology, Yale University)
JOHN CARLSON (Dept. of Biology, Yale University)

Nitric oxide (NO) is an important second messenger molecule produced by nitric oxide synthase (NOS) in neurons, endothelial cells, macrophages and other cells of vertebrates. NADPH-diaphorase (NDP) activity, which converts soluble nitroblue tetrazolium (NBT) to a blue insoluble formazan derivative, has a tissue distribution that agrees very well, if not perfectly, with that of NOS; moreover, NOS has been shown to have NADPH-diaphorase activity. We and others have detected NDP activity in the fly olfactory system, in the olfactory neurons of the antenna and maxillary palp and also in the olfactory glomeruli in the brain. We have also detected NDP activity in the developing olfactory system.NDP staining is not detected in olfactory tissues of lz^3, a mutant in which sensilla basiconica are completely lacking from the antenna and are defective in the maxillary palp.We screened a number of EMS mutagenized lines which had been enriched for olfactory mutants using a behavioral assay. One line, which shows defective olfactory response, shows reduced NDP activity in both antennae and maxillary palps, and also shows reduced levels of NOS. The mutant contains sensilla basiconica in the antenna. Molecular studies to determine whether the mutation lies in a structural gene for NOS are underway.

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Sequence Analysis and Expression of Inositol-1,4,5-Trisphosphate Receptor Amplicons from Chemosensory Tissues of Channel Catfish and Rat. JOHN ZIMMERMAN, CHANG-GYU HAHN, AND GREGORY SMUTZER. Department of Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Several lines of evidence suggest that in addition to cyclic AMP, inositol-1,4,5-trisphosphate (IP_3) functions as a second messenger in the chemosensory systems of both vertebrates and invertebrates. In response to stimulation by a ligand, IP_3 is hypothesized to bind to a plasma membrane-bound ligand-gated channel which activates cation flow into the neuron. Further evidence suggests the mRNA that encodes this receptor\channel is expressed at low levels in both olfactory and taste tissue of vertebrates. Reverse Transcriptase PCR was utilized to amplify IP_3 receptor cDNAs from catfish and rat chemosensory tissue. Primers were designed from the rat cerebellar IP_3 receptor cDNA sequence to amplify either a cytoplasmic, or a transmembrane, portion of chemosensory IP_3 receptors. These primers were used to amplify cDNAs from both olfactory and taste tissue of the channel catfish, Ictalurus punctatus. PCR products were cloned into an appropriate vector for both DNA sequence analysis and for production of RNA transcripts. Product sizes of catfish taste and olfactory IP_3 receptor cDNAs were similar to sizes predicted from published rat cerebellar IP_3 receptor cDNA sequence data. Sequence analysis of amplicons from both the transmembrane region and the cytoplasmic region of the catfish IP_3 receptor indicated a greater than 90 percent sequence identity between the rat cerebellar and catfish chemosensory IP_3 receptors. RNAse protection analysis of catfish olfactory RNA with a rat cerebellar IP_3 receptor cDNA probe indicated that the IP_3 receptor was expressed at low levels in catfish olfactory tissue. Furthermore, *in situ* hybridization studies using catfish olfactory tissue displayed low levels of labeling in olfactory neuroepithelium. Taken together, these results indicate that the IP_3 receptor is highly conserved at the nucleotide level among vertebrate species, and is expressed at low levels in chemosensory tissue of catfish.
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Neuromodulation of Transduction and Signal Processing in the End Organs of Taste. S. D. ROPER, D.-J. KIM, D. A. EWALD and R. J. DELAY (Rocky Mtn. Taste & Smell Center, Denver, CO 80262 and Dept. Anat. Neurobiol., Colo. State Univ., Ft. Collins, CO 80524)

Recent data suggest that a certain degree of signal processing takes place in taste buds. Synapses between taste cells have been documented with morphological and electrophysiological techniques. The most complete evidence to date indicates that **serotonin** (5HT) is a neuromodulator in taste buds. 5HT is localized to specific taste bud cells. 5HT is released in a Ca-dependent fashion by depolarization of these cells and elicits postsynaptic responses in adjacent receptor cells. These responses include membrane hyperpolarization (Ewald & Roper, '94a) and modulation of voltage-dependent Ca currents (*i.e.*, those currents involved in transmitter release; Delay, *et al.*, '94). The involvement of 5HT in gustatory end organs may bear upon side effects involving taste caused by certain drugs, such as tricyclic antidepressants, that are powerful blockers of 5HT uptake. There are also data supporting the hypothesis that **acetylcholine** (ACh) is a neuromodulator in taste buds. ACh-esterase is present in taste buds. Additionally, we have recently shown (Kim & Roper, '94) that choline acetyltransferase, a more reliable marker for cholinergic mechanisms, is present in taste cells, nerve fibers that innervate taste buds, and ganglion cells situated at the base of the vallate papilla. Axons from these ganglion cells can be traced to taste buds, suggesting that there may be cholinergic (parasympathetic) modulation of the end organs of taste. Additionally, ACh evokes muscarinic postsynaptic responses in taste bud cells (Ewald & Roper, '94b). Other neuromodulators, including catecholamines and peptides, are present in taste cells and may affect taste bud function (*e.g.*, Nagahama & Kurihara, '85; Wang, *et al.*, '95). Thus, signal processing in the peripheral end organs of taste may be modulated by a number of substances. This has implications for the basic neurobiology of sensory mechanisms, as well as for clinical medicine and for the food industry.

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Immunohistochemical localization of putative neurotransmitters in the nucleus tractus solitarii. Bruce E. Maley Department of Anatomy and Neurobiology, University of Kentucky Medical Center, Lexington, KY 40536.

The nucleus tractus solitarii (NTS) which receives visceral afferent information from the cardiovascular, respiratory, gastrointestinal and taste systems contains multiple neurotransmitters/neuropeptides throughout its rostral to caudal extent. The neurotransmitters/neuropeptides immunoreactivity was located predominately in varicose fibers and small puncta throughout the neuropil. In addition, immunoreactive NTS neurons for a variety of neurotransmitters/neuropeptides were present in subnuclear regions.

The neuroactive substances localized immunohistochemically in the NTS include acetylcholine; the neuropeptides, substance P, methionine- and leucine-enkephalin, β–endorphin, cholecystokinin, neurotensin, galanin, calcitonin gene related peptide, somatostatin, FMRMamide, neuropeptide Y, angiotensin II, vasoactive intestinal polypeptide, vasopressin, oxytocin, thyrotropin releasing hormone, luteinizing hormone releasing hormone, atrial natriuretic peptide; the catecholamines, dopamine, norepinephrine, epinephrine, serotonin, histamine and the amino acids; GABA and glutamate. As would be expected the pattern of innervation for each neurotransmitter and neuropeptide is not homogeneously distributed throughout the NTS. Each substance has a unique pattern within the NTS as well as each subnuclear region contains different immunohistochemical staining patterns and densities of fibers.

At the ultrastructural level both neurotransmitters and neuropeptides were present in synaptic terminals that were in contact with different parts of the neuronal membranes. Typically, the labeled terminals contained both small, clear vesicles and large, dense core vesicles with the exception of synaptic terminals containing acetylcholine, GABA and glutamate which did not typically have the large, dense core vesicles. The most frequent postsynaptic target were dendrites and spinous processes. Less frequently synaptic contacts were present on the cell soma.

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Neurotransmission in the Rostral Nucleus of the Solitary Tract. R. M. BRADLEY. (Dept. Biologic & Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078.)

The rostral nucleus of the solitary tract (rNST) is the first central relay in the gustatory pathway. While previous investigations have provided a wealth of information on the pattern of central terminations of gustatory afferent fibers, the morphology of synaptic connections of rNST neurons and responses of second order neurons to taste stimuli applied to the tongue, little is known regarding the neurophysiological characteristics of synaptic transmission in rNST. We have used an *in vitro* brain slice preparation of the rNST to study the intrinsic biophysical properties, neuropharmacology and synaptic responses of rNST neurons. These experiments have revealed that rNST neurons respond to the excitatory amino acid neurotransmitter glutamate as well as the inhibitory amino acid neurotransmitter γ amino butyric acid (GABA). By use of glutamate receptor agonists and antagonists we have shown that rNST neurons have AMPA/kainate and NMDA ionotropic glutamate receptors as well as matabotropic glutamate receptors. In addition rNST neurons respond to both $GABA_A$ and $GABA_B$ receptor agonists. The nature of the transmission at the synapse between primary afferent fibers and second order neurons in rNST has been examined by electrical stimulation the solitary tract to elicit post-synaptic potentials (PSP). Three types of monosynaptic PSP result from stimulation of the solitary tract: excitatory post-synaptic potentials, inhibitory post-synaptic potentials and a complex mixture of excitatory and inhibitory potentials. These new discoveries provide details about synaptic transmission in rNST and thereby clarify the underlying mechanism by which gustatory information is processed.

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Sensory afferent neurotransmission in caudal nucleus tractus solitarius - common denominators. M.C. ANDRESEN. Dept. of Physiol., Oregon Health Sci. Univ., Portland, OR 97201

The nucleus of the solitary tract (NTS) receives a wide range of sensory inputs including gustatory, gastrointestinal, and cardiorespiratory which are loosely segregated viscerotopically to subnuclei. NTS is the site of the first central synapse of important visceral homeostatic reflexes. Our lab has focussed on a dorsomedial area of caudal NTS (mNTS) which is critical for cardiovascular reflexes and receives a dense innervation from baroreceptors. We study primarily mNTS neurons mono-synaptically activated by solitary tract stimulation. The basic discharge properties of these mNTS neurons are similar to those reported throughout the rostral-caudal extent of NTS suggesting fairly similar ensembles of membrane ion channels. mNTS neurons show varying degrees of delayed excitation, spike frequency adaptation and afterhyperpolarizations. Sensory afferent transmission is mediated by glutamate acting at postsynaptic non-NMDA receptors to produce fast excitatory responses. Glutamate release depends on at least four different presynaptic calcium channels with N-type predominating. This profile of presynaptic calcium channels in NTS is also present at the peripheral soma but absent from the baroreceptor sensory endings. Many peptides are associated with these sensory neurons and several modulate glutamatergic transmission in mNTS. Angiotensin II facilitates excitatory responses to sensory afferent activation by a presynaptic mechanism. Somatostatin depresses tract evoked excitatory synaptic responses by both pre- and postsynaptic mechanisms. There appears to be a common framework of synaptic and cellular mechanisms in NTS and peptides may play a critical role modulating this framework to regulate overall reflex function.

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Taste Bud Number and Asymmetry in Fungiform Papillae. INGLIS J. MILLER, JR., FRANK P. BREWER (Wake Forest University, Winston-Salem, North Carolina, USA).

The number of taste buds on single fungiform papillae of humans, monkeys and rabbits varies from 0 to more than 20. While it is not known what controls the number of taste buds on a single papilla or an entire tongue, taste bud numbers are related to taste sensitivity (Arvidson and Friberg, 80); Miller and Reedy, 90; Bartoshuk, et al, 93). The number of taste buds on fungiform papillae seems to change over time (Miller, 93). Relationships between taste bud number, papillary size and shape, innervation and blood supply are examined in this presentation. Measurements are made from microscopic examination of sections from human (N=81), macaque (N=26) and rabbit (N=37) fungiform papillae. The number of taste buds in human fungiform papillae is significantly related to papillary diameter ($F = 4.2$, $p < .01$, N=48), but not height ($F = 1.3$, $p > .25$, N =48). The lengths and diameters of blood vessels within rabbit papillae increase with the size of the papilla, but there seems to be no apparent relationship between blood supply and the number of taste buds. Dermal cores occupy a volume of about $96 \pm 6.7\mu^3$ (N=81) within papillae, and secondary papillae comprise from 5 - 20 % of the dermal core. The proportion of dermal core comprised of secondary papillae is significantly related to the numbers of taste buds in macaque fungiform papillae ($F= 5.3$, $p < .05$, N=10). Counting of axons profiles in silver stained material shows that large papillae with more than 10 taste buds are supplied by multiple nerve bundles. The locations of taste buds in human fungiform papillae are frequently asymmetric in lines or ridges at apical axes of the papillae. Preliminary observations of spatial relationships between taste bud numbers, locations and papillary shapes suggest that papillary shape is influenced by the taste bud, itself, or by its associated innervation. This work is supported by NIH Grant DC 00230 from NIDCD.

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Metabotropic Glutamate Receptor Expression is Modulated by Free-Glutamate Content of Diet. NIRUPA CHAUDHARI[1], CYNTHIA LAMP[1] and STEPHEN D. ROPER[2] (Dept. of Physiology, Colorado State University, Ft. Collins, CO 80523 [1], and Rocky Mtn Taste and Smell Ctr, Univ. Colo. Health Sci. Ctr., Denver, CO 80262[2])

A metabotropic glutamate receptor, mGluR4, is expressed in vallate and foliate taste buds of the rat (Chaudhari *et al.*, 1994, *Chem. Senses* 19:452). We have now asked whether mGluR4 in taste buds might serve as a neurotransmitter receptor or whether it might instead represent a receptor for glutamate as a taste stimulus. We conducted two studies to examine this question. First, juvenile rodents are known to have a higher taste sensitivity to glutamate than adults. Using RNase protection assay, we found that mRNA for mGluR4 is present in tastebuds at several-fold higher concentration in pre-weaning rats as compared to adults. *In situ* hybridizations indicate that this difference between mRNA concentrations is more striking in vallate than in foliate taste buds. Second, the Na^+ content of diet is known to regulate the expression of transduction channels for salty taste (Hill, 1987, *J. Physiol.* 393:413). To determine if the titer of free glutamate in diet modulates the expression of the mGluR4 gene, we weaned rats onto a diet containing only cow's milk (128μM glutamate) or a diet supplemented with 10-15mM glutamate in water or milk. Rats were maintained on these diets for 2 weeks. Using the RNase protection assay, we found that rats fed low-glutamate diets expressed 2-4 fold higher concentration of mRNA for mGluR4 in vallate+foliate papillae. Thus, the free glutamate-content of diet appears to regulate the levels of mGluR4. These two experiments, comparing age-related and diet-related differences in receptor expression support the interpretation that mGluR4 serves as a receptor involved in the transduction of glutamate taste.

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Correlated Light and Electron Microscopic Immunolocalization of Cell-Surface Antigens on Taste Cells in the Rat Vallate Papilla.
CHENGSI YU, DAVID W. PUMPLIN, and DAVID V. SMITH (University of Maryland School of Medicine, Baltimore, MD 21201, USA)

Mammalian taste receptors express a number of molecules, including the adhesion molecules NCAM and L1, and several carbohydrates, including the 2B8 and 9-OE antigens and the A, B, H, and Lewis^b human blood group determinants. These molecules appear to be distributed on the cell surface, making them ideal candidates for roles in cell-cell recognition and adhesion. Because taste cells are continually replaced and there is continual synaptogenesis between newly formed taste cells and first-order gustatory fibers, cell recognition and adhesion are important in this system throughout life. We combined immunocytochemical with confocal and electron microscopy in order to investigate the distribution of these molecules within rat vallate taste buds. Rats were perfused with 4% paraformaldehyde/0.2% glutaraldehyde; tongues were removed, cut into 50 µm coronal sections with a Vibratome, and labeled with one of several primary antibodies, including those against the Lewis^b and H blood group substances, the 2B8 and 9-OE carbohydrate epitopes, and NCAM (using Mab 3F4). Antibody binding was visualized with a fluoresceinated secondary antibody for confocal microscopy or a biotinylated secondary antibody reacted with avidin-HRP (and DAB) for electron microscopy. Confocal microscopy showed that the Lewis^b epitope is distributed over the entire surface of a few spindle-shaped cells per taste bud. NCAM and the 9-OE epitope are expressed on the surface of greater numbers of cells. The H blood group antigen is expressed on most cells within each taste bud, as is the 2B8 carbohydrate epitope. Additional immunoreacted sections were embedded in Spurr resin and cut into alternate thick (0.5 µm) and thin (50 nm) sections for correlated phase contrast and electron microscopy, permitting us to view the ultrastructure of the same labeled cell in several planes. Classification of these labeled cells into dark cells, intermediate cells, and light cells will permit us to relate taste cell ultrastructure to expression of these antigens.

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Immunoelectron Microscopical Analysis of Gustducin in Taste Cells of the Rat.
SHOJI TABATA[1,2], HILDEGARD H. CROWLEY[1,2], BÄRBEL BÖTTGER[2,3], THOMAS E. FINGER[2,3], ROBERT F. MARGOLSKEE[4] and JOHN C. KINNAMON[1,2] (Department of Biological Sciences, University of Denver[1], Denver, CO, the Rocky Mountain Taste and Smell Center[2], Denver, CO, Department of Cellular and Structural Biology, University of Colorado School of Medicine[3], Denver, CO, and Department of Neurosciences, Roche Institute of Molecular Biology[4], Nutley, NJ)

Gustducin is a guanine nucleotide binding protein (G protein). It is thought to be involved in the transduction of bitter taste. In order to determine which taste cells might transduce bitter stimuli, we used the antisera to gustducin in circumvallate taste buds of the rat. Rat tongues were fixed with a mixture of glutaraldehyde and paraformaldehyde. Sections (50 µm thick) were obtained for pre-embedding immunocytochemistry using a vibratome or a cryo-microtome. These sections were reacted with rabbit anti-gustducin primary antibody, followed by a standard ABC protocol. Sections were then incubated in DAB solution and post-fixed with 1% OsO_4. The specimens were embedded in epon and sliced with a diamond knife. For post-embedding immunocytochemistry, animals were perfused with a mixture of glutaraldehyde and paraformaldehyde. Lingual tissues were then embedded in epon and sliced with a diamond knife at a section thickness of 0.12 µm. Sections were reacted with rabbit anti-gustducin primary antibody, followed by reaction with 12 nm colloidal gold conjugated with goat anti-rabbit secondary antibody. Using transmission electron microscopy, we observed immunoreactivity in a subset of taste cells in each taste bud. The immunoreactive taste cells possessed an ovoid nucleus and were devoid of dense granules in the apical cytoplasm. Using the post-embedding method, immunoreactivity was associated exclusively with the intermediate filaments in the cytoplasm. Surprisingly, there was little immunoreactivity associated with the apical microvilli. The significance of gustducin-like immunoreactivity in the intermediate filaments of taste bud cells is unknown at this time.

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Taste Buds In Macaques Show Protein Gene Product 9.5-like Immunoreactivity In Incoming Fibers
MIRIAM R. LINSCHOTEN, EDWARD W. JOHNSON, PAMELA M. ELLER and BRUCE W. JAFEK (Department of Otolaryngology and RMTSC; UCHSC; Denver, CO)

The present study was initiated to identify cytochemical characteristics of primate taste buds. Previous work in our laboratory has demonstrated calbindin-like immunoreactivity (-LI) in rat taste bud cells and fibers. Results from a current developmental study (see accompanying abstract by J. Kinnamon et al.) indicate protein gene product 9.5 (PGP) may be another useful marker of mammalian taste buds. Therefore, we have examined calbindin-LI and PGP-LI in macaque taste buds. In 7 and 11 week old macaques, fibers displaying PGP-LI were observed in all circumvallate papillae (CVP). A portion of these fibers entered the taste buds. No cells showing PGP-LI were seen within the buds. Calbindin-LI was not observed within any CVP. Ongoing studies include a 5 month old and adult macaques. In addition to the aforementioned markers, we will examine keratin 19 (which is an excellent marker for taste bud receptor cells in other mammals)-LI in macaque CVP and fungiform taste buds.

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Innervation Patterns of Fungiform Taste Buds in Adult Rats with a Regenerated Chorda Tympani Nerve.
MARSHALL G. SHULER, ROBIN F. KRIMM and DAVID L. HILL (University of Virginia, Charlottesville, VA 22903)

Since the earliest reports in the 1800s, it has been apparent that the morphological integrity of taste buds is dependent on their innervation. When gustatory nerves are sectioned, taste buds degenerate and subsequently regenerate upon reinnervation. Since the degenerating/regenerating taste system often is used as a model system for studies of trophic interactions between neural elements and target tissue, continued information detailing the trophic mechanisms will be of general biological interest. One of the issues unresolved is whether innervation patterns of single papillae are changed following sectioning of a taste nerve. For example, do the same numbers of fibers innervate a single fungiform before nerve sectioning as they do after section? This is the question addressed in this study. We have recently developed and perfected techniques whereby small amounts of fluorescent neuronal tracers can be iontophoresed into single papilla, taken up by neuronal processes in the papilla, and transported to their cell soma in the geniculate (taste) and trigeminal (somatosensory) ganglia. Therefore, we can analyze the number of geniculate and trigeminal ganglion cells that innervate a single papillae and determine the topography by which papillae map onto the ganglia. In fact, we know from experiments in intact adult rats that the number of geniculate ganglion neurons that innervate single fungiform taste buds are highly correlated ($r=0.91$; $p < 0.0003$) with the size of the taste bud. This function is used here as the standard to determine whether the same organization occurs following sectioning of the chorda tympani nerve and a subsequent regeneration period of 60 days. Furthermore, we are analyzing the topography of labeled ganglion cells in the geniculate ganglion. These results will provide the basis for further studies that examine the plasticity of the adult gustatory system.

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Recovery of the chorda tympani nerve following crush injury.
MICHAEL A.BARRY, AND PETER CAIN (Dept. of BioStructure and Function, University of Connecticut Health Center).

We are investigating the response of the gustatory system following chorda tympani nerve crush and regeneration in Golden Syrian hamsters. In this study, we examined the morphology of the chorda tympani nerve 2-16 weeks following unilateral nerve crush in the middle ear. Electron microscopy was utilized to examine axons in cross sections of the nerve distal to the crush site. In some cases, the nerves were fixed following recordings of the whole nerve response to taste stimulation. Axons were counted and the diameters of all myelinated fibers were calculated based on area measurements (not including the myelin sheath). At 2 weeks after nerve crush, almost all fibers were degenerated or missing. By 5 weeks, most of the nerve fibers appeared normal. There was, however, a reduction in the number of myelinated fibers. The number of fibers did not recover even 16 weeks after degeneration. In 4-16 week animals, there were 151.3 ± 20.4 myelinated fibers versus 377.7 ± 22.3 in intact nerves. The sizes of the regenerated myelinated fibers were similar to normal except that there were more fibers that were greater than 2 μm in diameter; 8.1% of axons in crushed nerves versus 3.8% in intact nerves. In one 10 week case, the number of myelinated fibers was counted: the numbers were about equal in the intact (1735) and crushed (1715) nerves. Recovery of the chorda tympani nerve correlates well with recovery of fungiform taste buds and physiological responses of the nerve to taste stimulation. The reduced numbers of myelinated fibers may partially account for the reduced strength of the whole nerve response of the chorda tympani after regeneration.

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Light Microscopic Analysis of Degeneration and Regeneration of Circumvallate Taste Buds in Rats.
STACIE ROUGAS, TERRI SHERMAN-CROSBY, HEIDI B. LINNEN, KATHY ZUBRZYCKI, HILDEGARD H. CROWLEY and JOHN C. KINNAMON (Department of Biological Sciences, University of Denver, Denver, CO and the Rocky Mountain Taste and Smell Center, Denver, CO)

Currently, we are elucidating the determinants of synaptic structure in rat taste buds. Specifically, we are examining taste buds from rats with cross-reinnervated fungiform and circumvallate papillae to determine whether synaptic structure in taste buds is influenced primarily by the nature of the receptor cell or by the type of innervation. The present study is a portion of the project described above, in which we are investigating the effect of denervation and regeneration of taste buds by the native nerve. This set of experiments constitutes an essential control for the overall project. Previous evidence suggests that degeneration of taste buds occurs within 0-14 days after denervation. If reinnervation is allowed to occur, regeneration of taste buds is usually visible after approximately 21 days. In the present study, both light microscopy and transmission electron microscopy are being utilized to characterize the events associated with taste bud degeneration following denervation and subsequent regeneration of taste buds. In 30 different rats, the glossopharyngeal (IXth) nerve has been cut and reattached. Ten time periods of three days each were used for these experiments, up to 30 days, consisting of three rats in each time period. Animals were perfusion fixed and lingual tissues embedded in epoxy for both light and electron microscopy. Our preliminary light microscopical results confirm the work of previous studies. Degenerating taste buds were visible up to nine days after surgery. From day nine to day 15, degeneration was complete and no taste buds were observed. At approximately day 18, developing taste buds became apparent at the light microscopical level. Beginning on day 21, some fully formed taste buds were visible, along with taste buds in various states of development. Regeneration continues after day 21 until the papillae appear normal at approximately day 30. Our next step will be to examine these sections using electron microscopy. The results from this study will then be compared with the results from the cross-reinnervation experiments to facilitate the recognition of ultrastructural differences in regenerating taste buds in rats in which the VIIth and IXth cranial nerves have been crossed.

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The Effects of Sialoadenectomy and Exogenous EGF on the Taste Bud Morphology and Maintenance.
R. SEGO, L. BRINKLEY, C. DOLCE AND J. MORRIS-WIMAN (University of Florida, Gainesville, Florida)

Taste buds on the dorsal tongue surface are continually bathed in a saliva rich in epidermal growth factor (EGF). The significance of the large excess of EGF in saliva, produced and secreted by the submandibular glands, is not yet known. In the following experiments taste bud number and morphology were monitored following submandibular removal (sialoadenectomy) to determine what role, if any, EGF plays in the maintenance and formation of taste buds. Adult male rats were divided into five groups: Sialoadenectomized (Ablated,AB, n=4); Sialoadenectomized with EGF replacement (AB+EGF, n=5); Sham operated (SH, n=5); Sham operated with exogenous EGF (SH+EGF, n=5);and unoperated controls (n=5). After a 3 week recovery, AB+EGF and SH+EGF animals were given 50 μg/day EGF (human recombinant, UBI)in their drinking water for 14 days. At day 14, the day of sacrifice, saliva was collected and the presence of EGF determined by western blot analysis. Tongues were removed and halved. One tongue half from each animal was embedded in glycol methacrylate, sectioned and examined for the presence and morphology of taste buds on fungiform and circumvallate papillae. The other tongue half from each animal was embedded in paraffin, sectioned and immunostained for the presence of EGF, TGFα (transforming growth factor alpha)and EGFR (EGF receptor). The fungiform papillae of AB animals had few taste buds and those observed were small and undeveloped. AB fungiform papillae often had spines characteristic of denervated papillae. The morphology of fungiform papillae and taste bud numbers were similar in AB+EGF, SH, SH+EGF and control animals. AB+EGF and SH+EGF had fungiform taste buds that were significantly larger than those observed in shams and controls. The number and morphology of taste buds on circumvallate papillae were similar in all groups examined. Immunostaining revealed the presence of TGFα and EGFR in both fungiform and circumvallate taste buds in both ablated and sham animals. EGF was localized mainly to superficial epithelial layers . TGFα was also observed within cells of von Ebner's glands. These results suggest that ablation of the submandibular glands results in the loss of fungiform taste buds and normal fungiform papilla morphology. These changes are prevented by the addition of EGF. The lack of effect of decreased salivary EGF on circumvallate taste buds may be due to the access of these taste buds to TGFα produced locally by von Ebner's glands.

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The Role of Chondroitin Sulfate Proteoglycans in the Morphogenesis of Gustatory Papillae.
J. MORRIS-WIMAN, R. SEGO AND L. BRINKLEY (University of Florida, Gainesville, Florida)

Gustatory papillae form from epithelial placodes on the dorsal surface of the tongue. The formation and differentiation of similarly placode-derived epithelial specializations, e.g., teeth, whisker follicles, feathers and scales, involve epithelial-mesenchymal interaction, often mediated by extracellular matrix molecules. Evidence exists that chondroitin sulfate proteoglycans (ChSPGs) may play a role in these interactions. To determine if ChSPGs play a similar role in gustatory papillae and taste bud patterning and formation, the temporospatial distribution of ChSPG was examined using standard immunofluorescent-staining techniques during papillae morphogenesis in the mouse (gestational day (gd) 11 to gd17). The distribution of NCAM (neural cell adhesion molecules) was also studied to demonstrate stage of innervation. On gd11, immunostaining for ChS was observed in both the epithelial and mesenchymal compartments of the merging lingual processes. After completion of tongue formation (gd12), immunostaining disappeared from the lateral aspects of the mesenchyme, but remained in the epithelium. Within the medial mesenchyme, ChSPG distribution showed a distinct pattern with areas of increased stain intensity underlying presumptive placodal epithelium. Immunostaining was gradually lost within the dorsal epithelium between gd12 and gd13 until positive staining was restricted to fungiform and circumvallate placodal epithelium. As fungiform and CV papillae matured, ChSPGs became restricted to the apical papillary epithelium where its distribution paralleled that of NCAM. The basement membrane of the papillary epithelium was heavily stained, particularly subjacent to the apical epithelium. The subpapillary mesenchyme subjacent to papillae remained more intensely immunostained than interpapillary mesenchyme. The observation that distinct temporospatial distributions exist for ChSPGs that correlate with stages in patterning and differentiation of gustatory papillae suggests that these molecules may play a role in papillae, and possibly taste bud, morphogenesis.

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Different populations of taste cells exhibit gustducin and serotonin immunoreactivities. B. BÖTTGER[1], T.E. FINGER[1], D.-J. KIM[2] AND S.D. ROPER[2] (Rocky Mtn. Taste & Smell Center; [1]Dept. Cell. & Struct. Biol. Univ. Colorado Sch. Medicine, Denver CO; [2]Dept. of Anat. & Neurobiol., Colo. St. Univ., Ft. Collins, CO).

Previous single-label studies in several laboratories show that some taste cells in every taste bud exhibit immunoreactivity to gustducin, NCAM, or serotonin. Whether the same cells are immunoreactive to more than one of the substances or whether the different immunoreactivities are present in mutually exclusive populations was not determined. For the current multiple-label experiments, tongue tissue was obtained from rats pretreated with 5-hydroxytryptophan (5-HTP) to intensify serotonin-immunoreactivity. The tissue was fixed in 4% buffered paraformaldehyde. Sections of vallate-, fungiform- and foliate papillae were prepared on a cryostat and processed free floating or on subbed slides. The sections then were incubated overnight at 4° C in either a mixture of: 1) mouse anti-serotonin (1:1) and rabbit anti-gustducin (1:500), or 2) rabbit anti-serotonin and mouse anti neural cell adhesion molecule (NCAM), both diluted 1:500. Following rinsing, the tissue was exposed to secondary antibody mixtures respectively of either: 1) fluorescein goat anti-mouse and rhodamine donkey anti-rabbit, or 2) fluorescein donkey anti-rabbit and rhodamine donkey anti-mouse. Gustducin and 5HT immunoreactivities were present in mutually exclusive populations of taste cells. The gustducin-ir cells were elongate cells of relatively simple morphology. In contrast 5HT-ir cells, although sometimes spindle-shaped, often exhibited a more complex morphology including tortuous processes or elongate extensions from the basal portion of the cell. No apparent correlation exists between NCAM-ir and either 5HT or gustducin-ir. That is, some NCAM-ir cells were immunoreactive for gustducin or 5HT; others were not. Whether cells with different immunoreactivites represent different stages of maturation, different cell types (light, dark, etc.), different functional states, or a combination thereof, remains to be determined.

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Long-term Culture of Taste Buds: Gustducin Immunoreactivity and Cell Division. COLLIN RUIZ[1,3], TATSUYA OGURA[1,3], BARBEL BOTTGER[2,3], THOMAS E. FINGER[2,3], AND SUE C. KINNAMON[1,3] (Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523[1]; Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262[2]; and the Rocky Mountain Taste and Smell Center, Denver, CO 80262[3])

Our previous studies defined conditions for maintenance of rat taste buds in defined medium *in vitro*. Cultured taste buds survive and maintain electrical excitability for at least 14 days following isolation from the tongue. In the present study we utilize BrDU and antibodies to gustducin to characterize the relative age, rate of proliferation and state of differentiation of the cultured cells. As described previously (Ruiz et al., Chem. Senses 18:622, 1993), taste buds were isolated from circumvallate, foliate and fungiform papillae and cultured at room temperature. After 2-14 days in culture, the cells were fixed in methanol and reacted with antibodies to either gustducin or BrDU. At all time points, numerous gustducin-immunoreactive spindle-shaped or oval cells were present in each surviving taste cell cluster. No systematic differences were detected between cultures from different papillae. When BrDU was injected *in vivo* 2 days prior to culturing of the taste buds, BrDU-labeled cells were found on the periphery of each taste cell aggregate fixed after 2-14 days in culture. These findings indicate that the youngest cells in taste buds are on the outside of the taste cell aggregates. When BrDU was added to the culture medium (15ug/ml for 48 hrs. after 2 days in culture), label was detected in one or two cells within only a few of the taste cell aggregates. These data demonstrate that cell division continues in the taste cell aggregates in culture albeit at a much reduced rate compared to the *in vivo* situation. In summary, taste cells maintained in long term culture exhibit biochemical traits of mature taste receptor cells and continue a slow rate of proliferative activity.

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In Vitro Development of Embryonic Taste Receptors. LINDA A. BARLOW[1], CHI-BIN CHIEN[2] and R. GLENN NORTHCUTT[1] [1]Neurobiology Unit, Scripps Inst. of Oceanography and Dept. of Neurosciences, [2]Dept. of Biology Univ. of California, San Diego, La Jolla CA 92093.

Taste buds arise from the local epithelium late in embryonic development. Typically, overt differentiation of taste buds is coincident with or slightly follows the appearance of cranial nerve fibers within the oropharyngeal epithelium. These observations have lead numerous workers to suggest ingrowing neurites induce the formation of taste buds. Using an *in vivo* amphibian model, in which the presumptive oropharyngeal region from one salamander embryo was grafted to the trunk of a host embryo, we have recently shown that innervation by cranial nerves is not necessary for morphogenesis of ectopic taste buds (Barlow and Northcutt, 1994 Soc. Neurosci. Abs.). However, oropharyngeal grafts were invaded by spinal nerve fibers, and thus embryonic development of ectopic taste buds may have been supported by contact with neurites, albeit inappropriate ones. We have now developed an *in vitro* system to examine the ability of oropharyngeal epithelia to generate taste buds in the absence of innervation. The presumptive oropharyngeal region was removed from stage 35-37 embryos, many days prior to nervous contact (stage 39) or overt taste bud differentiation (stage 40), and raised in organ culture until intact control embryos had reached stage 41 or hatching. After 6 days *in vitro*, the tissue was fixed and processed immunohistochemically with antisera against known markers of taste buds in salamanders. Specifically, a subset of receptor cells within each taste bud has been shown to be calretinin-immunoreactive (IR) (Barlow and Northcutt, 1994). In addition, serotonin is a marker of salamander tastebuds; Merkel-like basal cells possess this neurotransmitter. Calretinin-IR cell clusters, reminiscent of immature taste buds typically observed at stage 40, as well as occasional solitary serotonin-IR cells, were encountered in the endodermal epithelia of explants raised in culture. These results indicate that taste buds will differentiate in the apparent absence of innervation. We are currently using antisera against neurofilaments to ascertain that nerve fibers are not present in these explants. Our future goals include the determination of when taste cell precursors are induced to differentiate, and which tissues are responsible for this inductive event.

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Both Ectoderm and Endoderm Give Rise to Taste Buds in Mice. LESLIE M. STONE[1], THOMAS E. FINGER[1], PATRICK P.L. TAM[2] and SEONG-SENG TAN[3] (Univ. of Colo. Hlth. Sci. Ctr.[1], Denver, Child. Med. Res. Inst.[2], Australia, Univ. of Melbourne[3], Australia.

Taste buds in the lingual epithelium of mice and in the oral cavity of axolotls derive from local epithelial progenitors (Stone et al., 1994; Barlow and Northcutt, 1994). This indicates that in mice, ectoderm gives rise to lingual taste buds whereas in axolotls endoderm gives rise to oral taste buds. The present study examines whether, in mice, both ectoderm and endoderm give rise to taste buds in different taste fields, i.e. whether taste buds residing in endodermally-derived epithelium originate locally. To address these questions, we examined the epithelia in regions reportedly derived from endoderm: in the pharynx, epiglottis, upper esophagus and upper trachea. The female mice used in this study contain an *E coli. lacZ* gene, linked to a housekeeping promoter, on one of their two X chromosomes. This transgene results in the ubiquitous expression of ß-galactosidase (ß-gal) in all cells of the early embryo. However, at about the time of gastrulation, random X inactivation shuts off this enzyme expression in approximately half of the embryonic cells. Because the X inactivation status of each cell is a stable, heritable feature, the presence of ß-gal activity can be used as a cell lineage marker. In adult mosaic mice, the epithelia lining the pharynx, epiglottis, upper esophagus and upper trachea consist of patches of cells that express ß-gal, and patches that do not. Taste buds located well within such patches always match the surrounding epithelial cells in terms of ß-gal activity. Taste buds located on the border between a ß-gal positive (+) epithelial patch, and a ß-gal negative (-) patch may contain both ß-gal+ and ß-gal- cells indicating that at least two progenitors give rise to individual taste buds. In conclusion, our previous and current studies indicate that in mice, taste buds derive from local epithelium, whether ectodermal or endodermally derived. Accordingly, both ectoderm and endoderm are capable of forming taste buds in rodents.

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Developmental Features of the Human Fetal Taste Bud

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Morphological changes of the human taste bud (TB) primordium during the 5th to 20th week of gestation have been studied at the ultrastructural level. In addition, the innervation pattern of taste buds has been studied immunohistochemically using antisera against neural cell adhesion molecule (N-CAM), and neurone specific enolase (NSE). - Papilla-like elevations of the dorsal epithelium of the tongue develop from the 6th week of gestation, but regularly these comprise only an aggregation of epithelial cells without the characteristic convex indentation of the basement membrane. Many immunoreactive nerve fibers invade the papillary epithelium (8th week) and come into close contact with slender epithelial cells that are considered to be the TB´s progenitor cells. As seen in the electron microscope, nerve fibers form synapses with cells of the TB primordium. At the TB´s base, footlet-like cell processes containing dense-cored vesicles (120-200 nm ∅) occur, but these processes do not synapse to nerve fibers. The first taste pores are formed around the 10th week, but a major number develops around the 14th to 15th week. - The early presence of cells containing dense-cored vesicles suggests an at least dual function of fetal TBs: First, from the 8th until the 14th week, non-gustatory, paracrine functions should be considered. After the 15th week, when the taste pore has appeared, the TBs possibly start their gustatory function.

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Taste Bud Development in Turbot Larvae (Teleostei).

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In the Turbot (*Scophthalmus maximus L.)* taste bud (TB) development was studied in the transmission electron microscope by using 9 larval stages (kept at 19-21° C). The developing mouth cavity contains the earliest TB primordia at larval stage 8 (6 days old). In older stages the TB primordia already resemble to developed TBs (stage 9, 24 days old). Developmental details are: 1. Early TB primordia comprise only one type of cell and contain within their basal parts some nerve fibers. 2. Light, dark and basal cells are recognized before the TBs receptor area reaches the surface of the epithelium. 3. The TB's corium papilla is formed subsequently to the TB's primordium. 4. Basal cells seem to migrate into the TB primordium along with the innervating nerve fibers. 5. Younger TB primordia possess efferent synapses; afferent synapses occur on light and dark cells of elder TB primordia (stage 9). - In Turbot larvae, TB development is induced by nerve fibers growing out the cranial nerves (VII, IX, X). Light and dark cells derive from epithelial cells. Basal cells seem to be Schwann cells and therefore derive from the neural crest. This resembles TB development in regenerating TB-bearing appendices of the fish's body, as in the barbel regenerates of bullheads (Reutter 1978, Adv. Anat. Embryol. Cell Biol. 55, 1-98).

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Taste Receptor Topology: Correlation of Structure and Function

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Studies of rat chorda tympani NaCl responses under voltage clamp suggest that Na^+ ions stimulate taste cells via two routes: apical membrane channels and channels on the basolateral membranes (Ye et al. J. Neurophysiol. 70: 167, 1993). Functional studies suggest that both apical and basolateral membranes have amiloride-sensitive ion channels. If so, this would be a departure from the usual epithelial topographical segregation of components. Sorcher et al. (Amer. J. Physiol. 255: C835, 1988) have obtained antibodies against the amiloride-sensitive Na^+ channel. Simon et al. (Micr. Res. Tech. 26: 196, 1993) have demonstrated antibody binding to the basolateral membranes of canine circumvallate taste buds. Stewart et al. (ms submitted, 1994) have shown binding to both apical and basolateral membranes of rat fungiform taste buds. We have conducted a similar study and confirm that antibody against the epithelial sodium channel binds to both apical and basolateral membranes of rat fungiform papilla taste cells. Staining was confined mainly to intragemmal taste cells in agreement with Stewart et al. (1994) and similar to the keratin 19-like immunoreactivity reported by Wong et al. (Chem. Senses 19: 251, 1994). Filamentous structures below the lamina propria were also densely stained. These patterns resemble those labeled by lectin neuronal markers (Silverman and Kruger, J. Comp. Neurol. 292: 575, 1990). In conclusion both structural and functional studies support the presence of amiloride-blockable ion channels on both apical and basolateral taste cell membranes. Studies with Na^+-deprived rats suggest, however, that apical channels are Na^+ selective while basolateral channels do not distinguish Na^+ and K^+ ions. These results suggest that taste cell Na^+-K^+ pumps must be especially active to maintain stable cell resting potentials.

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Weak Acids Are Indiscriminable From One Another and From HCl.

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We previously reported that moderate concentrations of three acids, HCl, HNO_3 and H_2SO_4 are indiscriminable ('match') when their concentrations are adjusted appropriately (*Chem. Sens.*, **19**:1994). We have now conducted similar experiments to see if matches could be obtained among three weak (not fully dissociating) acids: citric, tartaric, and malic. The experiments consisted of a series of two alternative forced-choice (duo-trio) trials comprised of three sequential sip & spit exposures in which the subjects had to state whether the 1st or 3rd stimulus was different from the 2nd. Across sets of trials, the concentration of one acid was held constant while the concentration of the other acid was varied semi-randomly. The order of the solutions within the three cups was counter-balanced across trials. For all subjects (n=3), we found characteristic concentrations of the test-acids citric and malic that were indiscriminable from the standard, 3mM tartaric; test-acid concentrations that were higher or lower than the match point were discriminable following an inverted bell-shape function. Next, subjects were asked to discriminate between the two acids after the concentration of the standard, tartaric, was at least doubled. The subjects failed to discriminate for concentrations of the test acids that were scaled up an appropriate amount, and the match between these compounds was maintained. These acids were indiscriminable at different pH values. Thus the free proton concentration in solution was not the only aspect of the stimuli that contributed to their taste intensity even though they did not differ in quality. To determine whether the weak acids shared the same quality of sourness as did the strong acids, we determined whether subjects could distinguish between the strong acid, HCl, and the weak acid, citric. All subjects failed to distinguish between the acids at appropriate concentrations. We found no evidence for a qualitative difference in the taste of the acids tested. This suggests that, unlike the case for salts, anions play no role in determining the acid taste quality within the concentration ranges tested. We surmise that the tastes elicited by all of these acids are indistinguishable for specific concentrations because they act identically upon the relevant receptor mechanisms and give rise to indistinguishable neural signals.

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Judgments of "Mental mixtures" of sucrose and citric acid
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Prior investigations of memorial intensity judgments of "mental mixtures", where separate components of the mixture have previously been tasted alone and are mixed mentally, reveal a surprising consistency between responses to real and "mental mixtures". This ability may arise as "...people display far deeper chemosensory knowledge than they are either aware of or can intelligently articulate" (Algom et al., Chem. Sens., 1993, 18, page 159). We investigated this claim using Algom et al's method, by teaching subjects to associate certain card colours (red, blue, yellow and green) with 0.17M and 0.80M Sucrose and 0.005M and 0.05M Citric acid. After learning these associations to a preset criterion, subjects returned on the following day and were randomly assigned to receive either factorial combinations of the tastes or their equivalent in coloured cards. Crucially performance between the two groups was not significantly different. Unlike in previous studies of this kind subjects who received "mental mixtures" were then extensively debriefed (n=12). Asked how they had made their judgments, 58% of subjects made explicit reference to previous experiences where they had encountered mixtures of sweet and sour and claimed to have based their judgments on how they recalled these mixtures, a further 33% (not including any of the above), claimed to have used some simple heuristic to produce their responses. In conclusion, though these results are remarkable in the similarity of performance evidenced between real and "mental mixtures", they suggest that in judging "mental mixtures", at least in this case, probably involves both explicit memory and conscious control.

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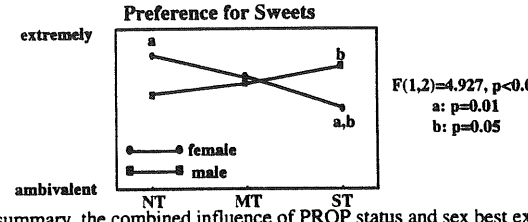
Preference for Sweets in Young Adults Associated with PROP (6-n-propylthiouracil) Genetic Taster Status and Sex. VALERIE B. DUFFY[1], HARVEY P. WEINGARTEN[2], LINDA M. BARTOSHUK[3] (University of Connecticut[1], Storrs, CT; McMaster University[2], Hamiliton, ON; Yale University School of Medicine[3], New Haven, CT).

Our aim was to associate suprathreshold PROP perception with food preference. We also examined the interaction of PROP and sex on food preference as women are more likely to perceive PROP as intensely bitter and have more fungiform taste buds than men (Bartoshuk et al, 1994). A sample of 55 females and 44 males (x=22±5 yrs) participated in taste testing to classify PROP status as non- (NT), medium- (MT), and supertasters (ST): PROP threshold and PROP/NaCl scaling. Ss indicated preference for 82 foods/beverages by marking the degree of liking/disliking on 200 mm line ("0" on the left, "extremely" at 132 mm, arrow at 200 mm). Foods were grouped into 20 taste groups. Regression analyses tested the PROP effect and ANOVA tested the PROP STATUS by SEX interaction. PROP Effect: No food groups associated significantly with PROP tasting. PROP by SEX Interaction: A significant interaction existed for preference of low and high fat sweets and for all sweets combined (shown below). In women, preference declined as PROP tasting increased. Men showed no effect, or showed an increase in preference as PROP tasting increased.



In summary, the combined influence of PROP status and sex best explained variation in sweet preference. Differences in preference response between women and men may be physiologic (i.e., women have more taste buds, perceive sweets as more intense and thus less pleasant) or psychologic (i.e., women exert more restrained eating even in making preference judgements).

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Potential Human Variants for Glucose Taste Suggest Separate Fructose and Glucose Mechanisms SHACHAR EYLAM, LINDA M. KENNEDY, and DAVID A. STEVENS (Clark University, Worcester, MA 01610)

Human psychophysical functions for sweetness are similar for sucrose and fructose, but different for glucose (Pangborn, 1963; Portmann et al, 1992), and suggest different mechanisms for fructose and glucose. Interestingly, *Drosophila adiastola* behavioral and neurophysiological thresholds, ranges and response functions for the three sugars vary in ways similar to the human data and indicate separate receptor cell mechanisms for the monosaccharides. Moreover, fructose 'nontasters' (NTs) and glucose NTs have been selected from a natural *D. adiastola* population (Kennedy and Poskanzer, 1994). Identification of human NTs for one or more of the monosaccharides and linkage of the variant traits to the responsible genes, would confirm separate mechanisms and could lead to identification of proteins involved in human sweet taste by molecular genetics techniques. Therefore, we attempted to identify human NTs by first obtaining response functions for sucrose, fructose and glucose from 10 males and 10 females. The subjects tasted pairs of water and various concentrations of the sugars (2 - 128 mM), and were asked to indicate the sweeter of the two solutions. Response functions (percentage of the population that correctly recognized the sugar as sweet [Recognition Index - RI] vs. the concentration), showed a marked difference between sucrose and fructose (compressing curves) and glucose (expanding curve). These results support, but do not prove, separate physiological mechanisms for fructose and glucose taste. At the highest concentration (128 mM) however, the RI values for all three sugars were the same (1.0). This result is consistent with the monogeusia reported by Breslin et al (1994) for concentrations higher than tested here. (That study does not rule out possible separate monosaccharide mechanisms, since comparisons were not made of sucrose vs. fructose.) Separate monosaccharide mechanisms are further supported by the isosweet concentrations we obtained for RI 0.7 for the 20 subjects: 8 mM sucrose, 10 mM fructose and 36 mM glucose. Potential NTs were suggested by shifts of their glucose curves and glucose concentrations for the RI 0.7 (60 mM), i.e. by showing elevated thresholds for glucose. In ongoing work, subjects will be asked to compare between the sugars at concentrations for RI 0.7. A statistically significant recognition (two-tailed binomial distribution) of one monosaccharide as less sweet, or not sweet, in comparison with the other will identify NTs. [Supported by a Clark Univ. Faculty Development Award and Research Grant Incentive Funds to LMK]

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6-N-Propylthiouracil Perception Affects Nutritional Status of Independent-Living Older Females. LAURIE A. LUCCHINA[1], LINDA M. BARTOSHUK[2], VALERIE B. DUFFY[1], LAWRENCE E. MARKS[3], ANN M. FERRIS[1]. (1. University of Connecticut, Storrs, CT; 2. Yale University, New Haven, CT; and 3. John B. Pierce Laboratory, New Haven, CT.).

The ability to taste 6-n-propylthiouracil (PROP) is genetically determined. PROP tastes bitter to "tasters", intensely bitter to "supertasters", and tasteless to "nontasters". Since PROP tasters perceive caffeine, saccharin, and potassium chloride as more bitter, sucrose as sweeter, and sour as more intense than nontasters, tasters may be more sensitive to primary taste qualities in foods. However, the effect of PROP status on diet-related behaviors and nutritional status has not been systematically examined. Methods: The effect of PROP suprathreshold intensity perception on nutritional status was assessed in 60 functional, independent-living females aged 65-95 years using a portable magnitude matching test. Perceived intensity ratings of randomized tastants [citric acid (range 0.001-0.032M), sucrose (0.032-1.0M), quinine hydrochloride (0.000032-0.001M), and PROP (0.0001-0.0032M)], and an odorant [n-amyl acetate (1-10,000 ul)], were assigned. Data were successfully normalized to sodium chloride taste, low frequency noise (band width 100-500 Hz; 55-85 dB levels), and a 5-point adjective scale (very weak-very strong). The nutrition indices included food behaviors and preferences, dietary intake, anthropometric measurements, body mass index (BMI), body fat, and serum total, LDL, and HDL cholesterol, and triglycerides. Results: Significance criterion: $p<0.05$ unless noted. PROP perception positively associated with cooking involvement and food interest, eating enjoyment, intake of pungent foods, and negatively with high-fat dairy product intake (Spearman correlations). PROP perception was consistently negatively correlated with BMI, body fat, and waist and hip circumferences. Also, PROP perception associated negatively with serum triglycerides ($p<0.01$) and positively with HDL cholesterol ($p<0.01$). These results suggest that PROP status may affect food behaviors and preferences, dietary intake, body composition, and biochemical parameters of nutritional status. The ability to taste PROP may provide an advantage in preventing chronic disease associated with obesity and altered serum lipid levels.

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Nutritional Information Influences Cognitive Attributes But Not Sensory Perceptions of Foods. BEVERLY J. TEPPER and AMY C. TRAIL (Rutgers University, New Brunswick, NJ)

Nutritional or health-related information can strongly influence the perception of foods, but studies assessing these effects are mixed. Both Shepherd et al. (1991) and Aaron et al. (1994) found that nutritional information shifted sensory judgements in a direction consistent with an individual's attitudes and beliefs. That is, sensory ratings and liking increased for the sample towards which subjects had a positive attitude. Presumably, incorrect information would violate a subject's expectations leading to a contrast effect. Upon tasting, ratings should shift in a direction away from the subject's expectations. To test this hypothesis, 297 adults participated in a consumer test. Subjects rated a high-fat (10%) and low-fat (2.5%) vanilla pudding for creaminess, vanilla flavor, fat content and healthiness using a 15 cm. line scale. Half of the subjects received samples correctly labeled (as "high-fat" and "low-fat"). The remaining subjects received samples with the labels reversed. Sensory ratings for creaminess and flavor were not influenced by the label information. In the correct label condition, subjects who perceived the low-fat sample as healthier rated the high-fat sample much higher in fat than the low-fat sample (difference score: high-fat minus low-fat=3.2; $p \leq 0.005$). This difference was not observed in subjects who perceived the high-fat sample as healthier (difference score=0.8; ns). Switching the labels strongly influenced judgements of perceived fat content for all subjects regardless of their health attitudes towards the samples. Low-fat samples which were incorrectly labeled as high-fat were rated higher in perceived fat content (difference score=3.1; $p \leq 0.001$). Thus, cognitive perceptions were manipulated by label information but the sensory ratings were not. No contrast effect was observed for the samples which were incorrectly labeled.

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Effect of ±2-(4-methoxyphenoxy)propionic acid on the Sweetness Intensity Ratings of Fifteen Sweeteners. SUSAN S. SCHIFFMAN[1], BREVICK G. GRAHAM[1], ELIZABETH A. SATTELY-MILLER[1], BARBARA J. BOOTH[2], B. THOMAS CARR[2], and MICHAEL L. LOSEE[2] (Duke University[1], Durham, NC and The NutraSweet Co.[2], Mt. Prospect, IL)

The purpose of this study was to determine the degree to which the substituted phenoxyalkanoic acid, ±2-(4-methoxyphenoxy) propionic acid, reduced sweet intensity ratings of fifteen sweeteners. This compound was patented as a sweetness reducing agent by Tate and Lyle Co. and is marketed as "Cypha" by Amstar Sugar Corp. A trained panel evaluated the effect of Cypha on the intensity of the following fifteen sweeteners: 3 sugars (fructose, glucose, sucrose), 3 terpenoid glycosides (monoammonium glycyrrhizinate, rebaudioside-A, stevioside), 2 dipeptide derivatives (alitame, aspartame), 2 *N*-sulfonylamides (acesulfame-K, sodium saccharin), 2 polyhydric alcohols (mannitol, sorbitol), 1 dihydrochalcone (neohesperidin dihydrochalcone), 1 protein (thaumatin), and 1 sulfamate (sodium cyclamate). Sweeteners were tested at concentrations isosweet with 2.5%, 5%, 7.5%, and 10% sucrose in combination with two levels of Cypha, 250 ppm and 500 ppm. Cypha significantly blocked sweetness intensity levels for 10 of the 15 sweeteners at both the 250ppm and the 500ppm levels. However, Cypha, at both the 250 ppm and 500 ppm level, failed to block monoammonium glycyrrhizinate, neohesperidin dihydrochalcone, and thaumatin at all isointensity levels. Additionally, Cypha did not block rebaudioside-A or stevioside as effectively as the sugars, sugar alcohols, dipeptide derivatives, *N*-sulfonylamides, and the cyclamate. The degree to which Cypha can suppress sweet intensity ratings may be related to the size of the sweetener molecule.

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Effect of Gymnemic Acid Rinse on a Taste Confusion Matrix. JANNEANE F. GENT[1], MARION E. FRANK[2], THOMAS P. HETTINGER[2], QUINTEROL MALLETTE[2] AND LAWRENCE E. MARKS[1] ([1]J.B. Pierce Lab., New Haven and [2]UConn Health Center, Farmington CT)

A sweet taste deficit was simulated by oral application of gymnemic acid (GA) and analyzed by the pattern of the resultant taste confusions. Subjects (N = 20) were asked to choose correct stimulus names for 10 stimuli (0.1M NaCl, 0.1M KCl, 0.1M Na glutamate [MSG], 0.1mM quinine HCl, 3mM citric acid, 0.3M sucrose, 3mM aspartame, and NaCl-sucrose, acid-sucrose, and quinine-sucrose mixtures) presented 10 times. A 'sip and spit' procedure, including the usual water rinses between each trial, was used. Subjects participated in 2 sessions of 100 trials and received an additional 2 min rinse prior to trials 1 and 51. In session 1, this additional rinse was water for all 20 subjects; in session 2, 10 Ss received water (control group) and 10 received 0.5% GA. For the control group the value of T, bits of information transferred, significantly increased from session 1 to 2 ($p<.01$) showing improved performance with practice. After GA, percent correct decreased for sweet stimuli ($p<.013$). Sucrose mixtures were mis-identified as the non-sucrose component 22% of the time after GA and never mis-identified in this way after water. GA rinses lost power over time: correct identifications of sweet stimuli were 36% for trials immediately following GA rinses, 59% later on. Sweet stimuli were correctly identified 63% of the time in the control group. The GA - treated group showed a slight decrease in the value of T from session 1 to 2 (n.s.) due to opposing effects of learning and the simulated taste deficit. On average, subjects treated with GA had a significantly lower T than controls ($p<.05$). We conclude that confusion matrices containing mixtures may be useful in discriminating taste deficits.

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Coding dimensionality for sweetness produced by nutritive and non-nutritive sweeteners.
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The question of whether sweet-tasting compounds are encoded as single or multiple signals was recently investigated by Breslin et al (1994)[1], using fructose, sucrose and glucose. They concluded that the taste for those compounds are monogeusic or identical in nature, and therefore probably bind reversibly to a single class of receptors in taste cells. Their protocol called for a single subject to perform around 600 taste comparison trials. We have adapted their protocol for use with a panel of 43 subjects who were asked to discriminate, in a duo-trio format, 9 concentrations of glucose ranging from 200mM to 600mM in 50 mM steps, from a standard 200mM fructose solution. Subjects were unable to discriminate the fructose standard from glucose solutions of 350 to 550mM. A line of best fit indicated that 450mM glucose was equivalent to 200 mM fructose. This is in agreement with Breslin et al's finding for glucose and fructose and suggests that the panel protocol is valid. The panel protocol makes the test for dimensionality of tastants more practicable. We will report the outcome of a study of non-nutritive sweeteners and discuss the application of the protocol to other tastants and the implications of it for identifying molecular receptors for tastants.

1. Breslin, P.A.S., Kemp, S. and Beauchamp, G.K. Single sweetness signal. Nature, 1994, 369, 447 - 448.

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Novobiocin And Magnitude Estimation of Saltiness In Humans.
ANN M. TENNISSEN (College of Saint Rose, Albany, N.Y.)

Novobiocin, an antibiotic, was tested for possible effects on the perception of saltiness in humans. Work by Feigin et al., (Am. J. Physiol. 266, (Cell Physiol. 35):C1165-C1172, 1994) showed an increase in activity to NaCl in amiloride-sensitive, sodium-specific (N-fibers) of the chorda tympani of rats. The present study sought to determine whether novobiocin would increase magnitude estimation ratings of saltiness of NaCl in humans. Subjects were tested in one session for amiloride sensitivity and in another session for responses to novobiocin and NaCl. Subjects were adapted to novobiocin for 3 minutes prior to testing and then dipped the anterior portion of the tongue, first, into 10 ml solutions of NaCl (0.00M, 0.05M, 0.15M, 0.30M). These were followed by solutions of QHCl (0.0001M), plus sucrose (0.01M), plus the 4 concentrations of NaCl. This mixture served as a control for the taste of novobiocin. A third set of solutions consisted novobiocin (0.002M) plus the 4 concentrations of NaCl. Subjects gave magnitude estimations for saltiness intensity for each of the solutions. Preliminary results, in amiloride-sensitive subjects, indicate no change in the intensity responses of saltiness with exposure to novobiocin.

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Amiloride and judgements of NaCl taste: No consistent effects on either time course of taste intensity or reports of salty taste. B. P. HALPERN, J. S. MELTZER, M. LEE, AND R. B. DARLINGTON (Cornell University, Ithaca NY 14853-7601).

Time-intensity tracking (visual feedback, 100 msec resolution, 8 sec total duration) and unrestricted taste quality descriptor (end of each trial) measurements of the effects of amiloride on NaCl were made in 6 practiced subjects over 8 data collection sessions. Solutions flowed for 4 sec thru a closed delivery system over 39.3 mm^2 of the anterodorsal tongue tip region, preceded by 10 sec H_2O and followed by 5 sec H_2O. Stimuli were 100, 250, 500mM NaCl in H_2O (pH ~6), in 10μM or 100μM amiloride, or in caffeine controls, or the caffeine or amiloride solutions in H_2O with no NaCl. Each subject selected caffeine solution concentrations to approximate amiloride tastes: 33μM-100μM caffeine for 10μM amiloride; 8.33mM-12.5mM caffeine for 100μM amiloride. Analyses were done with general linear model ANOVA and t-tests for each subjects. **RESULTS**: ANOVA: Latency, rise time, time to maximum amplitude (TMAX), time within 90% of maximum amplitude (T90MAX), and duration, for ≥ 4 subjects, had $F \geq 2.194$, $p \leq 0.040$. Salty and bitter taste quality descriptions for all 6 subjects had $F \geq 2.279$, $p \leq 0.033$. t-tests: With NaCl concentration, TMAX, T90MAX, and duration increased, $t \geq 2.2$, $p \leq 0.028$, for ≥ 4 subjects, and salty descriptions increased in all 6 subjects, $t \geq 2.845$, $p \leq 0.005$, while bitter descriptions decreased in all 6, $|t| \geq 3.296$, $p \leq 0.001$. With amiloride treatment, bitter descriptions increased in 4 subjects, $t \geq 2.226$, $p \leq 0.028$, but salty descriptions did not change significantly for any subject, $t \leq 0.618$, $p \geq 0.538$. **CONCLUSIONS**: 10 or 100μM amiloride mixed with 100, 250, or 500mM NaCl in H_2O alters neither the time course of tracked total taste intensity nor the incidence of salty descriptions. NaCl concentration does change the time course of tracked total taste intensity and the incidence of salty and bitter descriptions.

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The Effect of Bitter Inhibitors on Taste Perception of Urea, Quinine HCl, Magnesium Chloride, and Caffeine. SUSAN S. SCHIFFMAN[1], MARK S. SUGGS[1], BREVICK G. GRAHAM[1], ELIZABETH A. SATTELY-MILLER[1] and LARRY A. GATLIN[2] (Duke University[1], Durham, NC and Glaxo Inc.[2], RTP, NC).

The purpose of this study was to assess the following compounds for their efficacy as bitter blockers: *N*-(4-cyanophenyl)-*N'*-[(sodiosulfo)methyl]urea (4mg/ml), ±2-(4-methoxyphenoxy) propionic acid (500ppm), monoammonium glycyrrhizinate (456ppm), β-cyclodextrin (1.5%w/v), hydroxypropyl β-cyclodextrin (10%w/v), polydextrose (50%w/v), maltol (80ppm), ethyl maltol (50ppm), sodium chloride (0.3M), sucrose (60%w/v), and a mixture of sucrose (60%w/v) and ±2-(4-methoxyphenoxy) propionic acid (500ppm). Four bitter compounds that were mixed with the potential bitter blockers were the following: urea ($1.78X10^{-1}$M to 1.35M), QHCl ($8.68X10^{-6}$M to $2.11X10^{-3}$M), $MgCl_2$ ($1.04X10^{-2}$M to $3.33X10^{-1}$M), and caffeine ($2.94X10^{-3}$M to $4.83X10^{-2}$M). All compounds were dissolved in dH_2O. The intensity of each bitter compound mixed with a potential bitter blocker was evaluated by 15 subjects (mean age 26 years±0.91), consisting of 8 tasters and 7 non-tasters of phenylthiocarbamide (PTC). The subjects also evaluated the intensity of the same bitter compound alone as a control. All combinations of bitter compounds and potential blockers were assessed with the exception of QHCl, which was not tested with *N*-(4-cyanophenyl)-*N'*-[(sodiosulfo)methyl]urea or monoammonium glycyrrhizinate (MAG) due to solubility problems at suprathreshold levels. QHCl ($1.56X10^{-4}$M) and sucrose (7%) were used as mid-point references on 100 mm visual analog scales for bitterness and sweetness respectively. The potential bitter blockers that were the most effective overall in reducing the bitterness ratings for the bitter compounds were ±2-(4-methoxyphenoxy) propionic acid mixed with sucrose, sucrose alone, and NaCl. The average % decreases for the highest concentration of each of the 4 bitter compounds mixed with sucrose, ±2-(4-methoxyphenoxy) propionic acid with sucrose, and NaCl as compared to dH_2O alone were 47.4%±12.2%, 35.4%±12.5% and 32.4%±9.87%, respectively. Some of the other potential bitter blockers produced similar decreases in bitterness ratings. These results indicate that several compounds have bitter blocking potential in reducing the perceived bitterness of urea, QHCl, $MgCl_2$, and caffeine.

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Gustatory Sensitivity to PROP in Aging. YOSHIKO YOKOMUKAI[1,2], B. J. COWART[1] & G. K. BEAUCHAMP[1] ([1]Monell Chemical Senses Center, Philadelphia, PA; [2]Kirin Brewery Co., Ltd., Tokyo, Japan)

Cross-adaptation studies imply the existence of at least three classes of bitter compounds: one that includes the quinine salts, one that includes urea and one that includes PTC and related compounds (e.g., PROP) (McBurney et al., *Percept.Psychophys.*, 1972). Aging may differentially affect sensitivity to compounds representing these classes; e.g., threshold and suprathreshold quinine sensitivity declines with age, whereas sensitivity to the bitterness of urea does not (Cowart et al., *Physiol.Behav.*, 1994). Age-related change in responsiveness to compounds in the third presumed class of bitter stimuli has not been well-characterized. We obtained detection thresholds for PROP, as well as ratings of the perceived intensity of 5 suprathreshold concentrations (0.32-3.2 mM), from 49 young (<40 years) and 35 elderly (>65 years) adults. The threshold distribution of young subjects was more distinctly bimodal than that of the elderly, and overall, the young had lower PROP thresholds (Mann-Whitney U, $p<0.01$), although the percentage of non-tasters (as defined by the anti-mode of the young) did not increase significantly with age (24.5% vs 40%, $X^2=2.3$, $p>0.1$). There was no significant difference between the young and elderly in ratings of suprathreshold intensity. In both groups, however, mean ratings were significantly correlated with threshold sensitivity, and among tasters, the young tended to assign higher intensity ratings than did the elderly ($p<0.09$).

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Further Evaluations of the Utility and "Validity" of the Labeled Magnitude Scale. BARRY GREEN, PAMELA DALTON, BEVERLY COWART, GREG SHAFFER, KRYSTYNA RANKIN, JOHN PIERCE and JENNIFER HIGGINS. (Monell Chemical Senses Center)

The Labeled Magnitude Scale (LMS) is a semantic scale of perceptual intensity (Green et al., *Chem. Senses*, 1993, **18**: 683-702) characterized by a quasi-logarithmic spacing of its verbal labels and an upper bound of "strongest imaginable oral sensation." The LMS had been shown to produce psychophysical functions equivalent to magnitude estimation (ME) when gustatory, thermal and nociceptive stimuli were presented and rated together. It was unknown, however, whether the LMS could be used as a specific taste [or odor] scale if subjects were asked to rate intensity in the context of "the strongest imaginable *taste* [or *odor*] sensation". In Exp. 1, Ss who had no experience with either the LMS or ME rated taste sensations produced by six concentrations each of sucrose and NaCl. The stimuli were intermixed and presented three times each in two sessions: in one session ratings were made with ME and in the other with the LMS. Exp. 2 had the same design except the stimuli were ammonia and PEA delivered via sniff bottles. The LMS and ME yielded statistically identical psychophysical functions in both experiments. In Exp. 3 the LMS was evaluated for use with a *single* taste quality (sweetness). Unlike before, a significant interaction was found between scaling method and stimulus concentration [$F(5,95)=3.75$, $p<0.005$]: the LMS produced a steeper psychophysical function than ME. Together these results suggest the LMS produces data comparable to ME only when intensity ratings are made on perceptual continua that are bounded by painful sensations. Whereas in the first two experiments subjects were free to assume that the strongest imaginable tastes and smells included pungent and/or noxious sensations, in the third experiment the context was limited to "the strongest imaginable sensation of sweetness." Experiments are planned with other taste qualities that may (sourness) or may not (bitterness) be assumed to produce painful sensations at very high concentrations.

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Flavor Enhancers: A Psychophysical Approach
ATSUSHI OKIYAMA[1,2] and GARY K. BEAUCHAMP[1] ([1]Monell Chemical Senses Center and [2]Ajinomoto Co., Inc.)

The present study aimed to clarify the taste contributions of MSG in food systems, and to examine whether the taste dimensions are attributable to the sodium or to the glutamate or to both. Freshly cooked chicken soup was prepared with several concentrations (0.06 - 0.32 M) of added NaCl. In the first study subjects were presented with pairs of samples, each having the same concentration of salt but one of which contained 0.2% MSG. They were asked to judge which sample of the pair tasted best, why that sample tasted best, and subsequently, were given a test of discrimination. The subjects significantly preferred the sample with added MSG when the salt levels were low to moderate. The next 2 studies were designed to decide whether it was the Na or the glutamate that was responsible for this preference. In study 2, it was found that, by adding the amount of NaCl equivalent to the amount (0.01 M) of Na in 0.2% MSG to one of the two samples, subjects preferred the one with more salt at the two lower NaCl levels (0.06 vs. 0.07 M and 0.10 vs. 0.11 M). Thus, the Na alone may contribute to the enhancing aspect of MSG. However, a third study showed this is not sufficient to explain the effects of MSG. Here the concentration of Na was held constant by adding 0.2% MSG to one sample and 0.01 M NaCl to the other. Subjects significantly preferred the sample with glutamate to the one without. In discrimination studies subjects who were able to identify which sample contained the added MSG were most likely to prefer the MSG-added sample. Descriptions of these samples were "more flavor," "tastes better," etc. These studies, in sum, demonstrate that MSG increases palatability and that both the Na (salty) and the glutamate (umami) contribute to this enhancement.

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Development of an Automated Regional Taste Testing System.
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Traditionally, regional tongue taste testing has been performed in the clinic using either (i) small pieces of filter paper soaked in tastants, (ii) cotton applicators similarly soaked in stimuli, or (iii) drops of tastants applied via micropipettes. Recently, we developed an automated system for presenting spatially and temporally discrete stimuli to small regions of the tongue. This system, which is a further development of a system described by Matsuda and Doty (*Chem. Senses*, 1995, in press), presents stimuli or rinse to the tongue from 13 gravity-fed solution reservoirs. Temporal accuracy of stimulus presentation is approximately 100 msec. The system utilizes Teflon GVC solenoid valves controlled by an Atcom 64 Programmable Controller. The stimuli are delivered via glass applicators held to the tongue by mild vaccum surrounds. Currently, the sizes of the tongue areas that can be stimulated are 12.5 mm^2, 25 mm^2, and 50 mm^2. This system has the capability for computer control and automatic print out of results. Programs presently developed for psychophysical testing include a forced-choice staircase detection threshold procedure in which the stimulus concentrations are presented based upon subject response.

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Evaluation of Flavor Volatility Using a Retronasal Aroma Simulator
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The importance of retronasal aroma to flavor is best demonstrated by the decrease in overall flavor when the nose is pinched during eating. An instrumental simulation of flavor volatiles released during retronasal aroma perception is a needed tool for food analysis. A retronasal aroma simulator (RAS) was developed as a large scale dynamic purge-and-trap blender which included synthetic saliva addition, temperature regulation, and mixing at the shear rate of the mouth. Heating to 37 °C and mixing caused statistically significant increases in the volatility of 5 flavor compousnds in an acidic beverage. Different volumes of synthetic saliva were added to determine how the flavor profile would change as saliva increased the pH. At 1/4 volume saliva addition, the volatility of 2-methoxy-3-methylpyrazine, o-aminoacetophenone, methyl anthranilate, and 2-acetylpyridine increased 8, 12, 18, and 41 % respectively over the model neutral compound, 1,8-cineole. To test if these differences were detectable, a sensory line scale test using 1,8-cineole and 2-acetylpyridine in the sample acidic beverage and as standards for the ends of the line scale was performed. A statistically significant ($p < 0.00005$) flavor profile shift to 2-acetylpyridine was seen upon saliva addition. In addition, the volatility of a range of flavor compounds was tested in soybean oil and water matrices. The highly volatile nonpolar compounds, α-pinene, ethyl-2-methylbutyrate, and 1,8-cineole, with measured log P values of 3.75, 1.19, and 1.34, respectively, showed large decreases in volatility upon oil addition (7700, 130, and 100 fold). However, the more polar compounds, butyric acid and maltol, with log P values of 0.79 and 0.02, did not decrease in volatility upon oil addition. These results explain the often observed flavor changes in reduced fat products. The RAS provides a sensitive (to µg/L), reproducible (CV = 5%) and broadly applicable method for measuring retronasal aroma.

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Food Preferences in Individuals with Prader-Willi Syndrome

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This study investigated food preferences in individuals with Prader-Willi (PW) syndrome. The primary characteristic of the PW syndrome is hyperphagia which leads to morbid obesity if food intake is not strictly controlled. It's generally believed that the quality of the food seems less important than the quantity to the affected individual. The objective of this study was to learn more about eating behavior and food likes and dislikes of individuals with PW syndrome and how they compare to those unaffected by the syndrome. Affected and non-affected individuals tasted small amounts of 20 different foods, 10 familiar and 10 unfamiliar items. The four basic taste qualities of sweet, salty, sour, and bitter were represented equally among the food items. The subjects' task was to rank the foods in terms of preference. Contrary to expectations, PW individuals showed a similar pattern of food preferences as the non-affected counterparts: familiar foods were ranked as most preferred significantly more often than unfamiliar foods. PW subjects also showed preference for sweet and salty food items and clear dislike for bitter and sour foods. A better understanding of the sensory and cognitive attributes of food that influence eating patterns of PW individuals may aid in understanding the aberrant eating behavior associated with this syndrome.

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Chemosensory Complaints and Their Relationships to Malnutrition in ESRD Patients. H.J. DUNCAN[1], R.A. FRANK[1], J.R. KUES[1], L.E. JOHNSON[1], G.A. WARSHAW[1], P.M. HEACOCK[2], S.E. HEILE[2], P.A. NORTON[2], J.H. GALLA[1], University of Cincinnati[1] and Dialysis Clinics, Inc.[2], Cincinnati OH

Altered taste or smell sensations are common in End Stage Renal Disease (ESRD) patients. The relationship between these chemosensory complaints and malnutrition in ESRD patients has not been investigated. We compiled a "Chemosensory Complaint Inventory" (CCI), consisting of 35 statements about patients' subjective views of the taste or smell of food, as well as other questions about sensory function and attitude about food intake. In addition, assessments were made of patients' nutritional status, both subjectively and with respect to several biochemical and anthropometric measures of nutritional status. The CCI was also administered to healthy, elderly adults and healthy college students. Factor analysis identified three factors which accounted for 32% of the response variance, and complaints were found to be more common in ESRD patients than in either of two groups of controls; for example, on Factor 1 (12 items), ESRD patients had scores of 4.5, compared with 2.0 for students and 1.8 for the elderly subjects. In addition, when patients were divided into groups with and without nutritional difficulties (based on subjective impressions and need for dietary supplements), the CCI results showed significantly more complaints in the patients who were at risk for malnutrition (on Factor 1, 5.4 vs. 3.9 for healthy ESRD patients). Mortality was higher in the malnourished group of ESRD patients after 18 months, showing a correspondence between the subjective assessment of malnutrition and outcome. When others measures of malnutrition were considered, significantly higher proportions of patients with nutritional difficulties had clinically reduced levels of albumin and transferrin, but cholesterol levels, body mass index and mid-upper arm circumference did not distinguish between the two groups of patients. Thus, our finding of a correspondence between the CCI and malnutrition will be a useful additional diagnostic tool for identifying those ESRD patients who require nutritional intervention.

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Spontaneous Resolution of Dysgeusia. DANIEL A. DEEMS, DAVID M. YEN, ALLYSON KRESHAK, and RICHARD L. DOTY (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania Medical Center, Philadelphia, PA USA)

Dysgeusia is a debilitating disorder with 35% of dysgeusics experiencing mild to severe depression. Presently, most dysgeusics are untreatable, and no data are available for counseling them on the probability of recovery. In a recent follow-up of a small group of Center dysgeusic patients, we discovered that a number reported spontaneous remission of their symptoms. To explore this phenomenon in detail, we queried dysgeusic patients who had been evaluated at our Center from 1989 to 1994 regarding the current status of their problem. One hundred and seven patients were initially identified from medical records who presented with dysgeusia. Selection criteria included that the original taste distortion (i) be identifiable (e.g., bitter -- not distortion of "flavor" secondary to dysosmia) and (ii) be present in the absence of an oral stimulus (e.g., retronasal dysosmia). Approximately half (48) were able to be contacted and were willing to participate. The patient group (mean age = 62 yrs) contained more women than men (69% female). Symptom duration ranged from 11 to 110 months. Idiopathic dysgeusia was most common (n=19); however, dysgeusia was also related to upper respiratory infection (n=5), oral or dental procedures (n=5), head trauma (n=4), paranasal sinus disease (n=3), otologic surgery (e.g., secondary to chorda tympani disruption, n=2), cerebrovascular accident (n=2), periodontal disease (n=2), medication reaction (n=2), gastroesophageal reflux (n=1), radiation therapy (n=1), menopausal estrogen depletion (n=1), and toxic chemical exposure (n=1). Thirty patients (63%) reported experiencing complete or near complete resolution of dysgeusia on follow-up; 23 of these individuals (77%) reported spontaneous resolution, whereas seven reported resolution following medical intervention (e.g., treatment of periodontal and paranasal sinus disease and estrogen replacement therapy at menopause). Mean duration for spontaneous resolution was 10.4 months (+/-6.3; range 2 to 22 months). Interestingly, patients whose dysgeusia resolved had lower BDI scores on their initial visit to the Center than non-resolvers ($p < .05$), suggesting that resolution of the dysgeusia also helped to resolve the depression.

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Taste Intensity Performance of Edentulous Persons: Effects of Dentures and Dental Implants

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Conventional upper dentures cover the palate and may reduce the contribution of palatal receptors to taste experience. Inferences concerning the role of palatal receptors have been based on comparisons of taste tests with and without full upper dentures in place. However, removing the denture exposes not only the palate but other surfaces that are normally covered by the denture. A denture that does not occlude the palate can be installed if mounts are surgically implanted in the jaw. Stimuli reach the palate even when this denture is in place and its coverage of other oral surfaces is unchanged. To explore the taste effects of altered access of taste stimuli to the palate arising from these dental procedures, we studied 21 patients selected to receive implants. Each used full upper and lower dentures and had been edentulous for more than a year. Taste intensity judgments with and without conventional dentures in place were obtained for each of four taste substances at seven concentration levels by cross-modal matching. ANOVA of the judgments yielded highly significant main effects for stimulus quality and stimulus concentration but no significant differences between judgments with and without dentures. Findings from assessment 1 month after implant surgery are available for 7 patients. Effects due to stimulus strength and quality are significant but those reflecting differences between this and either of the two earlier assessments are not. Neither short or long term uncovering of the palate in edentulous denture wearers alters objective measures of taste intensity perception: Supported in part by VA Central Affairs, RR & D Grant #003

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Development Of A Reliable Method For Determination Of Secretory IgA In Saliva. IVANKA D. MILETIC, VOJISLAV D. MILETIC, and SUSAN S. SCHIFFMAN (Duke University, Durham, NC)

Immunoglobulin A (IgA) is the dominant immunoglobulin isotype on all mucosal surfaces where it acts as a first line of defense against microbial invasion. Recent investigations suggest that secretory IgA (sIgA) concentrations vary over the day due to a range of variables including mood and exercise. Saliva is the secretory fluid most commonly used for sIgA investigations, but, at present, there is no standard procedure for saliva collection and determination of IgA concentration. The main goal of this investigation was to develop a standard procedure for determination of sIgA concentrations that: 1) inhibits proteolytic enzymes normally present in high concentrations in saliva and 2) controls for salivary flow. Unstimulated saliva was collected three times during a two minute interval (t=0, t=1, t=2) in tubes containing the enzyme inhibitor cocktail (EDTA-EACA-benzamidine). Salivary flow was determined by weighing the tubes before and after saliva sampling. From aliquoted samples, IgA was determined by capture ELISA using an affinity purified polyclonal goat IgG anti-IgA, as a capture antibody adsorbed on the plate. The same antibody was used as a developing antibody after conjugation to peroxidase. As a standard, on each plate four different concentrations (11, 1.1, 0.1 and 0.01 ug/ml) of chromatographically purified secretory IgA were tested. Salivary samples were analyzed in triplicates and sIgA concentrations in ug/ml were converted to ug/min using pre-determined saliva flow.

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Determination Of Salivary IgA In Healthy People Of Different Age Groups. SUSAN S. SCHIFFMAN, VOJISLAV D. MILETIC, and IVANKA D. MILETIC (Duke University, Durham, NC).

In order to investigate the influence of daily mood, nutrition, disease, and other variables on secretory IgA concentration, it is necessary to establish reference values for healthy people in different age groups. Four groups of subjects participated in the study: young white subjects (19-36 years), young African-American subjects (19-35 years), elderly white subjects (68-79 years), and elderly African-American subjects (67-80 years). All young subjects were students and employees at Duke University. The white elderly subjects were residents of Methodist Retirement Home in Durham, NC. The elderly African-American subjects were residents of Durham Hosiery Mills Apartments in Durham, NC. Unstimulated saliva was collected into a tube containing the enzyme inhibitor cocktail (EDTA-EACA-benzamidine). Collections were made during a two minute interval twice daily at 7 am and 4 pm for seven consecutive days. At the same time, all subjects were asked to answer a questionnaire about daily mood, exercise, food intake, and food enjoyment. Salivary IgA was measured using capture ELISA. Younger subjects were found to have higher salivary IgA than elderly subjects independent of race. Differences between morning and afternoon levels of salivary IgA were significant in some but not all individuals.

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Cross-Cultural Differences & World-Wide Segmentation In Acceptance Of Flavors For Coffee - A Conceptual Approach. HOWARD R. MOSKOWITZ (Moskowitz Jacobs, Inc., White Plains, New York, USA)

Five hundred consumers (100 each from U.S., Germany, Italy, United Kingdom, Norway) rated their interest in different concepts for coffee. Components of these concepts were experimentally varied, to allow estimation of which elements promoted interest and which elements detracted from interest. Among these concept elements were a set of coffee flavors. A regression model revealed that many of these coffee flavors were not interesting to consumers. However, a segmentation of consumer responses revealed two major groups of consumers - experientials versus traditionalists. The experiential consumers were interested in several of the coffee flavors. The results suggest a segmentation of preferences (albeit conceptual in nature) which transcends cultures. These preference segments distribute in different ways across the five countries.

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Time intensity responses to single and multiple presentations of zingerone.
JOHN PRESCOTT and RICHARD J. STEVENSON (CSIRO Sensory Research Centre, Sydney, Australia)

There are few data on the psychophysical properties of zingerone, one of the primary pungent components in ginger, particularly in contrast to capsaicin. Green (Neurosci. Lett., 1993, 150: 44-48) has suggested that, unlike capsaicin, zingerone does not sensitize in response to multiple exposures within a session. Capsaicin sensitization is dependent upon the inter-stimulus interval (ISI) used. In order to estimate the optimal ISI for zingerone, its time intensity characteristics were examined using 12 frequent, 12 infrequent and 12 moderately frequent users of chili, who rated the intensity of whole mouth rinses of 1% zingerone at 6 sec intervals for 3 mins. Maximum intensity was reached within the first 18 secs while the zingerone was in the mouth. At this stage, there were no differences between groups. Following expectoration at 30 secs, frequent users showed a more rapid decline in rated intensity than did moderate users, who showed a more rapid decline than infrequent users. The fact that these differences emerged after maximum intensity was reached suggests that they were not due to contextual effects, but may represent different neural response to zingerone. It is unclear whether such differential responses result from long term effects of exposure to other pungent substances or pre-date such exposure. A second experiment examined whether desensitisation to zingerone could be demonstrated using ISIs based on the point of maximum intensity observed in the first study.

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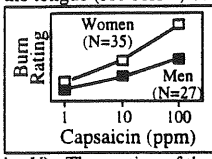
Women Perceive Greater Oral Burn from Capsaicin: Clinical Implications for Oral Pain. LINDA M. BARTOSHUK[1], VALERIE B. DUFFY[2], ANN BERGER[1], TRACY KARRER[3], and CLARENCE SASAKI[1] ([1]Yale University School of Medicine, [2]University of Connecticut, [3]International Flavors and Fragrances).

The ability to taste 6-*n*-propylthiouracil (PROP) shows genetic variation. Supertasters perceive intense bitterness, medium tasters, moderate bitterness, and nontasters, little or no bitterness from saturated PROP. The number of tastebuds in fungiform papillae varies with PROP status. Supertasters have the most tastebuds and perceive the most intense oral burn from capsaicin (chili peppers) possibly because these tastebuds are innervated by two nerves: the chorda tympani (VII: taste) and the trigeminal (V: touch, temperature, pain). We recently found that women are more likely than men to be supertasters. A reanalysis of data presented at AChemS 1992 (Karrer et al) shows that, on the average, capsaicin produced greater oral burn to women than to men on the tip of the tongue (see below) but not on the floor of the mouth (innervated only by V). The portion of the tongue innervated by VII and V extends from the foliate papillae at the extreme rear edge to the tip of the tongue (thus including the entire mobile tongue). We demonstrated that the higher burn experienced by supertasters in this area affects whole mouth experience with capsaicin by asking subjects (N=132) to rate (Natick 9-point scale) the bitterness of PROP paper (1" square filter paper containing 1.2 mg PROP) and the burn that resulted from consumption of candy containing 5-9 ppm capsaicin. A significant correlation resulted (Spearman Rho=.29, p<.001). Since the pain associated with oral lesions is produced primarily by neurons that respond to capsaicin, we predict that equivalent oral lesions will produce greater pain in supertasters (and thus disproportionately in women).



ANOVA: main effect of sex ($F(1,60)=10.932$, $p<.01$); interaction between sex and capsaicin concentration ($F(2,120)=5.570$, $p<.01$. T-tests: women gave significantly ($p<.05$) higher ratings of burn for 10 and 100 ppm capsaicin.

Research supported by NIH grant DC 00283 and the Yale University General Clinical Research Center.

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Astringency and Sourness of Buffered Acids. HARRY LAWLESS and PAUL GIASI (Department of Food Science, Cornell University, Ithaca, New York)

Acetic acid, lactic acid and citric acid solutions were adjusted to pH 3, 5, and 7 and evaluated for sourness, astringency and the astringent subqualities of drying, mouth roughing and puckery/drawing sensations. Twenty subjects made repeated category ratings of perceived intensity of all attributes at three time intervals, in quadruplicate. A strong pH-dependent effect was found for sourness, astringency and all astringent subqualities, with decreasing intensity for increasing pH. Acids were generally more intense in the order acetic > citric > lactic, although lactic acid was more drying and roughing in the mouth at pH 3. These results show the importance of a pH dependent effect of acids on astringency, implying that availability of H+ ions is as important or more important that anion effects. Althought the acids were generally similar, small differences in astringent subqualities are attributable to the effects of anions.

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Human Responses to Propionic Acid Vapor

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In order to proceed with efforts to integrate perceptual and respiratory measures of human response to odorants and irritants, it is necessary to examine inter-and intra-subject variability. In each of four 50-trial sessions, ten subjects (5 male, 5 female) were presented with clean air controls and a range of four logarithmically-spaced concentrations (0.08 to 80 ppm) of propionic acid, using an air-dilution olfactometer. This range of intensities was selected to extend from below the olfactory threshold to above the trigeminal threshold. Breathing data were collected in successive 15 sec periods just before and during stimulus presentation, after which the subjects entered ratings of odor strength (OS), nasal irritation (NI), annoyance and acceptability on unstructured line scales. Threshold estimates were computed for each session. These were used to examine session-to-session fluctuation in perceptual and respiratory responses for each subject and to assess the inter-subject variability in sensitivity. For OS, NI and total inhaled volume (TIV: total volume of air inhaled during odorant presentation) the within-subject range of thresholds averaged 0.20, 0.96 and 0.90 log units, respectively. Mean thresholds were computed for each subject, based on the four test sessions, and exhibited the following inter-subject spans: OS 0.09-0.23 ppm; NI 0.08-1.66 ppm; TIV 0.6-75.91 ppm. These results will be discussed in terms of the value of tidal volume as a measure of trigeminally mediated nasal irritation and the minimum amount of testing needed to characterize individual sensitivity to odorants or irritants.

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Antisense Oligonucleotides, Strategies and Successes: From the Dish to the Whole Organism. BARBARA R. TALAMO, Neuroscience Program, Tufts University School of Medicine, Boston, MA 02111

Macromolecules that appear to be important to particular biological functions have been identified by a variety of biochemical, molecular biology and immunological techniques. Proteins or messages that are localized to particular cells or tissues and that are regulated with a time course that suggests a critical role in development, differentiation or response of a system are good candidates for key players in the pathway of interest. However, to establish a functional role it is necessary to know whether the presence of that molecule is necessary or sufficient to support the process. A variety of techniques have been developed to interfere with expression or to overexpress the molecule of interest. These include genetic intervention through transgenic, transfection or gene knockout methods, and expression of dominant negative mutations that interfere with normal protein function. Strategies that utilize introduction of antisense oligonucleotides into cells in culture or into living organisms have been developed to try to interfere with expression of particular messages and hence with expression of the translated protein product in targeted locations. These methods have met with mixed success. Design of optimal approaches requires examination of how antisense oligonucleotides are efficiently taken up into cells and mechanisms by which they inhibit cellular processes, including the expected interference with mRNA translation as well as other unexpected actions. In this symposium, we will attempt to give an overview of approaches to optimizing interference with protein synthesis and confirming that the expected mRNA and protein have indeed been down-regulated as well as review some interesting systems where antisense oligonucleotide treatment has altered biological events. Specific and detailed examples will be presented for cell culture systems and for organisms, in which biological processes ranging from cellular behavior to whole animal behavior have been manipulated.

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Gene Inhibition Using Antisense Oligonucleotides

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Antisense oligonucleotides (ONs) have great promise as agents for the specific manipulation of gene expression. Until recently, nonspecific effects of ONs often confounded the interpretation of antisense studies. Improvements in ON chemistry and cellular delivery techniques now allow for more potent and specific gene inhibition. Recently, we described a potent class of antisense ONs which contained C-5 propynyl pyrimidines. The C-5 propyne substitution increased the binding affinity of an ON for RNA and correlated with improved antisense gene inhibition. Sequence- and gene-specific inhibition of a variety of genes, using RNA and protein analysis, show that this new class of ONs is far more versatile than previously described agents. Our data indicate that these agents allow one to turn off the translation of virtually any targeted gene in cell culture, thus re-introducing the antisense technique as a broadly applicable technology.

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Use Of Antisense Oligonucleotides In Neuronal Cell Culture

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Antisense technologies to suppress specific gene products are now a well-established means to gain useful insights regarding neuronal cell biology. We have administered antisense oligodeoxynucleotides to primary neuronal cultures from either the hippocampus or cerebellar primordium to identify molecules which are key to the formation of specific neuronal morphologies. The molecules we have targeted to date include tau, MAP2, kinesin heavy chain, synapsin II, and p53. A careful analysis of the phenotype after successful protein suppression can give information about the function of the suppressed protein. It is crucial to perform an extensive series of controls for any observed results to be meaningful, particularly because the specificity of the hybridization to a unique substrate may be at issue. These controls range from the use of a second non-overlapping antisense construct, which should replicate the results, to the rescue of antisense-treated cells by transfecting the suppressed gene product with a construct that contains substitutions in positions of codon redundancy at sites that are targeted by the antisense. In some cases other proteins will also be suppressed possibly because they form a macromolecular complex with protein targeted by the antisense. A useful complement to antisense strategies as applied to neuronal cell culture, is to determine whether cultures from knockout mice can replicate the phenotype observed when wild type neurons are treated with antisense to the targeted gene product.

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Direct Intrahypothalamic Applications of Antisense DNA and their Behavioral Effects.

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For the exploration of possible causal connections between molecular synthetic events and behavior, antisense DNA approaches are proving useful. These take advantage of the specificity of the genetic code to interfere with the function of the chosen messenger RNA's. In our experience behavioral effects are reversible. Furthermore, microinjections of small volumes into brain add site specificity. With 15-mers antisense to progesterone receptor messenger RNA we have significantly reduced lordosis behavior and achieved 80% reduction of courtship behaviors (Ogawa et. al. Journal of Neuroscience 1994). Likewise, with hypothalamic administration of antisense DNA against oxytocin receptor mRNA we reduced lordosis behavior under particular endocrine conditions (McCarthy et. al Neuroendocrinology 1994†). Internal controls using measures of food intake showed that reproductive behavior deficits were not due to damaged VMH neurons. In a surprising result, neonatal injections of estrogen receptor antisense DNA in the hypothalamus was able to reverse defeminizing effects of neonatal testosterone (McCarthy et. al. Endocrinology 1993), giving evidence that early antisense treatment could have behavioral effects enduring until adulthood. In methodologic experiments we see that oligos can be taken up rapidly in neurons and can survive for more than 5 hours. Alternate strategies and adequate control conditions will be discussed.

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A Mechanism for Signal Transduction in Bitter Taste.

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It is likely that a number of transduction mechanisms exist for bitter taste, one of which involves production of the inositol polyphosphate second messengers. Using the stimuli sucrose octaacetate (SOA) and denatonium benzoate (DB), and tissue derived from two strains of mice (both of which can taste DB, but only one of which can taste SOA),we have shown that the second messenger, inositol 1,4,5-trisphosphate (IP_3), is produced within a time frame relevant to taste transduction. Using quench-flow, DB (100 μM) induced a transient and rapid increase in IP_3, with maximal production near 75 msec after stimulation in both strains of mice. SOA (100 μM) brought about a similar increase in IP_3 only in SOA-taster mice. The response was potentiated by GTP and its analogs. IP_3 production in control tissue devoid of taste buds was not significant when the preparation was stimulated by either SOA or DB. Pretreatment of the taste tissue with pertussis toxin eliminated stimulus responses, whereas pretreatment with cholera toxin was without effect. Western blots of solubilized taste tissue probed with antibodies to the α-subunit of several G-proteins revealed bands reactive to antibodies against $G\alpha_{i1-2}$ and $G\alpha_o$, with no observable activity to antibodies against $G\alpha_{i3}$. It is proposed that the transduction of the bitter tastes of SOA and DB involves a receptor-mediated activation of a G_i-type protein which activates a phospholipase C to produce the two second messengers, IP_3 and diacylglycerol.

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Studies of the Taste-Cell Specific G-Protein α-Gustducin Gene in Transgenic and Knock-out Mice

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α-Gustducin, originally cloned from a rat taste cDNA library, is a member of the G-protein family which is highly homologous to both rod and cone transducin, and is uniquely expressed in taste buds of all taste papillae. The specificity of its gene expression pattern and its homoldgy to the transducins suggest that gustducin plays a role in taste transduction, in a manner similar to that of transducins in the visual system. We are using the transgenic mouse system to study the gustducin gene in two ways. To study the function of α-gustducin in taste cells, we have created a deletion in the α-gustducin gene in transgenic mice by homologous recombination. Homozygous knock-out mice have been obtained, and these mice are viable, appearing healthy. These mice are being analyzed for deficiency of gustducin expression, and functionally tested for behavioral abnormalities in taste perception using taste preference/aversion tests for sweet and bitter compounds. In these ways we hope to determine gustducin's specific role in taste transduction. To determine the requirements for taste-cell specific gene expression, we are creating transgenic mice with lacZ fusion genes containing sequences derived from the α-gustducin locus. Using DNA sequences from the 5'-end of the gene we have constructed a lacZ reporter gene containing 8.5 kb from the 5'-end of the α-gustducin gene. Initial experiments indicate that taste-cell specific lacZ expression is observed in these transgenic mice. These experiments are ongoing to clearly define the cis-acting sequences required for taste-cell specific expression.

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Paracellular Junction Potentials and Responses to Impermeant Cations and Potassium Salts in the Hamster *Chorda Tympani*.

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The **Diffusion Potential Model** of salt taste transduction (ISOT IX) proposes that the nerve response is determined by two variable potentials: the resting potential of cation-selective taste cell receptor membranes; and a liquid junction potential arising at the paracellular junctions, where the different electrolyte solutions of the interstitial fluid and the taste pore contents (that is, the stimulus) come in contact. In this model, excitatory current flows in through receptor membranes, spreads through cell interiors and out through lateral cell membranes, and returns to receptor membranes by way of the interstitial fluid of the taste bud, the paracellular junctions, and the taste pore. This electrical geometry places the two variable potentials in series. Previously (AChemS XIII), it was reported that the different responses to a fixed activity of Na^+ ions, paired with different anions (the "anion effect"), is a linear function of the liquid junction potentials, as the model predicts. The reciprocal slope of this function gives the voltage sensitivity of the response. It was also shown (AChemS XVI) that the difference in responses to NaCl and Na_2SO_4, throughout their entire concentration-response ranges, is given by the difference in their liquid junction potentials, scaled by the voltage sensitivity of the response. Further (AChemS XI), responses to a variety of impermeant cations (whose transduction mechanism is otherwise mysterious) are a linear function of their junction potentials. Here it is shown that the voltage sensitivity from this function, which is less than for the Na^+ salts, successfully scales the difference in responses to KCl and K_2SO_4. Taken together, these results suggest that excitatory currents from sodium salts have access to all ion-permeable receptor membranes in the taste bud, while currents from other salts are restricted to membranes permeable to K^+-like ions.

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Electrophysiological Support for Two Bitter Transduction Mechanisms within the Same Taste Receptor of an Insect

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Compared with other taste modalities, bitter taste is elicited by a large and structurally diverse range of compounds. To accommodate this diversity, it has been hypothesized that bitter taste is transduced by a multitude of specific mechanisms. Here, I provide direct experimental support for this hypothesis, using an insect model, *Manduca sexta* caterpillars, and two naturally occurring compounds, caffeine and aristolochic acid, which taste bitter to humans and elicit taste-rejection in many insects. In *M. sexta*, taste-rejection of these compounds is mediated by the stimulation of 4-6 taste receptor cells. I focused on one pair of these cells, the so-called deterrent receptors within the lateral sensilla styloconica. In the first experiment, I determined whether caffeine and aristolochic acid stimulate the same deterrent receptor, using the tip recording method. Because neural responses often contained spikes from several taste receptor neurons located within the same sensillum, I used spike classification procedures to identify deterrent receptor spikes. My experimental protocol involved stimulating a lateral sensillum with a mixture of caffeine and aristolochic acid at their respective R_{50} concentrations (i.e. that which caused the deterrent receptor to fire at 50% of its maximal rate). The mixture caused the deterrent receptor to discharge at a rate that was roughly twice that for each compound alone. In the second experiment, I used two approaches to determine whether caffeine and aristolochic acid act on different transduction mechanisms within the same deterrent receptor. First, I compared the temporal pattern of spikes elicited by the two compounds. There was a consistent 10-20 msec delay in the onset of maximal firing rate for aristolochic acid, but not for caffeine, suggesting the activation of different transduction mechanisms. The second approach examined the effects of 2 days of dietary exposure to a supra-threshold concentration of caffeine or aristolochic acid on the responsiveness of the deterrent receptor to both compounds. Exposure to the caffeine diet desensitized the deterrent receptor to caffeine, but not to aristolochic acid. In contrast, exposure to the aristolochic acid diet failed to desensitize the deterrent receptor to either compound. Taken together, these data suggest that caffeine and aristolochic acid act on different transduction mechanisms within the same taste receptor, and that the responsiveness of each mechanism can be modulated independently of the other. Supported by NIH DC02416

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Gustducin and Transducin couple Reconstituted Bitter Receptor to a Taste Tissue Phosphodiesterase

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The rod and cone transducins are specific G proteins originally thought to be present only in photoreceptor cells of the vertebrate retina. Transducins convert light stimulation of photoreceptor opsins into activation of cGMP phosphodiesterase. Gustducin is a transducin-like G protein that we identified and cloned from rat taste cells (McLaughlin et al., 1992). We have found that rod transducin is also present in vertebrate taste cells. We have isolated a novel phosphodiesterase from taste tissue that is activated by gustducin and transducin. We have also reconstituted a taste cell membrane fraction which in the presence of the bitter compound denatonium activates transducin. This taste receptor fraction also activates gustducin but not G_i. A peptide that competitively inhibits rhodopsin activation of transducin also blocks taste membrane activation of transducin, arguing for involvement of a seven transmembrane G protein-coupled receptor. The presence in taste cells of gustducin, rod transducin, a bitter responsive PDE and a bitter receptor that activates transducin and gustducin implies that transducin and gustducin may play similar roles in taste transduction; i.e. to couple bitter receptor activation to PDE activation. Additional support for this proposal comes from the discovery of a taste cell cyclic nucleotide regulated channel that may serve as the end target of this pathway (Kolesnikov and Margolskee, in press) and from transgenic mice deficient for gustducin expression (Wong and Margolskee, these abstracts). Further characterization of this taste receptor fraction is also presented (Ruiz-Avila and Margolskee, these abstracts).

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Hyperinnervation of Circumvallate Papillae in Keratin-14-NGF Transgenic Mice.

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We examined the innervation of the circumvallate papillae of transgenic mice in which nerve growth factor (NGF) expression was driven by a keratin 14 (K14) promoter. The aims of the current study were to localize K14 and NGF proteins in the tongue and to examine the density of protein gene product (PGP) 9.5- and tyrosine hydroxylase (TH)-expressing nerve fibers innervating circumvallate papillae by using immunocytochemical techniques. NGF immunoreactivity was localized in the K14-expressing basal cells in transgenic mice but not in controls. The density of PGP9.5-immunoreactive nerve fibers in the circumvallate papillae of the transgenic mice was substantially increased when compared with age-matched nontransgenic controls. The lamina propria of the adjacent non-taste epithelium of the transgenics received substantially increased innervation of PGP 9.5 nerve fibers as well. In contrast to a sparse distribution of TH-immunoreactive fibers in the circumvallate papillae of nontransgenic controls, transgenic mice contained thick subgemmal TH-immunoreactive fiber bundles and fine intragemmal fibers that projected into the taste bud *per se*. There were an average 2.4 TH-immunoreactive fibers per taste bud of the transgenics. These results indicate that target-derived NGF in transgenic mice exerts a neurotrophic effect on the innervation of circumvallate papillae.

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Subcellular Fractionation and Biochemical Characterization of Bovine Lingual Epithelium.

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Sweet and bitter taste chemoreception may function through modulation of the intracellular second messengers cAMP and IP_3 resulting in changes in receptor cell conductance and transmission of nerve impulses to the CNS. Since second messenger concentrations are often regulated by G proteins and their respective receptors, it has been hypothesized that sweet and bitter taste reception are mediated by G protein-coupled receptors. To test this hypothesis we have initiated a biochemical characterization of bovine lingual epithelial tissue. Membranes from striated muscle (SM), fungiform (FF), and circumvallate papillae (CV) were isolated by differential centrifugation and sucrose density gradients. Subcellular fractions were analyzed for various enzymatic activities, for specific immunoreactivity to anti-G protein antibodies, and for [γ-^{35}S]GTPγS binding in the presence and absence of various drugs and sweeteners. A CV membrane fraction was identified by its enrichment in 5'nucloetidase and adenylyl cyclase enzymatic activities. This fraction contained G_{α_s} as judged by positive immunoblot analysis and increased specific binding of [γ-^{35}S]GTPγS. The effects of Mg^{+2}, NaCl, GDP, and boiling on the binding of [γ-^{35}S] GTPγS were consistent with a G protein-mediated effect in this tissue. [γ-^{35}S]GTPγS binding to FF and CV membranes increased at millimolar concentrations but was inhibited at high concentrations (molar) of sucrose, maltose, and lactose. SM membrane preparations showed no effect of these sugars on [γ-^{35}S]GTPγS binding. Moreover, the various sugars tested showed differential concentration-dependent activation of [γ-^{35}S]GTPγS binding, suggesting a specific receptor interaction, and not an osmotic effect. These initial biochemical data are consistent with the hypothesis that G proteins and G protein-coupled receptors mediate sweet taste signal transduction.

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Taste-Responsive Neurons of the Chorda Tympani Nerve of the Pig.

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Responses of 29 single fibers of the chorda tympani (CT) nerve of the pig were recorded to taste stimulation with an array of 31 compounds. Stimuli included NaCl and LiCl (presented with/out amiloride), KCL, citric and ascorbic acids, QHCl, caffeine, sucrose octaacetate and denatonium benzoate. A group of umami compounds: monosodium glutamate (MSG) (alone or mixed with disodium 5'-guanylate (GMP) or disodium 5'-inosinate (IMP)) were presented. Sweet taste was addressed by the group of carbohydrates: sucrose, fructose, galactose, D-glucose, maltose, lactose. Other sweeteners were saccharine (with/out thaumatin), acesulfame-K, xylitol, D-tryptophane, stevioside, glycine and NHDHC. Based on responses to the four basic gustatory stimuli, single fibers were classified into 3 NaCl-best, 9 acid-best, 7 QHCl-best and 10 sucrose-best fibers. The average breadth of tuning for all fibers was 0.63+/-0.23, which shows that the pig CT fibers are tuned more broadly than CT fibers of other studied species. The hierarchical cluster analysis of the fibers' response profiles suggested five major clusters of fibers, characterized by predominant sensitivity to NaCl, MSG, sucrose, QHCl and citric acid.The comparison of the results from the pig CT fibers with data from the CT fibers of primates and other mammals shows that the taste world of the pig is quite different and some of the taste submodalities in this world may have to be redefined.

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Bilateral olfactory detection thresholds are lower than unilateral olfactory detection thresholds

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Performance on suprathreshold tests of olfactory function, including tests of odor intensity and memory, is greater under bilateral than under unilateral stimulation conditions. In this study, we determined detection thresholds for the odorant phenyl ethyl alcohol both bilaterally and unilaterally in 164 subjects stratified into four groups according to their performance on the University of Pennsylvania Smell Identification Test (UPSIT). On average, lower thresholds were observed under bilateral than under unilateral test conditions. This phenomenon was present for all four groups of subjects, and no meaningful sex or laterality differences were observed. These data suggest that central integration of olfactory processing occurs even at the threshold level. The findings are discussed in relation to a hypothesis that differential stimulation of the two sides of the nose alters overall odor intensity in a manner that provides information of use in chemotaxis.

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Development of Normative Data for the Modular Smell Identification Test. AVRON MARCUS and RICHARD L. DOTY (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania Medical Center, Philadelphia, PA USA)

The 40-item University of Pennsylvania Smell Identification Test (UPSIT) has become a popular tool for assessing olfactory function in both basic and applied test settings. Indeed, this test is now used in thousands of clinics in North America. In this study, we administered Booklet #1 of the Modular Smell Identification Test (MODSIT), a test derived from 12 internationally-recognized items of the the UPSIT, to several hundred subjects representing both sexes and a wide range of ages. Test scores were correlated to the same 12 test items obtained from age-, gender-, and race-matched subjects within a large UPSIT database to establish comparability. Since a high correlation was present between the MODSIT and these UPSIT fractions, we subsequently developed age- and sex-specific percentile norms for the application of the MODSIT. Although this test is not as sensitive as the UPSIT to subtle alterations in olfactory function, it is more rapidly administered and is particularly useful in settings in which administration time is limited, as in survey situations.

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Areas of Cortical Olfactory Activity in Man Identified with Event-Related Magentic Fields

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Among modern techniques of functional imaging of brain activity event-related magnetic fields (ERMF) are most suited to detect areas of cortical activity occurring in the time range of several hundred milliseconds after stimulus onset. Other techniques such as the positron emission tomography (PET) are characterized by comparably a bad time resolution in the range of many seconds to one minute. In order to detect the early activity mainly determined by the sensory characteristics of olfactory stimuli we used a new magnetoencephalographic system with 122 channels. The sensors were arranged in a way that recordings could be made from both hemispheres at the same time.
Six healthy volunteers (3 male and 3 female subjects; 26 to 51 years) participated in the experiments. Stimulants (phenyl ethyl alcohol; hydrogen sulfide) were presented with a stimulus duration of 200 ms and an interstimulus interval of 40 s. MEG was recorded with a 122 channel superconducting gradiometer (NEUROMAG) in a magnetically shielded chamber. In order to define time relations between magnetic and electric components of the responses olfactory event-related potentials (OERPs) were recorded at Cz referenced against A1. Records containing eye blinks were discarded from the average.
In accordance with previous findings data analysis indicated that the source of the OERMFs could be localized in the temporal lobe. We found that the activity responsible for the P2-peak in the olfactory event-related potential was located in the medial temporal gyrus while the N1-peak was generated in the temporo-polar area. As in our previous experiments we were unable detect neuronal activity in the fronto-orbital cortex within the time window of 1600 ms following stimulus onset. There were slight differences in the quality of source localizations depending on which hemisphere was analyzed and which nostril was stimulated.

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Emotion Experienced During Encoding Enhances Odor Retrieval Cue Efficacy. RACHEL S. HERZ[1] and SHARON B. ZEITLIN[2] (Monell Chemical Senses Center[1], Philadelphia PA, University of Toronto[2], Toronto Canada)

A theory was proposed that odor-associated autobiographical memories are formed when a novel odor is first encountered in an emotionally distinctive context. To test this theory the present research examined whether a heightened emotional state experienced during the encoding of information associated to an unfamiliar odor would enhance the efficacy of that odor as a retrieval cue. Three experiments were conducted in which an unfamiliar ambient odor (osmanthus 20% or violet leaf 10%) was either present during both the incidental learning encoding session for a list of neutral nouns and at the free recall retrieval session, or never present, and subjects were either in an emotionally heightened mood state during the encoding session or in a neutral mood. In experiment 1, a happy mood was induced using a continuous music technique. In experiment 2, an anxious mood was induced using a speech threat manipulation. In experiment 3, subjects who were naturally anxious prior to a mid-term exam were compared with subjects experiencing a regular class day. The results from experiments 1 and 2 revealed that word recall was higher when the same ambient odor was present at both encoding and retrieval than when no ambient odor cue was available. The experience of a heightened mood state during encoding did not further enhance recall, although in experiment 2 a trend for higher recall among anxious subjects was observed. The results of experiment 3, however, revealed that in addition to the facilitating cue effect of odor presence at both encoding and retrieval, subjects who were in a naturally anxious mood at encoding recalled significantly more words than subjects who were in a neutral mood at encoding. In sum, the present results indicate that a salient emotional state experienced during the encoding of information associated to an odor will augment the retrieval properties of that odor. This finding supports the theory that emotional context is a key component in the formation of odor-associated memory.

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A Rose by Any Other Name Does Not Smell as Sweet

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Earlier theories of olfactory memory for humans suggested that verbal information had little effect on retention of olfactory material. More recently, several researchers have provided evidence that semantic information about odors can enhance memory for them. The purpose of this present investigation was to seek evidence for the ability of semantic information to impair memory for odors. Following Cain & Potts (1992), it was found that semantic information can interfere with recognition of odors in certain conditions. A library of 20 pairs of odorants was compiled, with the constraint that each member of a pair had a similar, but distinct scent. The stimuli used were common household odorants. Examples of stimulus pairs are: cinnamon-nutmeg, spearmint-wintergreen, molasses-soy sauce. The basic experimental procedure was that in all conditions, subjects smelled one odor from each of the 20 pairs. Forty-eight hours later, subjects participated in a forced-choice recognition test, where they were to smell both members of the pairs and choose the odor which had been smelled previously. Level of semantic information was varied by providing the subjects with odor names. For 10 of the odors, this name was the veridical name of the odor (e.g., spearmint was called spearmint) and for the remaining 10 the name was that of the other member of the pair (e.g., cinnamon was called nutmeg). In the different conditions, the name was present at both acquisition and recognition, only acquisition, only recognition, or neither acquisition or recognition. The results indicated that subjects correctly recognized a higher percentage of the odors when given the veridical name (78% correct) than when given the given the name of the close associate (54% correct; baseline no-name performance was at 69% correct). In addition, performance was poorer when the incorrect name was given at acquisition compared to when given at recognition and best when the correct name was available at both occasions (all p's $< .05$). These results suggest that available semantic information plays a dominant role in the encoding and memory of odors. Implications for theories of memory for the chemical sense will be discussed.

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Task and EEG Alpha Effects on Olfactory Event Related Potentials. CARLO QUIÑONEZ[1], CHARLIE D. MORGAN[1], JAMES W. COVINGTON[1], DENNARD ELLISON[1,2], DERIN WESTER[3], STEVEN NORDIN[1,2], JOHN M. POLICH[4] and CLAIRE MURPHY[1,2] (San Diego State University[1], UCSD Medical Center[2], Naval Medical Center[3], San Diego, The Scripps Research Institute[4], San Diego)

The goal of this study was to identify sources of variability that influence olfactory event related potentials (OERPs) in normal individuals to facilitate development of optimal stimulating and recording protocols for application to individuals with olfactory dysfunction. An important consideration in this context is the effect of background EEG alpha activity, since it has been reported that this factor can influence OERP results. To assess these effects, three experimental conditions designed to manipulate the amount of alpha activity were employed during OERP recordings. In the first condition, subjects sat with the eyes closed; in the second condition subjects sat with the eyes open; in the third condition, subjects performed a tracking task in which a joystick was used to follow a randomly moving square across a computer screen. OERPs were elicited using amyl acetate stimuli (200 msec duration) presented with an inter-stimulus interval of 60 seconds from young adults and elderly subjects in two age groups: 21-36 yrs and 61-76 years. All subjects had complete ENT examinations to rule out the presence of nasal sinus disease. A battery of neuropsychological tests was administered to screen for dementia. OERP activity was recorded from the Fz, Cz and Pz midline electrode sites, referenced to linked earlobes, with a forehead ground, using an amplifier bandpass of 0.1 to 30 Hz (12 dB rolloff). Amplitude of the N1, P2 and N2 OERP components was found to be more variable in the eyes closed compared to the eyes open or tracking conditions, and young subjects demonstrated larger amplitudes compared to elderly subjects. Component latencies did not differ between experimental conditions or subject groups. These findings suggest that alpha does not contribute in a major fashion to the primary dependent measure of the OERP. The results will be discussed in terms of the clinical utility of OERP.

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The Influence of Perceptual Similarity on Cross-Adaptation in Urinous-Note Compounds. JOHN D. PIERCE, JR.[1], JONATHAN B. WEBB[1], RICHARD M. BODEN[2], & CHARLES J. WYSOCKI[1] ([1]Monell Chemical Senses Center, Philadelphia, PA and [2]International Flavors & Fragrances, Union Beach, NJ).

Cross-adaptation, a potential measure of the degree to which odors share common sensory channels, is affected by perceptual similarity. However, the precise relationship remains obscure. The present study was designed to assess how perceptual similarity affects cross-adaptation among urinous-note odorants. Androstenone (5α-androst-16-en-3-one) was tested for cross-adaptation with five other compounds (aldron, bacdanol, cassis ether, sandiff, and timberone) that share a urinous note with androstenone, but differ in their overall perceptual qualities. Subjects, in each of 10 sessions, provided magnitude estimates for two test odorants (androstenone and one of the other compounds) prior to, during, and following adaptation to an adapting odorant (either androstenone in 5 sessions, or one of the other compounds). Whereas all compounds showed significant self-adaptation, cross-adaptation was observed in only one pairing: Magnitude estimates for androstenone were decreased following adaptation to aldron. Cross-adaptation was asymmetric; adaptation to androstenone did not significantly affect the perceived intensity of aldron. In similarity ratings, aldron was judged significantly more similar to androstenone than were the other compounds. These results suggest that, with respect to perceptual similarity, simply sharing a common note is insufficient to produce cross-adaptation, provided the compounds retain unique perceptual characteristics. Rather, when odorants share perceptual notes, cross-adaptation is most evident among compounds that share all or many of their perceptual characteristics rather than a single trait.

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Dependency of Odor Discrimination upon Controlled Familiarization

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The role of controlled familiarization in the discrimination of odors was investigated. Thirty simple aliphatic and aromatic compounds were grouped in ten pairs of identical odors and ten pairs of slightly dissimilar ones. There were 110 subjects in the study. During a discrimination trial, they had to determine whether or not the second odor in a pair was identical in terms of quality to that presented first. The delay between successive presentations of two odors was 20 sec. A resting pause of 2 min was managed between odor-pair presentations. The subjects were distributed across eleven groups that differed by the number of familiarization sessions; none, one, two or three, taking place prior to the discrimination trials. A delay of 24 hours elapsed between each familiarization session and between familiarization and the discrimination test. The familiarized groups differed according to whether subjects were familiarized with either the first odor (target) or the second odor (distractor) or both. Pairs of identical odors showed no significant influence of familiarization on scores of correct recognitions (hits). However, a slight tendency for these scores to increase was found with increased number of sessions. For pairs of slighlty dissimilar odors, the most salient finding was a marked decrease in incorrect recognitions (false alarms) with longer familiarization. This result evidenced a positive effect of familiarization upon discrimination of odors of low discriminability. Moreover, these judgments did not depend on intensity. False alarm scores did not vary as to whether subjects were familiarized with the target or the distractor or both. The enhancement of subjects' discrimination abilities was ascribed to the interactions between two kinds of memory processes: A long-term memory of the familiarized odor when it was delivered again during testing and short-term memory of the non-familiarized odor. A positive correlation was found between discrimination performances and the number of odors which were correctly remembered as being presented during familiarization. All together, these results support the idea that familiarization induced a consolidation process of the memory trace of the familiarized odors.

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Pemenone and Androstenone Do Not Cross-Adapt Reciprocally. DAVID A. STEVENS[1] and ROBERT J. O'CONNELL[1,2] (Clark University[1], Worcester, MA and Worcester Foundation for Experimental Biology[2], Shrewsbury, MA)

Earlier we had shown that pemenone (PEM) and androstenone (AND) share a number of common perceptual characteristics. Among these are similarities in their odor quality, intensity ratings, and the fact that PEM is an efficient cross-adaptor of a subject's AND sensitivity. Here we evaluate the ability of AND to adapt the perceived intensity and quality of AND, PEM, and iso-valeric acid (IVA). Twenty four people including both those osmic and allosmic (n = 11) for the putrid odor quality of PEM were tested. Following training in odor quality and intensity rating techniques, adaptation testing began. Subjects were adapted for 2-min with a selected substance and then generated quality and intensity ratings for the 3 test stimuli. Two-way ANOVAs, done separately for the three test odorants (PEM, IVA and AND), were used to analyze the effects of adapting condition (blank vs self vs AND), and osmicity for PEM (osmic vs allosmic subjects). These analyses indicated that PEM-osmic people generally reported PEM and AND to be more intense than did PEM-allosmics, as expected. There was no difference in the intensity reports elicited by IVA in these subjects. There was significant self-adaptation of PEM and IVA in all subjects, but cross-adaptation by AND was not observed. There was significant self-adaptation of AND, but only in PEM-osmic subjects. This apparently was due to a "floor" effect; e.g. the allosmic subjects were relatively insensitive to AND and accordingly, the desensitization expected for AND self-adaptation was not detected. Collectively, these results contrast with those of our earlier study in which PEM was an efficient cross-adaptor of AND. Here, AND was no more efficient than the control material as an adapting substance for PEM, despite significant self-adaptation of PEM by itself. This lack of reciprocity in the effectiveness of PEM and AND as cross-adaptors is not related to differences in odor intensity, as the PEM and AND concentrations were adjusted, for each subject, to elicit comparable intensity reports. These results provide additional support for the notion that PEM, AND, and IVA share certain perceptual characteristics, but interact differentially with the several perceptual channels that are thought to give rise to the putrid odor quality.

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Odors Significantly Improve the Retrievability of Labels and Words

JODI L. DAY and RICHARD L. METZGER (The University of Tennessee at Chattanooga)

The authors gratefully acknowledge the support of the William H. Wheeler Center for Odor Research for the funding of this study.

In the past, several studies have been conducted to determine if odor memory is improved by providing the corresponding label. Little research has been done to find if memory for labels and words is improved by adding an odorant. The study presented here extends our consideration of odor memory by examining the retrievability of label only and word only conditions as compared to conditions in which an odor is presented with the label or the word. It was hypothesized that significant improvement would be seen in retrievability in the latter condition in which an odorant was presented with the initial presentation of the word or label. We first established the equivalence word and label lists; each word was matched to a label having the same word length and word frequency. We then determined that the lists were equally memorable. Subjects were randomly assigned to two testing conditions. In the first condition, subjects were initially presented with 30 labels paired with odorants matching the label, for example, the subject would hear the label rose and would then smell the odorant rose. The subjects were then tested on their ability to retrieve these 30 labels from memory by asking them to recognize these labels as ones they had already smelled or heard when presented with 60 odorants containing the initial 30. In the second testing condition, subjects were presented with 30 non-odor words presented with 30 odorants, for example the subject would be presented with the word steam and would then smell a lemon odorant. The subjects were then tested on their ability to retrieve the 30 words from memory by asking them to recognize the words from a list of 60 non-odor words containing the initial 30. As hypothesized, marginal improvement ($p < .08$) was seen in the subjects' ability to retrieve the non-odor words when paired with an odorant as compared to the word only. The labels combined with the odor were significantly better retrieved than the labels presented alone ($p < .05$) and were significantly better retrieved that the words paired with odors ($p < .05$). This study demonstrated that the retrievability of labels and words is improved by adding odorants to the encoding process. Further, we have shown that this improvement is not due to differences in the word and label lists, but instead to the odorants.

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Theoretical model of processing, recognition and discrimination of complex odors.

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A concept of odors as multidimensional vectors was used to understand phenomena of recognition and discrimination of complex odors. The model is based on the idea that an olfactory system consists of a number of independent information channels with peripheral receptors of various chemical specificity. Dimensionality of the natural frame for vectors representing odors corresponds to a number of independent olfactory channels available, while coordinates of a vector are signal intensities in each channel. It was shown that if odor intensity - concentration relationship can be described by a power law, such as Steven's law, the direction of a unit vector in a natural reference frame does not depend on concentration and therefore can serve as a unique descriptor for odor quality. As real systems always evaluate intensity with some error a real image of an odor includes all vectors whose directions are within some certain angle from the statistical mean determined during learning. This angle was called a dispersion angle. Our model assumes that if a measured angle between unit vectors is less than an established dispersion angle then the brain considers the two odors to be identical. Thus, for the purpose of analytical studies, a real olfactory image was considered as a multidimensional cone. The direction of the axis of the cone describes the odor quality, while a planar angle at the vertex reflects analytical capabilities. If an angle between two different olfactory images is less then a dispersion angle there is always a probability that a subject would perceive them as identical. In this study we were looking for functional relationships between this probability, dimensionality of the frame, angle between images, and dispersion angle. This function was found by considering common volume of overlapping multidimensional cones. With the function obtained many experimental facts concerning recognition of individual odors, complex mixtures and failures to isolate individual compounds responsible for social and individual odors can be explained. We also suggest an experimental paradigm estimating the number of olfactory channels involved in the discrimination of particular odor pairs.

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Orthonasal and retronasal identification of common substances presented as vapor phase stimuli. J. PIERCE AND B. P. HALPERN (Cornell University, Ithaca NY 14853-7601).

Subjects were taught to identify by assigned number each of four different common substances such as garlic powder, ground coffee, minced chocolate, oregano, or bar soap, when presented as vapor phase stimuli with the mouth closed. Following training, testing was done for ability to correctly identify by assigned number the four stimuli to a criterion of ≤ 1 identification error on each of 2 successive random order vapor phase presentations of the four stimuli 3 times each. Subjects who met this criterion for adequate training were tested with the 4 stimuli 3 times each in random order, 1st with the stimuli presented to the nares that differed from the nares used in training, and 2nd, to the nares that corresponded to the nares used in training. Breathing: Experiment 1: subjects breathed as they wished. Experiments 2 and 3: subjects were taught a form of diaphragmatic exhalation for use during retronasal presentations. **RESULTS**: Experiment 1: After orthonasal training, 13 of 15 subjects met the criterion with orthonasal presentations. Upon retronasal testing, median correct (mdn) = 10, semi-interquartile range (SIR) = 1.4; orthonasal testing, mdn = 12, SIR = 0; Wilcoxon Signed Rank $p = 0.0034$. The same subjects were next trained retronasally. 9 of the 13 subjects met the retronasal criterion; orthonasal testing, mdn = 12, SIR = 0.5, retronasal testing, mdn = 11, SIR = 1.0; $p = 0.038$. Experiment 2: 8 Subjects were trained retronasally and met the criterion; orthonasal testing, mdn = 12, SIR = 0; retronasal testing, mdn = 11.5, SIR = 2.0; $p = 0.066$. Experiment 3: After orthonasal training, all 7 subjects met the orthonasal criterion; retronasal testing, mdn = 10, SIR = 3.75; orthonasal testing, mdn = 12, SIR = 0.625. $p = 0.042$. The same subjects were next trained retronasally, 6 of the 7 subjects met the retronasal criterion; orthonasal testing, mdn = 12, SIR = 1.0; retronasal testing, mdn = 12, SIR = 3.0. $p = 0.655$. **CONCLUSIONS**: a) Orthonasal training permits correct retronasal identifications; retronasal, correct orthonasal. b) Retronasal identifications are less often correct, and are improved by adopting a form of diaphragmatic exhalation.

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Influence of ascending and decending trial presentations and the role of feedback on the odor detection threshold

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A popular means for assessing olfactory function is to measure the lowest odorant concentration detectable to a subject; i.e., the so-called detection threshold. In general, methods which employ ascending presentations of odorant concentrations are most popular, since they are assumed to induce less adaptation than procedures which employ descending presentations or random presentations of a wide range of stimulus concentrations. However, empirical evidence for the the superiority of ascending over descending stimulus presentations in threshold studies is limited and there is some suggestion from experiments employing staircase techniques that correct perithreshold responses occur primarily after long descending trial runs. In this study, we explored, in 128 subjects, the influences of ascending, descending, and randomized trial sequences on the detection threshold for the odorant phenyl ethyl alcohol. Trial sequences with equivalent numbers of odorants and blanks at each odorant concentration level were provided. To determine whether implicit feedback differentially influences thresholds determined from ascending and descending concentration sequences, subjects were tested under two conditions: (i) an "implicit" feedback condition where no information as to the subject's correctness of responding was provided by the experimenter; and (ii) an "explicit" feedback condition where the experimenter provided such information on all trials. The results of this study, which is in its final stages of completion, will be presented and discussed in relation to modern methods of testing olfaction in humans.

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Effect of Odors from Livestock Wastes on the Mood of Neighboring Residents. SUSAN S. SCHIFFMAN, ELIZABETH A. SATTELY-MILLER; MARK S. SUGGS, BREVICK G. GRAHAM (Duke University, Durham, NC).

The effect of environmental odors emanating from large-scale hog operations on the mood of nearby residents was determined using the POMS (Profile of Mood States). The scores for six POMS factors and the TMD (total mood disturbance score) for forty-four experimental subjects were compared to those of forty-four control subjects who were matched according to gender, race, age, and years of education. The results indicated a significant difference between control and experimental subjects for all six POMS factors and the TMD. Persons living near the intensive swine operations who experienced the odors reported significantly more tension, more depression, more anger, less vigor, more fatigue, and more confusion than control subjects as measured by the POMS. Persons exposed to the odors also had more total mood disturbance than controls as determined by their ratings on the POMS. Both innate physiological responses and learned responses may play a role in the impairment of mood found here.

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Implicit Memory of Odor After Being Exposed 16 Months Previously

TAKAFUMI TERASAWA, SAHO AYABE-KANAMURA (Inst. of Psychol., Univ. of Tsukuba, Japan), and SACHIKO SAITO (National Inst. of Bioscience and Human Technology, Japan)

It has been believed that memory of a minor experience could never exist for a long time. However, adopting Indirect Recognition Test (Terasawa & Ohta, 1993) and using words, we have detected some systematic effects that indicate the subject has retained part of an experience that had occurred several months earlier. Because the method is applicable for all the kinds of stimulus and, theoretically, odor stimulus is more suitable to replicate such a phenomenon, we have conducted an experiment using odor stimuli with a 16-month interval. Subjects attended two sessions with a 16-month interval. In the first session they smelled chemical odor one or three times during 2-weeks. In the second session, they were requested to learn a list of odors and accomplish recognition test about the list. The targets and distracters in the test list contained the two types of odors (presented once or three times) used in the first session. Hit and false alarm rates were calculated for each odor type and priming scores were computed by subtracting base-line of hit and false alarm of the control group(N=12), who joined only the second session, from those of the experimental group(N=12). A significant effect of the number of presentation (1 or 3) was detected in the priming scores for hit (.25, -.067, $F=8.75$, $p<.05$) and false alarm(-.15, .017, $F=4.84$, $p<.05$). The result suggests that an effect of a little experience with odor, as well as word (Terasawa & Ohta, 1993), continues to exist for long time as a style of implicit memory. These results suggest that human could store perceptual information permanently.

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Olfactory Stimuli and Verbal Recoding in Short-term Memory THERESA WHITE[1,2], DANIEL KURTZ[1], MICHEL TREISMAN[3], DAVID HORNUNG[1,4] ([1]Smell and Taste Disorders Clinic at the SUNY Health Science Center at Syracuse, [2]University of Warwick, [3]Oxford University, [4] St. Lawrence University)

Although the mechanism for remembering odor information in the short-term is unknown, there are three main possibilities: 1) a short-term store which deals with olfactory information in the form of an olfactory code. This possibility has been disputed by Lawless and Engen (1977). 2) Olfactory inputs are immediately converted into a non-olfactory form, usually assumed to be a verbal code. 3) There could be a dual system, in which information is held in an olfactory memory, but some or all of it may also be verbally recoded and retained. We attempted to distinguish between these alternatives by examining confusions in odor memory.

In a series of experiments, subjects were trained using feedback to apply specific labels to familiar odors. Each of a target set of odors were similar to 1 odor in quality, and to another odor in label, creating a number of confusion triads (e.g., Lime, Thyme, Sage). If the odorants are remembered via an olfactory short-term memory, then free-recall confusions should occur between similar odorants (e.g., Sage for Thyme). If odorants are transformed re-coded for storage in verbal short-term memory, then confusions should occur between similar odorant names (e.g., Lime for Thyme).

Although in the first experiment only odor confusions were evident, both acoustic and olfactory errors were made in later experiments. It may be concluded that although subjects make use of verbal re-coding where possible, a short-term store which includes an olfactory code is a feasible memory mechanism.

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CSERPs During Active and Passive Odor Administration.

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During normal breathing, olfactory stimulation is dependent on and correlated with inspiration. Because this relationship with inspiration can introduce non-olfactory artifacts, chemosensory event-related potentials (CSERPs) are usually obtained using stimuli presented randomly with respect to the respiratory cycle. Investigators such as Freeman have noted changes in olfactory bulb electrical activity synchronized with inspiration in rabbits. Should similar phenomena be characteristic of human olfactory responses, the phase of the respiratory cycle may act to "prime" the olfactory tract and produce differences in brain events related to inspired (active) versus passively administered stimuli. To test this hypothesis, CSERPs were recorded from thirty electrodes in twelve subjects. Each subject was exposed to two concentrations of butanol (2% and 4%) and a no odor control condition. Stimuli were administered in two phases: passively (as subjects breathed through the mouth) or actively (during nasal inhalation onset). Results indicated a significant interaction of administration technique, concentration, and electrode site suggesting that inspired/active CSERPs were topographically different from passive. It was also found that the largest CSERPs were obtained in the passive condition. These results suggest that studies of olfactory tract integrity may be best served by using the passive technique since the signal-to-noise ratio is best. Other studies, such as those relating the psychological dimensions of odors to CSERPs, may account for greater variance by using inspiration-based administration.

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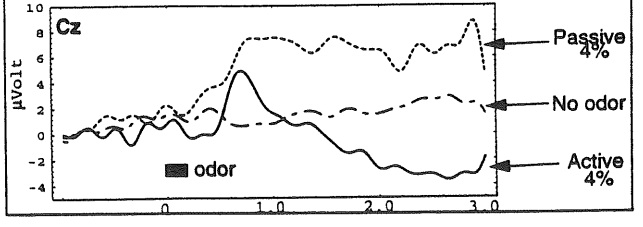

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Associations Between Olfactory Ability and Reported Dietary Intake in Elderly Subjects. W.L. SILVER[1], C.S. SILVER[2], D.B. WALKER[1] AND T.A. DOLECEK[3] (Wake Forest University, Winston-Salem, NC[1], Medicorp Nutrition Center, Winston-Salem, NC[2], American Dietetic Association, Chicago, IL[3])*

Clearly, the palatability of food, how the food "tastes" plays a role in dietary intake. This suggests that individuals with chemosensory deficits may be at nutritional risk. Since numerous studies have demonstrated a decline in olfactory ability with age, the elderly especially are thought to be at nutritional risk. Indeed, a study by Duffy et al., (1993, *Chem. Senses*, 18:549) reported that elderly women with a diminished sense of smell were at greater nutritional risk than women with normal smell ability. The present study examined the olfactory ability of 35 subjects ranging in age from 60-79 years. Subjects were part of a larger study and were healthy other than having high blood pressure treated with antihypertensive medications. There were 18 females and 17 males. Subjects' olfactory ability was determined using the Connecticut Chemosensory Clinical Research Center Olfactory Test (Cain et al.,1983, *Am. J. Otolaryngol.* 4:252). Prior to coming to the laboratory for olfactory testing, subjects filled out a semi-quantitative food frequency questionnaire. From this questionnaire daily intake estimates of 31 nutrients were obtained for each subject. In addition, a Cholesterol-Saturated Fat Index (CSI), which indicates the effect of these diet constituents on blood cholesterol, was determined for each subject. A stepwise regression analysis was conducted using the 31 nutrients. The two nutrients to emerge as predictors of the score on the Olfactory Test were saturated fat and polyunsaturated fat ($p < 0.05$). The lower the olfactory score, the higher the reported saturated and polyunsaturated fat intake. In addition, a discriminant function analysis demonstrated that the CSI was a significant predictor of high and low olfactory scores. The results of this preliminary study suggest that elderly individuals with lower olfactory function have higher saturated and polyunsaturated fat intake than those with normal olfactory function, which may put them at nutritional risk. These results are similar to those in the earlier study of elderly women by Duffy et al., 1993.

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An Application of "a Smell Test Based on Odor Cognition of Japanese People" for Aged People .

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SAHO AYABE-KANAMURA(Inst. of Psychology, Univ. of Tsukuba)

Recognition of odor may be affected by the experience of someone's life, so we reported "A Smell Test Based on Odor Cognition of Japanese People" at AChemS 1994. This test, named STAUTT(Smell Test of AIST, Univ. of Tsukuba and Takasago Int. Corp.) 3, was applied to aged people and compared to the results of middle-aged people. Prior to this test, 318 aged people were asked if they had experienced a decline in olfaction as part of a questionnaire of life quality, and 48 people (15.1%) reported some decline of olfaction. 58.3% of these people reported more forgetfulness in their ordinary lives than normal people(17.4%). STAUTT3 was applied to 21 aged people (including one people who reported a decline in olfaction) from 318 aged people. For 18 odors including the odor of soy sauce, Japanese traditional ink, Japanese cypress, nattou(fermented soybeans), the recognition of odor quality was determined by having the subjects identify odors, choosing from four possible answers. The intensity was measured on a scale of 6-points. The same test was applied to 21 middle-aged people (average age 35 years old). As a result, the average number of correctly recognized odor in the aged people was 11.8, and it was significantly smaller than the 14.4 in the middle age group. Average intensity of the aged person was 2.75, and it was also significantly lower than the 3.22 of the middle-aged people. But the number of correctly recognized odors in the aged person ranged from 3 to 18, individual differences were larger than middle-aged group.

Odor substances were offered by Takasago Int. Corp.

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Effect of Verbal Cue on Recognition Memory of Familiar and Unfamiliar Odors

SAHO AYABE-KANAMURA, TADASHI KIKUCHI (Univ. of Tsukuba, Japan) & SACHIKO SAITO (NIBH, MITI, Japan)

It was investigated whether verbal cue facilitated recognition memory of odors. As familiar odors, forty odor sources were used (ex. apple, soap, leather) and as unfamiliar odors, forty chemical substances were selected (ex. cis-jasmon, citral, iso-valeric acid). Subjects were allocated into two groups, a familiar odor group and an unfamiliar odor group. All subjects attended a learning and three recognition test phases. At the learning phase half of the subjects in each group were required to memorize twenty odors with experimentally presented verbal labels, while the remaining subjects smelled odors without the verbal labels. All subjects went through two test phases, one test phase was conducted 15 minutes after the learning phase. The other 1 week after the learning phase. The subjects were asked to recognize 10 learned odors among 10 unlearned odors. Moreover after 1 week from the second recognition test, the subjects performed the recognition test for the incidental learning, in which they answered "new" or "old" to twenty odors. As for the results, 1)the recognition performances for familiar odors were almost higher than for unfamiliar odors, 2) although the verbal labels did not facilitate both familiar and unfamiliar odor recognition performance for short retention interval and for the incidental learning, they were useful for the memory for familiar odors after 1 week, 3) the recognition performance decreased with time lapse, and this tendency was obviously shown in familiar odor group without verbal labels.

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Olfactory testing as an aid in the diagnosis of Parkinson's Disease: Development of optimal discrimination criteria. STEVEN M. BROMLEY, RICHARD L. DOTY and MATTHEW B. STERN (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, and Department of Neurology, University of Pennsylvania Medical Center, Philadelphia, PA USA)

Since olfactory dysfunction is among the first signs of idiopathic Parkinson's disease (PD), olfactory testing may aid in the early or "preclinical" diagnosis of this disorder. Indeed, the proportion of early-stage PD patients with olfactory dysfunction appears to be greater than the proportion of early-stage PD patients exhibiting some of the cardinal signs of PD. Because olfactory function varies in the general population and declines with age, empirically-based criteria are needed by the clinician to establish whether the degree of olfactory loss observed in a given patient is concordant with the presence of PD. In this study, we developed cutoff criteria for the optimal assessment of olfactory dysfunction in the evaluation of PD. Specifically, we determined scores for the University of Pennsylvania Smell Identification Test (UPSIT) that best discriminate between PD patients and age-matched controls. Receiver operating characteristic (ROC) curves, based upon sensitivity and specificity estimates, were computed for three age groups ($\leq$60 yrs, 61-70 yrs, and $\geq$ 71 yrs) and scores with highest sensitivity and specificity were determined. Sex- and age-related differences in the test scores were observed, with lower scores occurring for men and for the older patient groups.

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Olfactory Dysfunction in Patients with Minamata Disease

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We studied the olfactory functions of organic mercury poisoning (Minamata disease) by using the smell identification test and the olfactory detection threshold test (with phenyl ethyl alcohol). The subjects were 19 patients with Minamata disease who were treated in Meisuien, Minamata, Kumamoto, Japan and include cases that developed the disease in uterus. The mean age was 78.7 ±14.3 years old. Both smell identification and olfactory detection tests in the majority of patients decreased significantly compared with those of healthy elder subjects. A few cases showed normal olfactory identification and detection functions. The olfactory identification function decreased with advancing age. Correlation existed between detection threshold and background factors such as age and duration after documented Minamata disease was found. No significant relationship was identified between olfactory function and the typical symptoms of Minamata disease.

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Relationship Between CT Scan Findings and Sense of Smell

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There is an incomplete understanding of the relationship between CT scan findings and patients' symptoms. 202 patients who were having CT scans at Johns Hopkins Outpatient Center completed questionnaires on their symptoms at the time of the scan. Their scans were analyzed by a semi-objective rating system utilizing 5 physicians (2 otolaryngologists and 3 radiologists). Chi-square and T-tests were used to examined the relationships between the CT scan data and the symptom data. For the sense of smell, patients were asked to estimate their own olfactory ability as excellent, diminished, or absent. Results of the correlation are as follows: 1) Olfactory cleft size is larger in females, 2) Larger nasal cavity and larger olfactory cleft are associated with better sense of smell, 3) Increased opacity in the frontal recess is associated with a decreased sense of smell, 4) Increased opacity in the anterior ethmoid sinus is associated with a decreased sense of smell, 5) Increased opacity in the posterior ethmoid sinus is associated with a decreased sense of smell, 6) Increased opacity in the sphenoethmoidal recess is associated with a severe sense of smell dysfunction, 7) Increased opacity in the olfactory cleft is associated with a mild sense of smell dysfunction. These data generally support current understanding of how odorants reach olfactory receptors and the more anterior location of these receptors.

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Variation in the Olfactory Event-related Potential During the Menstrual Cycle. PHIOANH NGHIEMPHU, LIYING CUI and W. JAMES EVANS (University of California, Irvine).

Olfactory event-related potentials were recorded from normal female subjects aged 40 years or less at three times during the menstrual cycle, i.e., during menses, ovulation and the luteal phase. Estradiol and progesterone levels were drawn at the time of testing to confirm phase. Normal olfactory function was verified by the Smell Identification Test and odor detection thresholds for phenylethyl alcohol, isoamyl acetate and CA-phenone. Evoked potentials were recorded from Fz, Cz, and Pz, C3 and C4, referenced to A1, in response to amyl acetate and air control stimuli presented to the right nostril at a volume flow rate of 5 L/min, stimulus duration of 40 ms, and randomized interstimulus intervals of 6-30 s. Evoked potentials to amyl acetate were characterized by four reproducible components (P1, N1, P2, and N2). No reproducible evoked potential components were seen in response to the air control stimulus. Around the time of ovulation, the N1-P2 interpeak amplitude at Cz was observed to increase by approximately 100% from baseline measurements at menses. These data suggest that phase of the menstrual cycle is an important subject variable to be considered in interpreting olfactory evoked potential measures. Furthermore, this technique may be of value in studying central nervous system changes occurring during the reproductive cycle.

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Weber Ratios for Odor Are Greater for Elderly Than Young Persons JILL RAZANI[1] DIANE WILSON[2], and CLAIRE MURPHY[3*] (SDSU-UCSD Joint Doctoral Program[1], San Diego State University[2] and San Diego State University and UCSD Medical Center, San Diego, CA[3])

Previous research has shown differences in the odor intensity ratings between young and elderly individuals. However, due to the methodology used, these studies have been unable to show the degree to which the elderly individual requires an increase in stimulus concentration in order to perceive a difference in odor sensation. Further, they do not indicate whether the required increase in concentration for the elderly is constant across different odor concentrations. In the present study, intensity discrimination of butanol was assessed in young (mean age=33 years old) and elderly (mean age=73 years old) participants. The elderly subjects were all active community dwelling individuals and reported good to excellent health. The method of constant stimuli, in which comparison and standard stimuli are compared, was used. Three standard concentrations of butanol were used for determining Weber ratios: 63.3%, 20%, and 6.3% v/v in distilled water. These standards were each compared to six comparison stimuli: 70%, 82%, 94%, 106%, 118%, and 130% of the standard. Thus, there were three weaker and three stronger comparison stimuli for each standard. Just noticeable differences (JNDs; the increase in concentration necessary to notice a change in concentration) and Weber ratios (the ratios of JNDs to standard concentration) were calculated for the young and elderly groups. Analyses revealed significantly higher Weber ratios across all three standard concentrations for the elderly than for the young. In addition, differences were greater at the lowest concentration. The age-related differences in Weber ratios are larger for odors than those previously reported by our laboratory for taste. These results may have implications for the nutritional status of the elderly person since the perception of food flavor involves not only the taste but to a great extent the olfactory system. Poor odor discrimination may also affect the safety of elderly individuals, such as the inability to correctly assess spoiled and rotten foods, as well as detect harmful gases.

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Development of a Non-lexical Odor Identification Test for Alzheimer's Disease. JODI HARVEY, JILL RAZANI, and CLAIRE MURPHY (San Diego State University and UCSD Medical Center, San Diego, CA)

Odor identification deficits were the first olfactory functional impairment reported in Alzheimer's Disease (AD). The finding is robust across experiments. We have previously reported deficits in both lexical and non-lexical odor identification that occur in patients with very mild dementia. To enhance the potential for olfactory testing to contribute to differential diagnosis of dementia, this study was designed to develop an optimally sensitive odor identification test for AD patients. Participants were 24 persons who met the NINCDS-ADRDA criteria for probable AD, and 24 healthy elderly controls, matched for age, education, and gender. All subjects were diagnosed by two independent neurologists at the UCSD Alzheimer's Disease Research Center. In the case of AD patients, extensive neuropsychological and neurological tests were conducted to rule out alternative causes for dementia. Scores for Alzheimer's patients on the Dementia Rating Scale indicated moderate impairment. All subjects were asked to identify eight odorants of the non-lexical, Child-Odor-Identification Test. The odorants used were common household products: peanut butter, chocolate, coffee, baby powder, cinnamon, mustard, bubble gum, and Play-Doh. Two odorants, bubble gum and Play-Doh, were deemed not optimal for this age group and thus were excluded from analysis. Odorants were randomly presented, and subjects were required to identify each using a picture board containing pictures of the test stimuli as well as 12 distractors. A series of Chi-Square analyses revealed that for all odorants, a significantly greater number of normal controls than AD patients correctly identified them (all p values<.05). Scores were further subjected to logistic regression to determine the ability of the various odors to correctly classify patients and controls. Results suggest that the combination of all odors produces high predictive power, but that the test may be improved by the removal of specific odorant items.

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Colds as a Model System for Conduction Loss: Mechanisms for Changes in Odorant Identification in the OCM DAVID E. HORNUNG[1,2], DANIEL B. KURTZ[1], MARCUS E. MARTINEZ[2], THERESA L. WHITE [1] ([1]Smell and Taste Disorders Clinic at the SUNY Health Science Center at Syracuse, [2]St. Lawrence University)

Using the Odorant Confusion Matrix (OCM) we observed in college students, that relatively weak odorants with high water solubility (rose, ammonia, and cinnamon) were poorly identified at the height of an upper respiratory infection (URI). In contrast, relatively strong odorants with low water solubility (mothballs and licorice) were more easily identified. From this data, we hypothesized that the mucosal swelling which usually accompanies a cold accentuates the difference between highly and poorly sorbed odorants in the loss of odorant molecules to non-olfactory tissue. As a first test of this hypothesis, we increased the concentration of cinnamon (a weak odorant with high water solubility) and reduced the concentration of mothballs (a strong odorants with low water solubility), and again administered the OCM to college students with URIs. As predicted, cinnamon at the higher concentration was identified much more often. Mothballs was somewhat more difficult to identify, although still relatively easy for subjects with URIs to recognize. This is in contrast to informal experience with patients having an olfactory loss attributed to a neural loss. In these patients, it appears that the ability to identify odorants is insensitive to increases in odorant concentration.

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Title: Olfactory Manifestations of Multiple Chemical Sensitivity.

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Persons with Multiple Chemical Sensitivity (MCS) report low-level environmental chemicals as the cause of their symptoms which include headache, ocular and nasal irritation, cognitive effects, fatigue, and pulmonary complaints. These multiorgan symptoms were initiated by an acute chemical exposure or gradually developed. Subsequently, MCS patients report that low odor levels from different chemical classes provoke symptoms. No generallly accepted test of the organ systems validate the symptoms. 12 female and 2 male subjects, who met the above criteria, volunteered for this experiment. Odor testing in this experiment consisted of butanol threshold, Univ. of Pennsylvania Smell Identification Test (UPSIT), and a symptom-odor questionnaire. In addition to odor tests presented here, the subjects had a physical exam, blood tests, allergen testing, and psychological testing. Additionally, the subjects provided blood samples for pesticides and volatile organic compound (VOC) analyses. The results demonstrated that the body burdens of VOCs, PCB, and pesticides were at or below population levels. Their butanol thresholds were not different from controls and their UPSIT scores showed that they were within normal ranges for their ages. Odors that precipitated severe symptoms were turpentine, new carpet, paint remover, and diesel exhaust. Gasoline, fresh paint, cleaning materials, cigarette and cigar smoke, auto exhaust, particle board, new car odor and smoke from a barbecue resulted in moderate symptoms. Interestingly, food odors did not evoke symptoms and were regarded as pleasant or very pleasant. The odors that result in symptoms were hedonically unpleasant and may stimulate the trigeminal and olfactory nerves while those that did not produce symptoms were hedonically pleasant and low in trigeminal stimulatory capacity. The relationship between the hedonics and trigeminal stimulation may provide insight into this controversial syndrome. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

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Allergen Risk Ratios for a Community Sample With and Without Self-Reports of Chemical Sensitivity.

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Explanations for the psychophysiologic underpinnings of Multiple Chemical Sensitivity (MCS), as well as objective biological indices for accompanying symptoms, remain elusive. This study investigated asthma and hay fever reports, in combination with standardized forearm skin test results of a community-based sample (N=96) with and without self-reported Chemical Sensitivity. The Chemically Sensitive (CS N=39) and Non-Chemically Sensitive (NCS) were determined from a composite self-rating scale described in Bell et al. (1993). Chi-square was used to derive Relative Risk Ratios (ratios of proportions CS to NCS) positive (small=2-7mm/large=8mm and above) for each of 23 antigens. The Table shows Risk Ratios (RR) and hay fever/asthma reports of CS and NCS for antigens to which the CS were at least 50% more likely to react. Histamine response is included as another basis for comparison between groups.

				% WITH ASTHMA or HAY FEVER	
REACTIONS	CS	NCS	RR	(CS)	(NCS)
Cedar	22.2	10.7	2.22/2.96*	22.2	10.0
Cricket	11.1	5.0	2.22	11.1	5.0
Dema (Mite)	33.3	20.0	1.67	27.8	12.5
Der.f (Mold)	33.3	21.6	1.54	27.8	15.8
Histamine	27.8	47.5	0.59**	16.7	32.5

*Risk Ratios reactions of 8mm or greater. **p =.08

Other allergens/risk ratios of note included Weed-mix, Mesquite, and Mixed Smut (each at 1.48); Blatta (1.47); Cat (1.34); Careless Weed (1.33); Alternaria (1.11/1.27*), Mulberry (1.11); Arizona Ash (1.04/1.39*), Goosefoot (1.04/1.33*); and Clados (0.89/1.48*). Housedust, Bermuda, Treemix, Rabbit Bush, Olive, Ragweed, and Barley were not related to CS. Data support the increased reports of allergic rhinitis among CS sufferers; suggest the CS may be at greater biological risk to allergens than previously thought; and based on the Histamine result, suggest the NCS and CS may be at risk to specific allergens, but not to histamine-mediated responses per se.

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Olfactory Performance In Early Alzheimer Patients.
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Although the olfactory system is prominently involved in Alzheimer's disease, the nature of olfactory dysfunction in the early phase of the disease remains unclear. Thus, the present study examined odor detection, odor quality discrimination and odor identification in 13 patients with Alzheimer's disease (12 Probable, 1 Possible), chosen for the mildness of their condition, and 18 healthy elderly controls of similar age, sex and education. Odor detection was assessed with standardized Clinical Smell Test Kits (Olfacto-Labs), employing pyridine and phenylethyl methyl ethyl carbinol (pm-carbinol) as test odorants. Odor discrimination and odor identification were evaluated by means of suprathreshold materials (pure essences of various familiar odors) and two-alternative forced-choice procedures designed specifically for the dementing elderly. ANOVA revealed that the patient groups' mean thresholds for pyridine and pm-carbinol were significantly increased relative to those of controls. Both groups demonstrated significantly lower mean thresholds for pyridine than for pm-carbinol, largely due to the trigeminal properties of pyridine. Nonparametric analyses indicated that the patients' odor discrimination and odor identification scores were significantly reduced compared to those of controls and performance within groups across suprathreshold measures did not differ significantly. The patient group revealed a pattern of well-established mild deficits in olfactory sensitivity with some preservation of trigeminal sensitivity relative to olfactory sensitivity at the threshold level; their odor discrimination and odor identification deficits were shown to be marked and similar in degree. Such evidence suggests that odor detection deficits are present early in Alzheimer's disease, along with odor discrimination and odor identification deficits, and may differ from the latter two only in severity. Further empirical investigation is required to determine just how early in the disease odor detection deficits may be identified. Olfactory testing may be useful as a supplementary diagnostic index for early detection and monitoring of Alzheimer's disease. Development of a suitable olfactory battery appears essential.

This study, partially supported by NSERC Grant A-0679 to Dr. J. M. Lacroix, constituted the doctoral dissertation research of the first author.

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The Role of Rhinomanometry in Evaluating the Results of Endoscopic Sinus Surgery in Patients with Chronic Sinus Disease.
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The measurement of nasal airway resistance (NAR) by rhinomanometry has proven to be useful in evaluating the results of surgery for nasal obstruction due to deviated septum; however, data supporting its usefulness in monitoring patients undergoing endoscopic sinus surgery (ESS) has been lacking. Patients with chronic sinusitis also often complain of nasal congestion and obstruction and these symptoms have been shown to improve markedly three months after endoscopic sinus surgery by subjective, self-evaluation ratings using a scale of 0-100, where 100 represents total obstruction and severe congestion (Maricio, et al, AChemS 1994). Having an objective method for evaluating nasal obstruction in this group of patients would be useful. In this study, total NAR was measured by an anterior, active rhinomanometric technique in 21 patients with chronic sinus disease, before and three months after ESS. Results indicate that total NAR decreased significantly ($p<.03$) after ESS. Furthermore, the decrease in NAR following ESS correlated significantly with decreased subjective ratings of nasal obstruction reported by patients, ($p<.008$). The measurement of NAR by rhinomanometry is, in our experience, a sensitive test for objectively measuring improvement in nasal breathing function after ESS in chronic sinusitis patients.

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MR of Patients with Congenital Anosmia.
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Often the site of anatomic abnormality in patients with congenital anosmia is inapparent. Twenty two patients with congenital anosmia were evaluated by olfactory testing, sinonasal endoscopy, and magnetic resonance (MR) imaging. The MR evaluation included surface coil imaging of the olfactory bulbs, tracts and subfrontal cortex by contiguous 3 mm T1-weighted and T2-weighted MR sections. A head coil was employed to evaluate the temporal lobes and hippocampal regions with 3 mm sections. Unilateral olfactory bulb and tract and temporal lobe volumes were determined from a three dimensional data set by 2 reviewers to assess interobserver variation on 2 occasions to assess intraobserver variation. These analyses were correlated with olfactory test results. The most common MR finding in individuals with congenital anosmia was complete absence of the olfactory bulbs and tracts bilaterally (64%). In 4 cases we found a tract without a bulb but never the opposite. In 4 of 22 patients, both olfactory bulbs and tracts were visible and two of these had volumes comparable to those of normal controls. Temporal lobe volumes were similar to those of the control group. Of the 22 patients, 6 had definite Kallmann's syndrome and all of these had complete absence of olfactory bulbs and tracts. Interobserver and intraobserver variation ranged from 3-5% for temporal lobe volumes, and from 15-20% for olfactory bulb and tract volumes (owing to their very small size). Based on the University of Pennsylvania Smell Identification Test, 19 of 22 patients were anosmic. This study indicates that bilateral olfactory bulb and tract absence is the most common MR finding in patients with congenital anosmia with or without Kallmann's syndrome. The temporal lobes and hippocampi have normal volumes in these patients.

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Use of the Chicago Smell Test (CST) as a Predictor of Depression
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Since the olfactory lobe is part of the limbic system, their respective functions may be assumed to be interrelated. Diseases which affect olfactory ability have been shown to effect emotional state. Previously, ability to detect PE Phenol had been correlated with levels of depression based on the Beck Depression Inventory (BDI). This study was performed in order to determine if olfactory identification ability also predicted depression. METHODS: 27 volunteers (18 female, 9 male), 18-81 years old (mean 30) participated in this IRB approved study. All underwent CST[1], a test of olfactory identification and two tests of depression severity: BDI and the Zung Depression Scale (ZDS). Statistics were independently analyzed using Pearson Correlation Coefficient by the University of Illinois School of Public Health. RESULTS: Ability to identify odors on the CST predicted degree of depression. A direct correlation was found between the CST score and level of depression with the ZDS ($r=.54$; $p=.004$) and the BDI ($r=.38$; $p=.05$). Mean test results ranges were as follows: CST 4.0 (3-5), ZDS 42.4 (30-73) and the BDI 7.4 (1-30). CONCLUSION: Olfactory identification ability as determined by the CST appears to be a predictor of level of depression, further cementing the link between the functions of the olfactory and limbic systems.

[1] Hirsch, A.R. and Cain, D.R.: "Evaluation of the Chicago Smell Test in a Normal Population," Chemical Senses, Vol. 17, No. 5, 1992, p. 642-3.

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Cyclin-Dependent Kinases Regulate Postnatal Cell Division in the Main and Accessory Olfactory Bulbs of Corn Snakes. D.A. HOLTZMAN, LINDA LEIMBACH and D.T. KUHAR (Neuroscience & Biopsychology Program, Oberlin College, Oberlin, OH 44074)

This study shows that cells of the ependymal/granule cell (E/GC) layer of the main (MOB) and accessory (AOB) olfactory bulbs of postnatal corn snakes can incorportate 3H-thymidine and migrate to give rise to cells in other layers. In addition, we show that disruption of the production of cyclin-dependent kinases (CDKs) impairs 3h-thymidine uptake by cells in the E/GC layer. Twelve 4-6 month old corn snakes (*Elaphe guttata*) were anesthetized with sodium brevitol and were divided into three groups for injections into both lateral ventricles: 5 received an antisense oligonucleotide for the PSTAIRE region of CDKs, 5 received a scrambled oligonucleotide consisting of the same bases, and 2 were injected with saline vehicle only. 23 h post-ICV injection, all snakes were injected with 1 μCi of 3H-thymidine/g of snake. All animals were then anesthetized with a lethal dose of sodium pentobarbital 1 h post-3H-thymidine injection, fixed in 4% paraformaldehyde, embedded in paraffin, and processed for standard emulsion autoradiography. For analysis, the total number of labelled cells and the average number of labelled cells/mm^2 were calculated for the E/GC layers of the MOB and AOB for each snake. In the AOB, there were no significant differences between the average number of labelled cells/mm^2 between groups ($p>0.05$), but there are more total labelled cells in the scrambled vs. antisense group ($p<0.03$). In the MOB, a significant difference was found between groups for the average number of labelled cells/mm^2 ($p<0.01$), and more total labelled cells were found in the scrambled vs. antisense group ($p<0.02$). In addition, mitral and periglomerular cells were labelled with 3h-thymidine in all three groups. These data suggest that the E/GC layers of the MOB and AOB in postnatal snakes can give rise to neurons all cell layers 1 h after incorporating 3H-thymidine. In addition, the antisense data suggest that CDKs are necessary for normal cell division to take place in the postnatal MOB and AOB.

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Transmitter phenotype of olfactory bulb interneurons derived from neuronal progenitor cells of the neonatal subventricular zone.

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It has recently been shown using retroviral lineage tracers encoding lacZ (Luskin, *Neuron* 11:173, 1993) that the anterior part of the neonatal subventricular zone (SVZa) generates an immense number of neurons in the first postnatal week that migrate to the olfactory bulb (OB) and differentiate into granule cells (~74%) and periglomerular cells (~26%), the two major interneuron subtypes. The classification of lacZ-positive neurons was based on their laminar distribution and morphological features. To further characterize the SVZa-derived neurons in the OB, immunohistochemistry was used to determine their transmitter phenotype. To identify SVZa-derived cells, we stereotaxically labeled SVZa progenitor cells at postnatal day 2 (P2) with the cell proliferation marker BrdU. (For convenience, cells labeled with BrdU will be referred to as cells generated on P2). At P20, when most of the SVZa-derived cells have reached their final destination, BrdU-labeled cells were localized using immunohistochemistry and their neurotransmitter phenotype was assessed using antibodies against γ-aminobutyric acid (GABA) and the dopamine-synthesizing enzyme tyrosine hydroxylase (TH). Using simultaneous indirect immunofluorescence to detect the presence of single and double-labeled cells, we found that 10% of the SVZa-derived cells were both BrdU- and TH-positive in the glomerular layer and that approximately 67% and 46% of the SVZa-derived cells in the granule cell layer and the glomerular layer were GABAergic (GABA- and BrdU-positive), respectively. We also determined that when analyzed at P20, 28% and 12% of the periglomerular cells, that arose from a P2 injection of BrdU, were TH- and GABA-positive respectively. Similarly at P20, 11% of GABAergic neurons in the granule cell layer were generated on P2. These results indicate that the neonatal SVZa is a source of dopaminergic cells destined for the glomerular layer and also a source of GABAergic cells for the granule cell and glomerular layers.

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A Reversible Technique for Unilateral Naris Occlusion. DIANA M. CUMMINGS, HEATHER E. HENNING, and PETER C. BRUNJES (Neuroscience Program, University of Virginia, Charlottesville, VA)

A large body of evidence indicates that permanently closing a single external naris drastically alters the development of the ipsilateral olfactory bulb. For example, naris closure on the day after birth in rats results in a 25% reduction in the volume of the ipsilateral bulb by postnatal Day 30 (P30). We have recently adapted the method for reversibly blocking nares described by Kucharski and Hall (*Science*, *238*, 1988). Nose plugs, constructed of polyethylene tubing and surgical suture thread, were inserted into pups' right nares on the day after birth. Graded sizes of polyethylene tubing (PE10-PE90) were used to construct a variety of sizes of nose plugs. Every 2-4 days plugs were replaced to accommodate the growing naris. On P30 animals were sacrificed, their olfactory bulbs removed, and serial Nissl sections produced. Volume measurements were made of the glomerular (GLM), external plexiform (EPL), and granule cell layers (GCL) of both bulbs using a computer planimetry system. These measurements indicated a 20% reduction in the overall size of the olfactory bulb ipsilateral to the plugged naris as compared to the contralateral control bulb. The greatest decreases in laminar volume occurred in the EPL (26%) with less drastic reductions in the GLM (17%) and GCL (17%). Thus, naris occlusion by nose plugs results in a profound reduction in bulb size, with a pattern similar to that observed following permanent closure. Current studies address the question of whether or not the volumetric reductions seen after naris occlusion can be "recovered" once nose plugs are removed. Preliminary results suggest that reductions after naris occlusion for 20 days are reversed following a long (11 week) but not short (10 day) recovery period. These results indicate that naris occlusion using nose plugs will be very useful for studying the plasticity of the olfactory system during postnatal development.

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The Ferret Olfactory Bulb in View of the Postnatal Development of Granule Cell Spines. ELKE WEILER, University of Tübingen, Institute of Zoology, Dept. Animal Physiology, Auf der Morgenstelle 28, 72076 Tübingen, Germany

In altricial mammals, like the European ferret, brain structures are not mature at birth. This is also true for the phylogenetically old olfactory bulb. As in all mammals, the olfactory bulb of the ferret is arranged in layers; maturation of neurons takes place in all layers. The last neurons to mature are the granule cells, interneurons responsible for processing the olfactory information. Granule cells do not have an axon, however, there are spines on the soma and dendrites. Golgi studies reveal, that the number and density of spines vary with age. Somatic spines reach their maximum density around postnatal day 30, with an average of 6.7 spines per soma. Spine density is reduced afterwards. While somatic spine density decreases, dendritic spine density still increases. Spine density on apical dendrites is highest during a sensitive phase between postnatal day 60 and 90, during a time, when food odor imprinting takes place. The following normal reduction depends highly on the amount of incoming information during the sensitive phase. Dendritic spines seem to be part of a specific filter for information processing.

Looking at the olfactory system we find a correlation between the maximum density of somatic granule cell spines, the maximum number of mitral cells and the increase in receptor cells. We assume that the information influx increases dramatically prior to completion of the filtering ability of the dendritic spines. To protect the system during this vulnerable phase against overexcitation, the somatic spines of the inhibiting granule cells form a temporal unspecific filter until the specific filter of the dendritic spines has matured.

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Two Populations of Cells Expressing GnRH-Like Immunoreactivity Migrate From the Olfactory Organ to the Central Nervous System in the Zebrafish

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The olfactory placode of vertebrates gives rise not only to the primary sensory neurons of the olfactory system, but also to the gonadotropin releasing hormone (GnRH) cells that migrate from the olfactory placode to the hypothalamus during development. Using two different antibodies against GnRH, one recognizing the salmon form of GnRH (sGnRH) and one recognizing the chicken II form of GnRH (cII) we showed that during development these antibodies label two populations of cells. The first population expressing sGnRH-like immunoreactivity is apparent at the lateral edge of the olfactory organ 48 hours after fertilization (h). A second, later appearing population of cells expressing cII immunoreactivity is evident at the medial edge of the olfactory organ at 56 h. These two populations of cells migrate along different pathways; the first population passing behind the eye to the ventral CNS and the second group of cells following the anterior commissure in the telencephalon. To learn whether or not these cells expressing GnRH-like immunoreactivity were actually migrating from the olfactory placode, we labeled olfactory placodes with Di I at 48 h and 52 h and then viewed them at 52 h and 57 h respectively. Di I labeled cells left the labeled placodes and traveled along the pathways where we had observed the cells labeled with the GnRH antibody. In addition, we ablated the olfactory placode and showed that there are no GnRH cells migrating into the brain of these fish. These data suggest that the cells observed with the anti-GnRH antibodies migrate from the olfactory placode into the CNS. To examine the differentiation and migration of GnRH cells, we have transplanted olfactory placodes to ectopic locations in the fish. Preliminary evidence shows that axons originating from the ectopic nose grow into the CNS and we observe cells apparently migrating away from the transplanted nose. We are now confirming the identity of these migrating cells. Using both behavioral and morphological assays, we are screening for olfactory system mutants.

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Modulation of Amino Acid Receptors by Transition Metals and Carnosine. PAUL Q. TROMBLEY and GORDON M. SHEPHERD (Section of Neurobiology, Yale Medical School, New Haven, CT 06501)

Synaptic transmission in the glomerular layer of the olfactory bulb is mediated primarily by amino acid transmitters. Glutamate, GABA, and glycine activate distinct receptors with specific modulatory sites and functional properties. Among the modulators are zinc (Zn) and copper (Cu), which are found in high concentrations in the olfactory bulb. Although the precise localization of Cu is unknown, Zn is co-localized with glutamate in olfactory receptor neuron (ORN) terminals. In addition to Zn and glutamate, ORN terminals contain high concentrations of carnosine, although no specific function for carnosine has been identified. We have shown previously that Zn and Cu are potent modulators of specific subtypes of amino acid receptors. We now report evidence that the modulatory actions of Zn and Cu are dependent not only on the type of amino acid receptor, but also on the state of the receptor. To explore a function for carnosine, we have tested several hypotheses including whether carnosine has direct affects on either olfactory bulb neurons or on amino acid receptors. Furthermore, because it has been proposed that carnosine can chelate Zn, we tested the hypothesis that carnosine can modulate the actions of Zn and Cu. Olfactory bulb neurons from E18-P3 rat pups were grown in primary culture and examined using whole cell voltage clamp recording techniques. Carnosine (≤1 mM) had no direct affect on olfactory bulb neurons nor did it affect currents evoked by glutamate, NMDA, GABA, or glycine. Carnosine (0.1-1.0 mM), however, potently blocked the effects of Cu (30-100 μM) but only had a small affect on Zn (30-100 μM). It has been demonstrated that Zn, Cu, and carnosine can be released from nerve terminals by depolarization. The high concentrations of Zn, Cu, and carnosine in the olfactory bulb may suggest that they play a role in odor information processing through activity-dependent modulation of amino acid-mediated synaptic transmission.

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More Evidence That The Human Vomeronasal Organ Has Unique Epithelial Cells: Calbindin-, NSE-, OMP-, and PGP-like Immunoreactivity In Two Fetuses

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We have shown that epithelial cells lining the vomeronasal organ (VNO) of a newborn human exhibit calbindin-like immunoreactivity (-LI). Others have shown that the human VNO has cells that express neuron-specific enolase (NSE)-LI and protein gene product 9.5 (PGP)-LI. Olfactory marker protein (OMP)-LI has not been detected in the human VNO. A small sample of VNO sections were obtained from two human fetuses: a female of 24 weeks and a male of 31 weeks of gestation. A number of epithelial cells surrounding the lumen expressed calbindin-, NSE- and PGP-LI. One cell showing calbindin-LI had a thin axon-like process. PGP-LI fibers were in the surrounding lamina propria (LP), but none appeared connected to VNO cells. A few VNO cells displayed OMP-LI and fibers in the LP showed OMP-LI. No cells or fibers showed serotonin (5HT)-LI. Overall, these results indicate neuroendocrine and chemosensory cells in the human VNO. Additionally, we observed cells in the olfactory and respiratory LP and near the cribriform plate which exhibited calbindin-LI. Based on morphology, these cells appear to be migrating cells and may be part of the LHRH system. We are examining all tissue sections to determine if these cells also express NSE-, PGP-, OMP- or 5HT-LI. Additional VNOs will be surveyed as they become available.

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Patch Recorded Isolated Adult Human Vomeronasal Cells are Electrically Excitable and Respond to Skin Steroidal Substances: Androsta-4,16-dien-3-one and Estra-1,3,5(10),16-tetraen-3-ol.

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We previously reported that low concentrations of certain airborne steroidal substances, isolated from human skin (1), can stimulate the human vomeronasal organ (VNO). Epithelial cells from the VNO of young adult men and women, were maintained in a monolayer short term cell culture, using a technique recently developed in our laboratory. The cells survived for 10-15 days, and did not reproduce in culture. Neuroepithelial cells were identified using neuron-specific enolase antibodies. These cells showed distinctive bipolar asymmetric morphology, had a long (30-70 μm) neurite, and did not have cilia. Whole cell and patch recorded neuroepithelial cells (n=54) were electrically excitable and had a mean R_m=3.1GΩ, ±1.4 (R_s=1.0 to 6.5 GΩ). The cells were exposed to various substances: androstadienone, estratetraenol, testosterone, estradiol, a mixture of olfactants (cineole, amyl acetate, acetophenone and phenylethyl alcohol), and saline. All substances (conc.=10^{-4} to 10^{-7}), and saline, were pulsed from micropipettes located 20μm from the recorded cell. When androstadienone and estratetraenol were delivered to the apical cell process, both induced a dose dependent inward current in neuroepithelial cells (latency= 10-40ms), but not in other cell types present in the culture.This current showed adaptation to long lasting pulses of stimulus. I/V relations for the inward current, holding the membrane from -80 to +40mV, while pressure injecting the skin steroidal substances, showed a linear slope. The current reversed near +5 mV, and was inactivated with cationic blockers. Testosterone, estradiol and olfactants did not have significant effects on VNO neuroepithelial cells. Also, ciliary cells present in the culture were not affected by any of the substances used in our experiments. We conclude that human VNO cells have receptor sites for androstadienone and estratetraenol. Also, stimulation of these receptor sites activates a cation current that is the earliest electrical event in the transduction process.

Work supported with funds from Pherin Corporation.

(1) D.L. Berliner. (1994) U.S. Patent 5,278,141.

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Anterior Distribution of Human Olfactory Neuroepithelium

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The distribution of human olfactory neuroepithelium is not clearly understood. The goal of this study was to examine both the functional and the anatomical distribution of olfactory epithelium in the human nasal cavity. Twelve healthy, trained volunteers (6 female and 6 male), ages 18-48, participated in functional testing with recordings of electro-olfactogram (EOG) (See Hummel et al for details). The location of the electrode placement was determined using thin fiber endoscopy and was documented photographically. Ten patients undergoing nasal or sinus surgery had sampling of healthy appearing mucosa from anterior nasal and olfactory cleft areas. Specimens were fixed in Bouins solution and processed using previously described methods. After examination with light microscopy, each specimen was catergorized as 1) containing olfactory epithelium or evidence that it contained olfactory epithelium in the past, 2) no evidence of olfactory epithelium, 3) too distorted to make judgements. Results from the functional testing showed at least one positive vanillin response from 8 of the 12 subjects. These response-positive locations were clustered above the anterior insertion of the middle turbinate on the lateral wall and opposite this location on the septum. The biopsied tissue showed a similar tendency to have olfactory epithelium 1 or 2 cm anterior to the olfactory cleft on both the septum and the lateral wall. These studies suggest that functioning olfactory tissue is located more anteriorly than previously thought. These findings help to explain previous clinical findings of intact olfactory ability in patients with obstructed olfactory clefts.

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The Peripheral Input Signal to The Olfactory System in Man: The Electro-Olfactogram. Hummel T. [1], Knecht M.[1], Wolf S.[2], and Kobal G.[1] ([1] Dept. of Pharmacology, Krankenhausstr. 9, [2] ENT Clinic, Waldstr. 1, Univ. of Erlangen-Nürnberg, 91054 Erlangen, Germany)

The major objective of this investigation was to test changes of the electro-olfactogram (EOG) in relation to both stimulus concentration and length of the interstimulus interval (ISI) with pairs of olfactory stimuli. In addition, the EOG's relationship to the perceived stimulus intensity and the corresponding chemosensory event-related potentials (CSERP) should be investigated.
Forty-seven healthy volunteers (21 male and 26 female subjects; 18 to 32 years) participated in one or more sessions performed on separate days. Duration of recordings ranged between 30-120 min. Suprathreshold stimulation was performed with substances believed to exclusively excite olfactory receptor cells (vanillin, 0.4-1.2 ppm; H_2S, 1.3-2.8 ppm; duration 1000 ms). In addition, pairs of stimuli were applied at ISIs of either 2 or 6 s (stimulus duration 1000 ms). EOG was recorded by means of tubular electrodes (cutaneous reference contralateral bridge of the nose; impedance < 10 kΩ; DC to 70 Hz, sampling rate 125 Hz). EEG was recorded from 3 positions (Fz, Cz, Pz; referenced against A1+A2); eye blinks were monitored via the Fp2 lead. EOG could be recorded in 18 of 58 trials (31%). In all subjects EOG amplitudes increased in relation to an increase in stimulus concentration. When subjects adjusted the recording electrode themselves, success rates were lower (11 of 39; 28%) compared to endoscopical placement of the electrode (7 of 18; 39%). Only in 2 of 18 trials clear responses to both olfactory stimulants (H_2S, vanillin) could be recorded in the same location although subjects always perceived a strong olfactory sensation in combination with the occurance of CSERPs. In line with immunohistochemical findings *(Strotmann et al. 1994, Cell Tiss Res 278:11)* these results indicate that odorant receptors are not dispersed homogenously throughout the olfactory epithelium.

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An fMRI Study of Human Brain Response to Attractant and Aversive Odors

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We sought to identify regions of human brain active in processing food attractant and/or aversive odors. Stimulus-related changes in cerebral blood flow were measured with fMRI at 1.5T. Eight coronal slices were imaged beginning anterior to the orbits and extending through the temporal lobe. Odors were presented through an olfactometer connected to both nares. Twenty images were acquired for each slice during 42 sec periods of continuous odor presentation or pre stimulus baseline. Voxels were considered active if t-values comparing signal prior to and during odor were above threshold (usually t=2) for two presentations of the odor. Initial studies compared peppermint (P, n=3), coconut (C, n=3) and birch tar (BT, n=2). P and C always activated lateral inferior frontal gyrus, subcallosal and septal areas, lateral amygdala and prepiriform cortex, and caudate. BT never activated the subcallosal area and inconsistently activated the other areas. Subsequent studies (n=5) compared blank trials and varying concentrations of one odor. Caudate and septal areas only activated with strong sensations of odor. Other areas were sometimes active in the absence of stimulus when subjects may have expected and searched for odor. We are now comparing responses to attractant and aversive stimuli at concentrations well above threshold. Results thus far indicate fMRI can identify activation in a distributed olfactory response system, and may be able to identify differences in response to different types of odors. Results are preliminary and require larger samples and investigation of possible artifact.

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Functional Imaging of Olfactory Cortical Activity using Electrical and Magnetical Recordings in Combination with Magnetic Resonance Imaging

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We were able to demonstrate specific olfactory event-related potentials (OERP) in man recorded with an array of electrodes attached to the surface of the skull. Using different stimuli topographical analysis revealed that olfactory responses could be separated from trigeminal responses. While the peak amplitudes of the olfactory potentials were found at the parietal electrode Pz trigeminal responses had their maximum at the vertex elctrode Cz. The aim of the present study was to localize the underlying generators of the olfactory event-related potentials and analyze the size and orientation of the electric dipoles in order to explain the topographical distribution of the potentials recorded from the surface of the skull. Recently magnetoencephalographical methods have been developed and used to record olfactory event-related magnetic fields (OERMF). Although both, electric potentials and magnetic fields are epiphenomena of the transmembrane currents, ERMFs seem to be better suited for the localization of neuronal activity in the brain. Therefore the electrical recordings were combined with magnetoenecphalographic recordings and the coordinates of the identified dipoles were projected into the magnetic resonance image of the individual subjects.
Two types of magnetic recording systems were used. Both systems (a 37 channels magnetoencephalograph KRENIKON and a 122 channels magnetoencephalograph NEROMAG) were installed in a magnetically shielded chamber. 26 healthy volunteers (20 to 51 years) participated in the experiments. Stimulants (phenyl ethyl alcohol; hydrogen sulfide; vanillin) were presented with a stimulus duration of 200 ms and an interstimulus interval of 20-40 s. Records containing eye blinks were discarded from the average.
Analysis of the responses indicated that the dipoles in the cortical areas activated by olfactory stimuli were located in the temporal lobe and orientated towards the parietal electrode Pz, thus explaining the topographical distribution and, also, confirming the specificity of the olfactory event-related potentials.

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MR of Patients with Post-traumatic Olfactory Deficits. DAVID M. YOUSEM, RENA GECKLE, RICHARD L. DOTY (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery. University of Pennsylvania, Philadelphia, PA USA)

The sites of injury in patients with olfactory deficits after head trauma may be manifold and have not been readily evaluated by magnetic resonance (MR) imaging. Twenty patients with smell dysfunction after closed head injury were evaluated by olfactory testing, sinonasal endoscopy, and MR. The MR evaluation included surface coil analysis of the olfactory bulbs, tracts and subfrontal cortex by contiguous 3 mm T1-weighted and T2-weighted MR sections. A head coil was employed to evaluate the temporal lobes and hippocampal regions with 3 mm sections. Unilateral olfactory bulb and tract and temporal lobe volumes were determined from a three dimensional data set by 2 reviewers (to assess interobserver variation) in 2 separate settings (to assess intraobserver variation). The scans were also analyzed qualitatively by a neuroradiologist for damage to the olfactory bulbs, tracts, gyrus rectus-subfrontal regions, hippocampi, and temporal lobes. The volumetric and qualitative analyses were correlated with olfactory test results. Interobserver and intraobserver variation ranged from 3-5% for temporal lobe volumes, and from 15-20% for olfactory bulb and tract volumes. Bilateral olfactory bulb and tract injury was present in 85% of cases. Concurrent subfrontal injury was present in 75% of cases and temporal lobe injury was noted in 20%. The qualitative assessment of the olfactory bulbs and tracts correlated less well with scores on the University of Pennsylvania Smell Identification Test and an odor detection threshhold test than did quantitative values. Those patients with cortical injuries had greater deficits in smell discrimination than did those with only bulb and tract injury.This study demonstrates that olfactory bulb and tract injury with atrophy is the most common MR finding in patients complaining of post-traumatic smell dysfunction. Volumetric analysis has limited ability to predict sidedness to olfactory deficits or UPSIT scores, but is more accurate than a qualitative assessment. MR detects abnormalities in this population at a very high rate (90%), but the ability to predict recoverability of olfactory dysfunction by MR findings remains undetermined.

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Expression and Analysis of Subunits of the Olfactory Cyclic Nucleotide-gated Channel.
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Olfactory receptor neurons respond to odorant stimulation with a rapid and transient increase in intracellular cAMP that opens cyclic nucleotide-gated (cng) cation channels. Cng channels in rat olfactory neurons are activated by cAMP in the low micromolar range and are outwardly rectifying. The cloned rat olfactory cng channel (rOCNC1), however, is much less sensitive to cAMP and exhibits very weak rectification. We have investigated this discrepancy between native and cloned channels and have cloned a new rat cng channel subunit, denoted rOCNC2. rOCNC2 does not form functional channels when expressed alone in HEK 293 cells. When rOCNC1 and rOCNC2 are coexpressed, however, an outwardly rectifying cation conductance with cAMP sensitivity near that of the native channel is observed. In situ hybridization with probes specific for the two subunits shows they are coexpressed in olfactory receptor neurons. These data indicate that the native olfactory cng channel is likely to be a hetero-oligomer of the rOCNC1 and rOCNC2 subunits [Bradley et al. PNAS 91 8890 (1994)]. We have also obtained evidence that this hetero-oligomeric channel is permeable to Ca2+. Quantitative RT-PCR and in situ hybridization indicate rOCNC1 and rOCNC2 are also expressed in the olfactory bulb, cerebellum, cortex and hippocampus. We have used subunit specific antibodies to show localization of the channel proteins in sections from rat brain and in cultured hippocampal neurons. In addition, we have recorded cng channels with high sensitivity to cAMP and cGMP in excised inside out patches from these cultures. This is of interest in the hippocampus, because cAMP elevation has been implicated in one form of long-term potentiation (LTP). Nitric oxide (NO) is thought to be involved in another form of LTP, and cGMP can be elevated in response to the binding of NO to its effector guanylyl cyclase. Thus, the "olfactory" cng channel may be an electrophysiological sensor for cAMP or cGMP elevation, and Ca2+ flow through this channel could be a component of synaptic plasticity in the hippocampus.

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TITLE: Modulation of Olfactory Cyclic Nucleotide-Gated Cation Channel by Calcium-Calmodulin

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ABSTRACT: The apparent affinity of cyclic nucleotide for the olfactory cyclic nucleotide-gated cation channel (OCNC) is reduced by Ca^{2+} in the presence of a soluble factor (Kramer and Siegelbaum, Neuron 9, 897, 1992), constituting a mechanism for olfactory adaptation. Our experiments suggest that this soluble factor is likely to be calmodulin. Using excised, inside-out membrane patch recordings on both the cloned rat OCNC (rOCNC) expressed in HEK 293 cells and the native OCNC from rat olfactory receptor cells, we have found that Ca^{2+} reduces the apparent affinity of the channel for cyclic nucleotides by up to 20-fold in the presence of calmodulin. This affinity change appears to involve a direct interaction between Ca^{2+}-calmodulin (CaM) and the channel. Using chimeric channels composed partly of rOCNC and partly of the human retinal rod cyclic nucleotide-gated channel subunit 1, which does not show any CaM effect, we have identified the cytoplasmic N-terminal segment of rOCNC to be necessary for the CaM action. Using deletion mutants of rOCNC, we have further identified a small domain on this N-terminal segment that, when deleted, results in a decrease in cyclic nucleotide affinity comparable to that produced by CaM on the wild-type channel; moreover, this mutant loses its responsiveness to CaM. Separately, gel-overlay experiments have indicated that ^{125}I-labelled calmodulin binds to a fusion protein corresponding to the N-terminal segment of rOCNC in the presence of Ca^{2+}. Inspection of the amino acid sequence of the N-terminal segment suggests the presence of a putative CaM binding site that coincides with the important domain indicated by physiological experiments. Indeed, when this putative CaM-binding site was deleted, the fusion protein no longer binds CaM. Our interpretation of the above results is that there is a domain in the N-terminal segment of OCNC that is important for tight cyclic nucleotide binding, but its influence becomes removed when CaM binds to it or its vicinity. Finally, based on experiments with cAMP as ligand, it appears that the change in ligand affinity of the channel due to Ca^{2+}-CaM results from a change in the channel gating step, which is tightly coupled to the ligand binding step.

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Gaseous Messengers in Olfaction. RONNETT, GABRIELE, V.[1], ROSKAMS ANGELA-JANE[2], INGI, TATSUYA[3] (Johns Hopkins University School of Medicine, Baltimore, MD.)

ABSTRACT: Nitric oxide (NO), a short-lived free radical gas, is a biological messenger molecule that can activate soluble guanylyl cyclase by binding tightly to the heme moiety of the enzyme. Recent evidence suggests that CO, a chemically stable gas, may also function as a messenger molecule in the CNS. Like NO, CO combined to the iron in heme can activate guanylyl cyclase. HO consists of two homologous isoenzymes, type 1 (HO-1) which is induced by heme and most abundant in spleen and liver and type 2 (HO-2) the constitutive form highly expressed in brain. To investigate the potential roles of NO and CO in olfactory transduction/development, specific antibodies were used to localize the enzymes and assays were performed to investigate their regulation. Whereas neuronal nitric oxide synthase (NOS) is absent from mature adult neuroepithelium, HO-2 is present in olfactory receptor neurons (ORN's). These findings were confirmed by enzyme assay. However, NOS is highly expressed at early embryonic ages in ORN's and immunoreactivity diminishes prior to birth, and is replaced with immunoreactivity to HO-2. The potential consequences of this expression will be discussed. To investigate the generation of CO by neurons and its physiological functions, primary cultures of olfactory receptor neurons were studies as they contain a high density of heme metabolizing enzymes, including HO-2, and do not contain detectable amounts of NOS. To directly monitor the production of CO by neurons, olfactory receptor neurons are incubated [2-^{14}C] glycine, a heme precursor, to label CO produced by the action of HO-2. In ORN cultures, there is an immediate sharp increase in ^{14}CO release within the first days of culture. This peak in ^{14}CO production parallels cGMP levels, and both peaks are inhibited by zinc protoporphyrin 9 (ZnPP-9) a potent inhibitor of HO function. On the other hand, nitro arginine methyl ester (NAME), a potent inhibitor of NOS failed to significantly alter either parameter. Further studies were done to investigate the physiological significance of CO production. Collectively these results suggest that in the absence of NOS, CO may act as a regulator of cGMP levels. These results will be contrasted with evidence using cerebellar granule cell cultures, which express NOS, and HO-2. In this system, CO may have quite a different function. These results are discussed, as is the utility of the olfactory system in dissecting the roles of these gaseous messengers.

KEY WORDS: transduction
development
mammal

TOPIC 1: N Workshop abstract, Chairperson Kai Zinn

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Olfactory signalling and olfactory development in *C. elegans*.
CORI BARGMANN, JOE CHOU, CARA COBURN, PIALI SENGUPTA, and JEN ZALLEN (The University of California, San Francisco)

To understand the basis of olfaction in a simple system, we have been conducting genetic and cellular analysis in the soil nematode *C. elegans*. *C. elegans* has eleven types of chemosensory neurons which are known to respond to pheromones, attractants, and repellents. Chemotaxis to olfactory attractants is controlled by just two pairs of olfactory neurons, called AWA and AWC neurons. The AWA and AWC neurons each recognize several types of volatile attractants, including molecules that are discriminated by the animal.

Mutant nematodes that were unable to chemotax to particular odorants have been identified in direct genetic screens. In these studies we identified *odr-7*, a gene required for sensory function in the AWA neurons. *odr-7* encodes a transcription factor in the steroid receptor superfamily. *odr-7* appears to be directly involved in determining the sensory specificity of the AWA olfactory neurons, perhaps by controlling the expression of olfactory receptors and signalling molecules in the AWA neurons.

We have also identified four genes that are required both for olfactory signalling and for normal axon outgrowth of the chemosensory neurons. Our studies indicate that sensory activity contributes to axon outgrowth and target recognition in this simple olfactory system.

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Distribution and characterization of functional amiloride-sensitive sodium channels in rat tongue. RICHARD E. DOOLIN and TIMOTHY A. GILBERTSON (Pennington Biomedical Research Center and the Department of Zoology & Physiology, Louisiana State University, Baton Rouge, LA)

The role of amiloride-sensitive (AS) Na channels in the transduction of salty taste stimuli in rat fungiform taste buds has been well established. Evidence for the involvement of AS Na channels in salt transduction in circumvallate and foliate taste buds is, at best, contradictory. In direct contrast to Na salt responses recorded in the glossopharyngeal nerve which exhibit little or no amiloride-sensitivity[1], the presence of AS Na channel mRNA and protein has recently been localized to the circumvallate papillae using *in situ* hybridization and immunohistochemistry[2]. In an attempt to resolve this apparent controversy, we have begun to look for the presence of *functional* AS Na channels using giga seal whole-cell recording in taste receptor cells (TRCs) isolated from fungiform, foliate and circumvallate papillae of male Sprague-Dawley rats. Cells which exhibited voltage-dependent currents reflective of TRCs were subsequently tested for amiloride-sensitivity. TRCs were held at -70 mV and steady-state current and input resistance were monitored during superfusion of Na-free saline and salines containing amiloride (0.1 μM-1 mM). Greater than 90% of all TRCs from each of the papillae responded to Na replacement with a decrease in current and an increase in input resistance, reflective of a reduction in electrogenic Na movement into the cell. The mechanism for the Na influx appears to differ between taste bud types. Approximately 65% of fungiform, 35% of foliate, and <10% of circumvallate TRCs were amiloride sensitive. All amiloride sensitive currents had apparent K_d's in the submicromolar range. These results agree with afferent nerve recordings and raise the possibility that the extensive labeling of the AS Na channel protein and mRNA in the circumvallate papillae may reflect, at least in part, a pool of non-functional channels.

[1]Hanamori et al. *J. Neurophysiol.* 60:478, 1988; Frank *J. Neurophysiol.* 65:1459, 1991; Formaker and Hill *Physiol. Behav.* 50:765, 1991. [2]Li et al. *Neurobiol.* 91:1814, 1994; Simon et al. *Microsc. Res. Tech.* 26:196, 1993.

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Specificity of hamster fungiform taste cells. CHRISTI DANIELS & SUE C. KINNAMON (Dept. of Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 80523, Rocky Mountain Taste and Smell Center, University of Colorado Health Sciences Center, Denver, CO 80262)

Previous microelectrode studies have revealed that individual amphibian and mammalian taste cells are broadly tuned to various taste stimuli (Kimura & Biedler, J. Cell Comp. Physiol. 58:131-9, 1961; Sato, J. Cell Physiol. 80: 207-18, 1972); however, single fiber studies have shown that single afferent nerve fibers do show selectivity, particularly for salt and sweet tastes (Frank et al, J. Gen. Physiol. 91: 861-96, 1988). We have re-examined taste cell specificity using giga-seal whole cell recording of individual cells of hamster taste buds isolated according to the method of Béhé et al (J. Gen. Physiol. 96: 1061-84, 1988). Cells were held at -80 mV, and short hyperpolarizing voltage pulses were used to monitor membrane conductance to bath applications of taste stimuli. Only taste cells exhibiting voltage-dependent Na^+ and K^+ currents, characteristic of mature mammalian taste cells, were used to insure that all cells tested were taste receptor cells. We have previously shown that the synthetic sweetener NC-00274-O1 (NCO1) blocks a resting K^+ conductance; therefore, a decrease in K^+ conductance was indicative of a sweet response (Cummings, et al, J. Neurophys. 70: 2326-36, 1994). Salt taste is known to be mediated by amiloride-sensitive Na^+ channels; therefore, a decrease in membrane conductance in response to amiloride application was used to assess salt sensitivity. Tetraethylammonium chloride (TEA) was used as a control to test cell viability since it is known to block the resting K^+ conductance in all cells. Other than one cell which had a small response to both NCO1 and amiloride, many cells responded to amiloride or NCO1, but not both. A large subset of cells did not respond to either NCO1 or amiloride, however, all cells responded to TEA. These preliminary results suggest that specificity of taste responses may begin with individual taste cells.

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Field Potential- and Competition-Mediated Suppression of Chorda Tympani Responses to Mixtures of Sodium and Potassium Salts. Robert E. Stewart, Gerard L. Heck, and John A. DeSimone (Virginia Commonwealth University, Richmond, Virginia, USA)

Mechanisms of taste mixture interaction have generally eluded elucidation. With new understanding of taste transduction processes, these mechanisms are beginning to yield to investigation. Access of K^+ to basolateral transduction sites depends on electroneutral diffusion of K^+, and accompanying anions, through tight junctions into the paracellular space. Consequently, chorda tympani (CT) neural responses to K^+ salts exhibit practically no sensitivity to applied voltage perturbations. For example, when applied to the anterior tongue, 0.25M potassium gluconate (KG) induces a significant, positive transmural field potential, but does not stimulate a neural response. Due to the high voltage sensitvity of CT responses to Na^+ salts, we predicted that the large electropositve field potential associated with KG would act to suppress CT responses to sodium gluconate (SG), when applied in mixture. Indeed, CT responses to a mixture of 0.10M SG in 0.25M KG were reduced by 50% versus responses to 0.10M SG. However, attempts to compensate this suppression using in situ lingual voltage clamping indicated that field potential could account only partially for the response suppression observed. Remarkably, analyses of CT responses to SG alone and to SG+KG as a function of holding potential revealed that largely voltage-independent competition of K^+ with Na^+ for apical ion channels mediates predominantly suppression of SG responses in mixture with KG. These findings are the among first to describe a specific mechanism for a peripheral taste mixture interaction. Furthermore, these data indicate that taste buds may act as 'peripheral filters' due to the restricted topological distribution of transduction sites for different taste stimuli. Such 'filter' properties could have significant ramifications for both quality and intensity coding in the peripheral taste system.

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Effects of adapting the tongue with NaCl on taste-nerve responses. B.I. MACKINNON, A.J. SESSA, and M.E. FRANK (UCONN Health Center, Farmington, CT USA)

Recent data demonstrate effects of ions in saliva on the sensitivity of the taste receptors innervated by the *chorda tympani* nerve of rats and hamsters. In particular, in golden hamsters *(Mesocricetus auratus)*, adaptation of the tongue to salivary ions virtually eliminates multi-unit responses to quinine hydrochloride (QHCl) (Rehnberg *et al.*, 1992). Also, adding 3-30mM QHCl results in little increase in the magnitude of multi-unit responses to 50-250mM NaCl (Formaker & Frank, 1992). In the NaCl-QHCl mixture interactions, 50mM NaCl, as well as higher concentrations of NaCl, essentially eliminates evidence of a response to 3-30mM QHCl. We recorded the whole-nerve response of the hamster *chorda tympani* to 5mM, 10mM and 15mM QHCl after adapting the anterior portion of the tongue for 60s with deionized water, 0.05M NaCl, 0.10M NaCl, 0.15M NaCl or 0.30M NaCl. Our standard was the transient response to 0.05M NaCl. Adapting to 50mM or more concentrated NaCl solutions resulted in a transient response to QHCl that was 45% of the response to QHCl after water adaptation. The effect was similar for the three concentrations of QHCl. Thus there was a significant cross-adaptation between NaCl and QHCl. Adaptation to increasing concentrations of NaCl (0.0M, 0.01M, 50mM, 0.1M, 0.15M) resulted in decreasing transient responses to a 50mM increment in NaCl concentration. Thus, the transient did not accurately measure stimulus increases. For example, the response to 100mM after 60s adaptation to 50mM was 59% of the magnitude of the response to 60mM NaCl after adaptation to 10mM. The response to 150mM NaCl after adaptation to 100mM NaCl was 31% of the response to 60mM after adaptation to 10mM. Thus, in contrast to the NaCl-QHCl cross-adaptation, NaCl self-adaptation increases with the concentration of the adapting stimulus. Supported by NIH RO1 DC00058.

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The Chorda Tympani Nerve Response to Salt Stimulation is Temperature and Anion Dependent in Rats. ROBERT F. LUNDY, JR. AND ROBERT J. CONTRERAS (Florida State University, Department of Psychology, Tallahassee, FL., 32306-1051)

Prior studies demonstrate two important aspects of NaCl taste transduction: (1) amiloride-sensitive sodium channels play a major role; and (2) the response magnitudes of the chorda tympani nerve to sodium salts are temperature dependent. Few studies extend these findings to nonsodium salts differing in "quality" and associated anion. We investigated the effects of tongue adaptation temperature (25 vs. 35 °C) on the summated responses of the whole chorda tympani nerve to a range of salts mixed with and without 100 μM amiloride hydrochloride in male Sprague-Dawley rats. The stimuli consisted of 500 mM concentrations of NaCl, Na_2SO_4, sodium acetate (NaAc), NH_4Cl, KCl and potassium acetate (KAc). Each stimulus was presented twice: by itself for 45 s followed by a 90 s water rinse (protocol A), and secondly with the first 15 s by itself, the next 15 s mixed with amiloride, and the last 15 s by itself again with uninterrupted flow (protocol B). Percent amiloride suppression was calculated as the response ratio at times: A30/A15 ÷ B30/B15 X 100. In the absence of amiloride, the phasic response magnitudes of the chorda tympani nerve were smaller for all stimuli except KCl and KAc at 25 °C than at 35 °C. Similarly, the tonic response magnitudes to the same stimuli, except NaCl, were also smaller at 25 °C than at 35 °C. Amiloride suppressed the chorda tympani nerve responses to all stimuli so that NaAc>Na_2SO_4=KAc>NaCl>KCl=NH_4Cl. However, the degree of amiloride suppression for three salts, NaCl, Na_2SO_4, and NH_4Cl was greater at 25 °C than at 35 °C. Amiloride completely suppressed the chorda tympani nerve responses to NaAc at 25 and 35 °C. Substituting sulfate for chloride in sodium salts, and acetate for chloride in in both sodium and potassium salts increased amiloride suppression. However, the acetate anion in sodium salts abolished the temperature dependence of amiloride suppression. Taken altogether, the findings suggests that: (1) the degree of amiloride suppression of the chorda tympani nerve to salt stimuli is a function of lingual mucosal temperature and the associated anion; and (2) amiloride's ability to block ion channels is either not as specific as previously suspected or many salt stimuli express various affinities for amiloride-sensitive Na^+ channels.

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Chronic Recordings From Fibers of Rat Glossopharyngeal Nerve Regenerated Through a Micromachined Sieve Electrode Array. R. M. BRADLEY[1], T. AKIN[2], S. GURKAN[1], X. CAO[1], and K. NAJAFI[2]. (Dept. Biologic & Materials Sciences, School of Dentistry[1] and Center for Integrated Circuits and Sensors, College of Engineering[2], University of Michigan, Ann Arbor, MI 48109-1078.)

We have shown that when a thin, micromachined, silicon diaphragm containing a large number of 2μm holes is implanted between the cut ends of the glossopharyngeal nerve, fibers will regenerate through the holes to functionally reinnervate gustatory and somatosensory receptors on the posterior tongue. We have now recorded from electrode sites surrounding 5 of the holes, connected via a silicon ribbon cable that is integrally fabricated with the sieve electrode and bonded to a headcap on the rat's skull. The impedance of the electrode sites is measured by passing a 1 kHz sine wave through the animal and measuring the amplitude of the signal transmitted via the electrode sites. Every week after implantation we briefly reanesthetize the rat and measure electrode impedance and noise levels. To date we have implanted 37 animals and recorded from the electrode sites in 20 of these for an average of 12 weeks (range 6-17) prior to termination. The remaining animals have been implanted for less time or terminated for various technical reasons without any attempt to record. Electrode impedances remain stable over the entire period of recordings and noise levels range from 20-30 μV. After 12 weeks of implantation evoked responses have been recorded from all the electrode sites to mechanical stimulation of the tongue. Rapidly adapting, few fiber responses are recorded when the tongue is stroked with a blunt rod. These responses can be recorded on subsequent weeks. In some animals in which evoked recordings could not be obtained after 12 weeks of implantation it was found that the implant did not contain a nerve. To date no evoked responses have been recorded to thermal or chemical stimulation.

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Single Fiber Recordings from the Chorda Tympani and Glossopharyngeal Nerves of Rhesus Monkeys. GÖRAN HELLEKANT,YUZO NINOMIYA+, VICKTORIA DANILOVA,and THOMAS ROBERTS (University of Wisconsin and Wisconsin Regional Primate Center, Madison, USA. +Asahi University, Gifu Prefecture, Japan).

Responses were obtained in approximately 30 single taste fibers in the chorda and glossopharyngeal nerves of rhesus monkeys during stimulation with about 25 different tastants representing the salty, umami, sour, bitter and sweet taste qualities. While the specificity of the fibers was not as pronounced as in the chimpanzee, the fibers were clearly grouped according to the above taste qualities. Interestingly a majority of the chorda tympani fibers belonged to the salty and sweet groups while only two fibers gave a response that was best to the bitter compounds and 3 fibers responded best to sour stimuli. This is in contrast to observations in the glossopharyngeal nerve where an overwhelming majority of the fibers responded best to sour and bitter compounds, while only a few fibers responded to NaCl and none could be designated as only sweet fiber. This shows clearly that in the monkey sensitivity to the four taste qualities are distributed in different areas of the tongue. If the same partition occurs in the taste nerves from the human tongue, these findings could be considered as the neurophysiological basis for the division, based on introspective and psychophysical observations, of the tongue into an anterior area, primarily sensitive to sweet and salt and a posterior sensitive to sour and bitter.

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Novel Mediation of Peripheral Taste Function by the Immune System. LYNNETTE M. PHILLIPS and DAVID L. HILL (Univ. of Virginia). Taste receptors newly formed after unilateral nerve cut in adult sodium-restricted animals exhibit abnormal function. Specifically, the chorda tympani (CT) nerve of adult rats that receive CT section and maintenance on a low sodium diet (0.03% NaCl) shows a selective and profound reduction in taste responses to sodium salts after neural regeneration. Perhaps even more surprising is that the function of the contralateral, intact CT nerve is also altered. Shortly after nerve section, the uncut CT demonstrates attenuated responses to sodium stimuli. However, the response of the intact nerve to sodium progressively increases until it reaches a supersensitive level approximately seven weeks after the contralateral nerve section. We hypothesized that this interaction between the intact and denervated taste receptors is related to the local release of immune system-derived factors following nerve section and subsequent degeneration. While control animals are able to utilize the immune system to promote normal gustatory responses following neural degeneration, rats on a low sodium diet may not maintain normal immune function. Thus, if immune function could be augmented in sodium-restricted rats, the reduction in the intact nerve response to sodium soon after contralateral CT section should be ameliorated. To examine this possibility, adult rats received unilateral CT section and were placed on a sodium-restricted diet, as in the original experiment. However, the following day rats were also injected with 100 µg (i.p.) of lipopolysaccharide (LPS), an endotoxin of gram-negative bacteria that induces activation of immune-derived cells and cytokine production. CT recordings from the intact nerve were then performed 4-9 days after contralateral nerve section and LPS injection. Indeed, stimulation of the immune system with LPS reversed the functional changes normally induced by nerve section and sodium restriction, so that experimental animals displayed normal sodium sensitivity. These results are the first that we are aware of to demonstrate an interaction between gustatory function and the immune system.

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Spectral Tuning Properties of the Walking Legs of the Red-Jointed-Fiddler Crab. THOMAS J. TROTT, RAINER VOIGT, AND JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

The red-jointed fiddler crab *Uca minax* (Le Conte) is one of the most abundant macroinvertebrates inhabiting salt marshes of the temperate western Atlantic coast. *Uca minax* is semiterrestrial and prefers low salinity to fresh water habitats. It feeds on a wide variety of food items including meiofauna and, unlike other species of *Uca*, decomposing flesh. Chemical stimulation of the dactyls of the walking legs initiates feeding behavior. However, nothing is known about the spectral sensitivities of chemoreceptor cells on the walking legs of *U. minax*. Therefore, we measured the responses of chemoreceptor cells on the second and third pair of walking legs with compounds present in the crab's natural diet. In addition we tested structural analogues of these compounds. Tuning properties were assessed as the number of action potentials elicited by a 50 µl aliquot of each compound at 10^{-3} M; standard electrophysiological methods were applied using suction electrodes on afferent axons. Chemoreceptor cells responded strongest to L-glutamate followed by glucuronic acid, citric acid, and ammonium chloride. Amines were moderately stimulatory. Surprisingly, in contrast to behavioral observations, carbohydrates, i.e., hexose sugars, elicited only small responses. This study is the first attempt to describe the spectral tuning properties of dactyl chemoreceptor cells of *U. minax* electrophysiologically with compounds known to be behaviorally stimulatory in other fiddler crabs.

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Citrate enhances behavioral and cellular gustatory responses to sweet and amino acid stimuli in mammals. TIMOTHY A. GILBERTSON[1,2], DONNA M. GILBERTSON[1] W. TODD MONROE[1], JODI R. MILLIET[1], and JOHN CAPRIO[2] ([1]Pennington Biomedical Research Center and the [2]Department of Zoology & Physiology; Louisiana State University, Baton Rouge, LA)

The role of citric acid as an acidulant in food processing has been well established, acting both to enhance flavor and to buffer pH. However, there is little or no information about how citrate ions act or the mechanism by which they affect taste perception. In two- and four-day two bottle preference tests, addition of citrate (1-5 mM; pH 7.0) significantly enhanced the preference for sucrose and saccharin in a dose-dependent manner in both rats and hamsters. Citrate, however, did not affect the preference for, nor the avoidance of, salty (NaCl), bitter (quinine, denatonium), or acidic (HCl, acetic acid) taste stimuli and was itself neither preferred nor avoided relative to water. Citrate did enhance significantly the preference for the " sweet " amino acids, alanine and glycine. When faced with a 24 hour " forced choice " between sweet compounds in the absence or presence of citrate, those containing citrate were significantly preferred. Water-deprived rats, conditioned to avoid saccharin by pairing it with interperitoneal LiCl injections, completely rejected saccharin and, to a lesser extent, sucrose. Addition of citrate to these solutions reduced the rejection of these compounds, suggesting that citrate may not be merely intensifying sweet perception. In an attempt to elucidate the cellular mechanism, if any, for the effect of citrate, we have begun giga-seal patch clamp recording on isolated rat fungiform taste receptor cells (TRCs). In current clamp recording, applications of saccharin (20 mM) and glycine (50 mM) elicit action potentials in isolated TRCs. Addition of citrate to these solutions causes increases in action potential firing compared to the control response. In voltage clamp mode, citrate increases the holding current at -80 mV, consistent with causing a net depolarization of the TRCs. We are currently investigating the mechanism by which citrate depolarizes rat fungiform TRCs.

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Citrate Enhances Glossopharyngeal Taste Responses to Arginine in the Largemouth Bass.
K. OGAWA AND J. CAPRIO (Louisiana State University, Baton Rouge, LA. 70803)

Citric acid is used extensively in food processing as an acidulant, serving a variety of functions including that as a flavor enhancer; however, little is know concerning its specific physiological effects on the sense of taste. We report here that glossopharyngeal (IX) taste responses of the largemouth bass, *Micropterus salmoides*, to the highly stimulatory amino acid, L-arginine (Arg) is selectively enhanced by the citrate ion (Na_3citrate), which is itself poorly to nonstimulatory at the pH and concentrations tested. Enhancement of integrated multiunit IX taste activity occurred in response to binary mixtures of Arg (0.1 - 1.0mM) and citrate (0.1mM-1.0mM) at pH 7-9. The pH of the test solutions was critical for the enhancement effect as taste responses to the same binary mixtures tested at pH 4-6 were not significantly different than responses to Arg alone. Similarly, the concentration of Arg within the binary mixture was equally critical as 10mM or 100mM Arg mixed with 1mM citrate was only as stimulatory as Arg alone. Of eight other hydroxy acids tested at 0.3mM in a binary mixture with 1mM Arg, only cis-aconitic and isocitric acids resulted in taste responses of greater magnitude than that to Arg alone; however, taste enhancement induced by these other hydroxy compounds was only approximately 70% of that produced by citrate. Under continuous adaptation to 1mM citrate (pH 9.0), taste responses to ca. 0.1-1.0mM Arg solutions were enhanced similarly as with the previously described binary mixtures (i.e. brief simultaneous applications of Arg and citrate); however, under continuous adaptation to 1mM citrate, taste responses to 10mM and 100mM Arg were significantly suppressed compared to the taste response to Arg alone in the absence adaptation to citrate. The mechanisms for these two distinct effects of citrate are currently unknown.

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Expression of Sweet Protein Brazzein by *Saccharomyces Cerevisiae*.
ZHENGYU GUAN, GORAN HELLEKANT, WEI YAN (University of Wisconsin, Madison, WI.).

Brazzein, is a protein purified from the fruit of *Pentadiplandra brazzeana* Baillon, a tropical plant from west Africa, and has commercial potential as a high-potency sweetener. Due to among other facts difficulties attaining raw material, we decided to express brazzein in yeast *Saccharomyces cerevisiae*, a preferred host for production of protein for consumption. Synthesized brazzein gene SW-3 in the plasmid pGEX-3X from the E. coli strain was cloned into the shuttle vector pRD56 and expressed in *S. cerevisiae*, yielding GST (Glutathione-S-Transferase)-brazzein fusion protein. Purification was achieved by: passing yeast extract through affinity glutathione column; eluting GST-brazzein with the treatment of glutathione (reduced form) ; thrombin cleavage; and once more passing through the glutathione column. This is a modification of the conventional one step purification procedure because thrombin can not cleave the fusion protein when it is bound to the column. This may due to the configuration of the fusion protein. Polycolonal antibody and protein sequencing verified that the expressed brazzein was the correct gene product. We are in the process of producing more brazzein for sensory analysis.

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Time-Course of Bitter-Induced Levels of IP_3 and cAMP in Mouse Taste Tissue. GULSHAN SUNAVALA, MAX DASSO and ANDREW I. SPIELMAN (New York University College of Dentistry, New York).

Bitter taste is elicited by a diverse group of chemicals, and it is thought that several mechanisms of signal transduction exist for this taste modality. Previous studies have shown that IP_3 is one of the second messengers in bitter taste of sucrose octaacetate and denatonium.

Using a rapid quench flow module (QFM5) we have screened a number of bitter compounds including strychnine (STR 10 mM), caffeine (CAF, 50 mM), denatonium (DEN, 10 mM), guaifenesin (GUA, 10 mg/ml) and cetylpyridinium HCl (CET, 0.9 mg/ml). We have monitored production of IP_3 and cAMP over a time course of 25-500 msec in taste and nongustatory tissue taken from C57BL/6J mice. All 5 compounds induced an increase in IP_3 but the peak of maximum production varied from 50 msec to 200 msec, dependent on the compound. GUA induced an IP_3 peak around 50 msec, STR, and CET at around 75-100 msec, while CAF induced a peak at around 200 msec. Denatonium at 10 mM induced the production of a double IP_3 peak, one at 50 msec and another at 100 msec. Neither the basal nor the nongustatory control tissue elicited a significant change in the time course of IP_3.

The variation in peak IP_3 levels induced by diverse bitter compounds and their time-course imply different peripheral mechanisms of signal transduction for these compounds.

When denatonium and strychnine (10 mM each) were combined the IP_3 levels equaled the sum of the two individual stimulations, demonstrating independent mechanisms.

cAMP levels were also monitored for DEN, STR, GUA and the combination of DEN+STR, and found not to be affected in any of the compounds.

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Forskolin Closes Potassium Channels In Taste Cells Via A cyclic AMP Independent Mechanism. XIAO-DONG SUN, YUSHE CHEN, and SCOTT HERNESS. Indiana University School of Medicine, Ctr. Med. Ed., BSU, Muncie, IN 47306.

Forskolin (FSK) is commonly used to increase cytosolic levels of cAMP via its stimulatory effect on adenylate cyclase. Increased cAMP in taste cells causes closure of potassium channels and depolarization of the membrane potential. However, in many cells FSK is known to directly block potassium channels independent of adenylate cyclase or cAMP. We investigated this possibility in dissociated posterior rat taste cells using the patch clamp recording technique in the whole cell configuration. Various methods of raising intracellular cAMP levels (cAMP, di-butyl cAMP, IBMX) result in approximately 33% diminution of potassium currents over tens of minutes time scale. FSK (50 μM) however, causes almost complete blockage of the current within a few minutes of administration. Moreover, blockage is complex, leaving a large unaffected transient portion followed by a mostly blocked sustained portion. Unlike other cAMP manipulations the FSK affect was easily reversed with washout. This pattern is very reminiscent of OPEN CHANNEL block of potassium channels by intracellular TEA originally proposed by Armstrong (1969). We tested the FSK analogue 1,9 dideoxy FSK which does not stimulate adenylate cyclase but does produce direct channel blockage. The analogue produced current records virtually identical to FSK application. Finally since cAMP blockage of potassium channels is thought to depend on phosphorylation, then if FSK operates via cAMP its effect should be blocked by inhibitors of protein kinase A, such as H8. H8 was effective in blocking cAMP effects in taste cells. The diminution of potassium currents produced by IBMX (100 μM) could be prevented by prior exposure to 100 μM H8. However, this same concentration of H8 was without effect on the FSK response. We conclude that in taste cells FSK has its major effect directly on potassium channels.
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Partial Purification of a Bitter Receptor that Activates Gustducin and Transducin from Taste Membranes. LUIS RUIZ-AVILA and ROBERT F. MARGOLSKEE (Roche Institute of Molecular Biology, 340 Kingsland Street, Nutley, NJ 07110).

Bitter and sweet tastes are believed to be transduced through heterotrimeric G proteins, which couple taste cell membrane receptors to intracellular effector enzymes. The taste receptors have not yet been functionally characterized nor molecularly cloned. Taste cells preferentially express a few G protein alpha subunits; notably transducin and gustducin (which is absolutely taste specific). These two proteins are closely related at the amino acid level, and have been shown to activate a taste-specific phosphodiesterase (McLaughlin et al., submitted), thereby regulating the concentration of cyclic nucleotides inside the taste cells and consequently modifying the activity of cyclic nucleotide-regulated channels also present in taste (Kolesnikov and Margolskee, *Nature*, in press). We have reconstituted a taste responsive system using a bovine circumvallate papillae membrane preparation and purified bovine rod transducin or recombinant gustducin as exogenously added reporters. We show here that both transducin and gustducin are specifically activated by receptors present in taste membranes. A preliminary characterization of these receptors will be presented.

L. R-A. is recipient of a postdoctoral fellowship from the spanish MEC-Fulbright program.

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A Cyclic Nucleotide Suppressible Conductance is the Target of the Transducin-PDE based Taste Transduction Cascade

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We have found that rod transducin is present in mammalian taste receptor cells where it is activated by taste receptor and in turn activates a phosphodiesterase (PDE) (see Ruiz-Avila et al, these abstracts). Introduction into frog taste receptor cells of peptides derived from transducin's PDE interaction region caused an inward whole-cell current in a subset of cells. The peptides' effects were reversibly suppressed by IBMX and forskolin, suggesting a transducin-activated PDE. Cyclic nucleotides (cNMPs) suppressed the whole-cell current, indicating that cNMPs may regulate taste receptor cell conductance. IBMX modified taste receptor cell responses to two taste stimuli (saccharin and NC-01), implicating PDE in taste transduction. Submicromolar cNMP directly suppressed the conductance of inside-out patches derived from the taste receptor cell plasma membrane independently of protein phosphorylation. The channels are unusual in that they are suppressed, rather than activated by cNMP. We propose the following model: tastant activated receptor activates transducin; activated transducin in turn activates cAMP specific PDE. The ensuing drop in TRC cAMP concentration activates the cNMP suppressible channel leading to depolarization, increased Ca^{2+} influx and elevated intracellular Ca^{2+}. Our model contrasts with proposals for sweet taste transduction that implicate G_S, adenylyl cyclase, elevated cNMP and K^+ channel phosphorylation. It may be that both of these pathways are utilized in different subsets of TRC. Alternatively, saccharin and NC-01 may stimulate bitter transduction in frog taste cells. We are presently testing mammalian taste receptor cells to determine if they also contain the cNMP-suppressible channel.

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Characterization of Inward Rectifying Potassium Current in Rat Taste Receptor Cells. SCOTT HERNESS, XIAO-DONG SUN, and YUSHE CHEN. Indiana University School of Medicine, Ctr. Med. Ed., BSU, Muncie, IN 47306.

Inward rectifying potassium channels are unique from other potassium channels because their conductance increases with hyperpolarization rather than depolarization and they pass inward rather than outward current. They are important in setting the resting potential and in permitting long depolarizing responses. We describe inward rectifying currents carried by potassium from dissociated posterior rat taste cells using patch-clamp recordings in the whole cell configuration. Cells were held at zero current potential and hyperpolarizing or depolarizing voltage steps applied under voltage-clamp conditions. Shifts the voltage dependence of gating occurred with changes in external potassium (3mM, 10mM, 30mM, or 100mM). Currents displayed an e-fold *decrease* in conductance for each 5 to 11 mV of depolarization or an equivalent gating charge of 2.3 to 4.8 elementary charges placing them closer to classical steep inward rectifiers rather than mild. Current magnitude was highly dependent on external potassium concentration with inward current magnitudes reaching up to -2 nA. The reversal potential closely followed a Nernstian relationship to external potassium and varied from -97 mV to -9 mV. Whole cell conductance varied from 2 nS to 10 nS. The inward rectifying current was blocked by both external barium and cesium at concentrations of 10 μM and higher. The block demonstrated concentration and voltage-dependence with an asymmetric 'U'-shaped current-voltage relationship with a nadir occurring in the range of -100 to -120 mV, typical for FLICKERING BLOCK of a multi-ion pore. Changes in external sodium, which has been demonstrated to influence gating in some inward rectifiers, had little effect on these currents. Inward rectifying currents carried by potassium appear to be common in taste receptor cells and may play an important role in maintaining the resting potentials of these cells.

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Basolateral Chloride Conductance in Rat Fungiform Taste Cells. STACEY L. WLADKOWSKI[1], WEIHONG LIN[2,3], MARTHA MCPHEETERS[2,3], SUE C. KINNAMON[2,3], AND SHEELLA MIERSON[1] (Univ. of Delaware[1], Newark, DE, Colorado State Univ.[2], Fort Collins, CO, and Rocky Mountain Taste and Smell Center[3], Denver, CO.)

Chloride channels are found in other sensory receptors, but have not been observed previously in mammalian taste cells. We report here that niflumic acid (NFA) and flufenamic acid (FFA), nonsteroidal anti-inflammatory aromatic compounds known to inhibit Cl^- conductances, affect transepithelial and whole-cell currents in rat fungiform taste cells. In the intact *in vitro* dorsal anterior rat tongue epithelium under voltage clamp in an Ussing chamber, both NFA and FFA reversibly inhibited the transepithelial short-circuit current (I_{sc}) when added to the submucosal but not to the mucosal solution. For both compounds, I_{sc} was inhibited over the entire mucosal NaCl concentration range (0.01-0.5M). With Krebs-Henseleit buffer on both sides of the tissue, 100μM NFA decreased I_{sc} by 30±5% (mean & S.E.M., n=8). In whole-cell voltage clamp recordings from isolated fungiform taste cells, current was recorded in response to voltage ramps in the presence and absence of NFA. In Tyrode's solution containing normal Cl^- (136 mM), 100μM NFA caused a reversible decrease in outward current and in membrane conductance. In both Ussing chamber and patch-clamp experiments, the effects of NFA were mimicked by replacement of bath Cl^- with methanesulfonate or gluconate. In low Cl^- Tyrode's (18 mM), the effect of NFA on whole-cell current was reduced. Based on the effects of NFA and FFA in other types of cells, it is likely that rat taste cells contain a basolateral Ca^{2+}-dependent Cl^- conductance, perhaps similar to that found in *Necturus* taste cells (Taylor & Roper, *J. Neurophysiol.*, 72:475-478, 1994). Further experiments will be required to determine the role of this conductance in taste transduction.

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Responses to Monosodium Glutamate in Mouse Taste Cells.

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The taste system of C3H strain mice is sensitive to the umami substances, monosodium glutamate(MSG) and 5'-ribonucleotides. Recordings from bilayers containing membrane fragments from vallate and foliate taste papillae of C3H mice suggested that the taste response to MSG may be mediated by cation channels directly activated by MSG (Kumazawa, et al., AChemS abstract 1994). We have recorded voltage-dependent and MSG-activated currents from vallate and foliate taste cells enzymatically isolated from tongues C3H mice. MSG-induced changes in intracellular Ca^{2+} ($[Ca^{2+}]_i$) concentration were recorded from some taste cells using Fura-2. Stimuli were applied from a puffer pipette. Whole-cell recordings revealed two types of taste cells; some cells displayed both inward and outward voltage-dependent currents, while others displayed only outward currents in response to depolarizing voltage steps from -80 mV. MSG (10 mM) induced membrane depolarization under whole-cell current-clamp in some cells. MSG induced changes in $[Ca^{2+}]_i$ were also observed in some taste cells. In the cell-attached configuration, MSG induced increases in outward current in 3 of 17 cells.

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Artificial sweeteners and denatonium increase $[Ca^{2+}]_i$ in isolated hamster taste cells. TATSUYA OGURA[1,2], PETER GUTHRIE[1] & SUE C. KINNAMON[1,2] ([1]Dept. Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523, and [2]the Rocky Mountain Taste and Smell Center, University of Colorado Health Science Center, Denver, CO 80262)

An increase in $[Ca^{2+}]_i$ has been proposed as a trigger of transmitter release as the final step in the mechanism of transduction in taste cells. In this study, we used Ca^{2+} imaging with fura-2 to detect $[Ca^{2+}]_i$ changes in isolated taste cells from hamster fungiform papillae in response to taste stimuli. Whole taste buds were isolated by the method of Béhé et al. (1990) and loaded with the membrane permeable Ca^{2+}-sensitive dye, fura-2 AM. Two artificial sweeteners, NC-00274-01 (NC01) and SC-45647 (both 300 µM), increased $[Ca^{2+}]_i$ in a subset of taste cells. After stimulation, $[Ca^{2+}]_i$ increased approximately 10-50 nM from a resting $[Ca^{2+}]_i$ level of 50-100 nM. The cells that responded to sweeteners also responded to 8cpt-cAMP (1 mM), a membrane permeable analog of cAMP. This result is consistent with our previous electrophysiological data which showed that both NC01 and cAMP depolarize taste cells (Cummings et al., *J. Neurophysiol.* 70:2326-2336, 1993) by decreasing K^+ currents (Cummings & Kinnamon, *Chem. Senses* 16:511, 1994). These data support the hypothesis that cyclic nucleotides are involved in the sweet transduction mechanism. Interestingly, more than half of the cells that responded to sweetener also responded to 300 µM denatonium, a bitter substance. Previous behavioral experiments showed hamsters drink water containing 500 µM denatonium (unpublished data). These results suggest that bitter stimuli may be perceived differently in hamster as compared to other animals.

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Changes in Intracellular Ca^{++} in Isolated Catfish Taste Cells Induced by Changes in Extracellular K^+ and Na^+.

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Substitution of K^+ for extracellular Na^+ is used routinely to induce membrane depolarization in taste cells (Akabas et al., Science 242:1047-1050, 1988, Orola et al., Acta Otolaryngol 112:120-127,1992). We measured changes of intracellular Ca^{++} ($[Ca^{++}]_i$) in isolated catfish taste cells upon the introduction of isomolar solutions containing various concentrations of Na^+ and K^+. Some taste cells exhibited the expected increase in $[Ca^{++}]_i$. However, most of the cells, exhibited a decrease rather than an increase in $[Ca^{++}]_i$. Similar decreases were observed when $NMDG^+$ was used in place of K^+. Since the high $[K^+]$ solutions contained low $[Na^+]$, these observations suggest that the responses were elicited by the removal of Na^+ rather than the increase of K^+. Valinomycin, a K^+-specific ionophore, was used to clamp the membrane potential to E_K. When added in normal fish Ringer's solution (110 mM K^+), valinomycin induced increases in $[Ca^{++}]_i$. Elevation of extracellular $[K^+]$ to 30 mM (80 mM Na^+) in the presence of valinomycin consistently caused a further increase in $[Ca^{++}]_i$. The apparent differences between the responses induced in the presence and absence of valinomycin, together with the similarity between the responses in high K^+ solution and $NMDG^+$ solution in the absence of valinomycin, can be explained assuming that the membrane potential of catfish taste cells is normally more negative than E_K and that $[Ca^{++}]_i$ increases upon cell depolarization.

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Glutamate Chemoresponse In Paramecium. X. LI[1], W. Q. YANG[3], J. YANO[1], C. BRAUN[2], H. PLATTNER[2], W. E. BELL[1], J. VAN HOUTEN[1], [1]Dept. of Biology, University of Vermont, Burlington, VT 05405 USA, [2]Universität Konstanz, Germany, and [3]NHLBI, Bethesda, MD.

Glutamate is an attractant of *Paramecium tetraurelia*. Cells swim smoothly and rapidly to congregate in areas of glutamate. We have shown that the cells bind glutamate specifically and saturably and that there are multiple glutamate binding sites. Therefore, we are taking a molecular genetic approach to the identification of a receptor for glutamate. In order to design primers based on literature descriptions of neurotransmitter receptors, it seemed necessary to determine whether the receptor could be G protein coupled (metabotropic) or ion channel receptor (ionotropic). We had previously found that intracellular cAMP increased rapidly with glutamate stimulation, implicating involvement of metabotropic receptors. The pharmacology of binding and cAMP response do not help us to distinguish between potential metabotropic or ionotropic glutamate receptors and we were limited in our measurements of cAMP at that time to a first time point of 30 sec. It was imperative to measure cAMP, at much shorter time periods if we were to verify that cAMP could rise rapidly enough to be part of the stimulus pathway as opposed to desensitization pathway, thus making G protein coupling to adenylyl cyclase a possibility. We found that cells rapidly increase intracellular cyclic AMP in response to glutamate. Within 30 msec there is a measurable increase in cAMP, and a seven fold increase by 100 m sec. This gradually declines onver time to basal levels by 10 min. Hyperpolarization increases intracellular cAMP and was used as a control. The kinetics of the increases induced by glutamate stimulation and hyperpolarization in low K were quite different. There was no apparent change in cGMP levels.

We have generated primers for both classes of receptors. To date, we have found candidate PCR products for ionotropic receptors receptors only.

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Saline Induced C-Fos-Like Expression In Sodium Restricted And Replete Rats. B.R. WALKER and D.L. HILL (University of Virginia, Charlottesville, Va 22903)

Previous work from our lab has shown that dietary sodium restriction instituted early in prenatal development produces anatomical and functional changes in the developing gustatory system. In particular, neurophysiological recordings from the chorda tympani nerve (CT) demonstrate that whole nerve responses to NaCl are reduced in restricted rats while responses to non-salt stimuli are unaffected. Furthermore, early sodium restriction causes a rearrangement of the CT terminal fields within the nucleus of the solitary tract (NTS) in restricted rats, while glossopharyngeal (IX) terminal fields remain similar to controls. Developmental sodium restriction also produces postsynaptic changes in the NTS. For example, neurophysiological NTS recordings of restricted rats repleted with sodium show hyper-responsive activity to only sodium salts, while Golgi techniques have revealed dendritic alterations of the presumptive projection neurons from the NTS.

To examine early postsynaptic cellular events related to ingestion of NaCl, immunocytochemical techniques were used to detect the expression of *c-fos-like* in the rostral pole of the NTS following ingestion of 0.9% saline in control and developmentally, sodium-restricted and replete rats. Previous results (Soc. Neurosci. Abstr. 20:117, 1994) show a similar topography of *c-fos-like* labelled cells between restricted and control rats; however, sodium restricted rats show 5X more labelled cells as compared to controls. The topography and number of labelled cells in sodium replete rats were compared to these previous groups. These results support the neurophysiological data of our model of central nuclei alterations in response to developmental sodium restriction.

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Distribution of calbindin D-28k immunoreactive neurons in gustatory NST and PBN of the rat. THERESA A. HARRISON, GERNOT S. DOETSCH AND NANCY W. MILLER. (Medical College of Georgia, Augusta, GA)

The calcium binding protein, calbindin D-28K (CaBD), has been identified within structures of the taste pathway at all levels of the CNS (Celio, 1990). We have examined the detailed distribution of neurons expressing CaBD immunoreactivity within the rostral nucleus of the solitary tract (NST) and in the parabrachial nucleus (PBN). Paraformaldehyde-fixed frozen sections of male rat brains were reacted with anti-CaBD (Sigma), using standard immunocytochemical procedures. CaBD-reactive cells were visualized with HRP or alkaline phosphatase, using Vector kits. In the rostral NST, labeled cells were sparse; the most rostral tip of the nucleus was virtually devoid of reactive cells. More caudally, scattered cells were seen, but the majority were located in the lateral subdivision of the nucleus, with few in the more medial, taste-responsive zone. In the PBN, in contrast, areas showing high levels of anti-CaBD reactivity were found which corresponded closely to those regions known to respond to gustatory stimulation. These same PBN regions receive projections from gustatory NST (Travers, 1993) and label with anti-Fos antibody after electrical stimulation of taste nerves (Harrison & Miller, 1994). In other experiments, NST output neurons were retrogradely labeled by injecting fluorescent latex microspheres or biotinylated dextran into the PBN gustatory responsive zone. After subsequent processing for CaBD, very few double-labeled cells were found in the NST. Thus, while calbindin D-28k -containing cells are abundant in the gustatory zone of the PBN, such cells are seldom observed in those regions of the NST which are most responsive to gustatory input, and which project rostrally to the CaBD-rich PBN.

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Immunohistochemical Localization of Protein A1 in Geniculate Ganglia and Medulla. PHILLIP S. LASITER[1] and G. W. PERRY[2] (Department of Psychology[1], Center for Complex Systems[2], Florida Atlantic University).

Our recent studies have shown that damage to fungiform receptors alters the concentration of protein A1 in geniculate ganglion neurons. Protein A1 has been characterized in our studies as a glycoprotein (*Mr* 62-64 kD; *pI* 4.8 - 5.2) that is relatively abundant in geniculate ganglion neurons, as compared to gasserian ganglion neurons, cerebellum, anterior pituitary, optic nerve, and cervical spinal cord. Specific aims of the present study were to generate immunological probes to identify the histological location of protein A1 in anterior tongue, geniculate ganglia, and other central neural regions. Protein A1 was purified from anterior tongue epithelium homogenates by first performing 2D-PAGE procedures. Following staining/fixation and destaining, protein A1 spots were excised, equilibrated with SDS, electroeluted, and dried under TRIS/MeOH buffer (20% MeOH; pH 6.8). BALB/c mice received between 10 and 15 immunizations with approximately 140-280 ng of purified protein A1/injection. Western blot and immunohistochemical studies were performed with polyclonal sera obtained during the course of immunizations, and when maximal immunologic responses were obtained spleen immunocytes were fused with mouse myeloma cells for production of monoclonal antibodies. Current results have been obtained by the use of *polyclonal antisera* that reacts with purified protein A1 and 2-3 three distinct bands (Mr 50 kD - 70 kD) isolated from anterior tongue epithelium homogenates. In the CNS, anti-protein A1 reacts with a limited subset of astrocytes, mainly at somatic membranes and distal portions of their processes; cytoplasmic staining in CNS astrocytes is usually not observed. Within the geniculate ganglia anti-protein A1 is visualized in perisomatic regions resembling the cell membrane and in neuropil processes. Cytoplasmic staining of geniculate neurons and glial cells is rarely observed. The pattern of staining obtained with anti-protein A1 does not resemble that obtained by the use of either anti-GFAP Mab or anti-S100 Mab. Results obtained with Mab anti-protein A1 will be presented at the meeting.

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Cyto-, myelo-, and chemoarchitecture of the insular cortex of the Syrian golden hamster. R.G. WEHBY[1] and J. A. LONDON[1,2] (Center for Neurological Sciences[1] and Department of BioStructure and Function[2], University of Connecticut Health Center, Farmington, CT 06030).

The architectonics of the insular cortex of the Syrian golden hamster (*Mesocricetus auratus*) was investigated. We examined cyto- and myeloarchitecture, as well as the distribution of acetylcholinesterase, NADPH diaphorase, and Zn^{2+} in synaptic vesicles. These features were analyzed at five rostrocaudal levels of the insular cortex. Four major observations have emerged from the present investigation. First, we find that chemoarchitecture can reflect cytoarchitectonic borders in the hamster insular cortex. Second, to the best of our knowledge, this is the first report of NADPH diaphorase activity in the rodent insular cortex. We find a cluster of granularly-filled NADPHd-containing cells in the superficial layers of the dysgranular and agranular insular cortices. Third, the layers containing the dense acetylcholinesterase label appear to alternate with the layer containing the cluster of granularly-filled NADPH-diaphorase-containing cells. Fourth, this cluster of granularly-filled NAPDH-diaphorase-containing cells coincides with the superficial part of the stain for Zn^{2+} in synaptic vesicles.

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The Ventromedial Hypothalamic Neuronal Responses to Iontophoretic application of Amino Acids in Lysine-deficient Rats

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The plasma and brain concentrations of essential L-amino acids decrease few hours after the animal has been exposed to a diet deficient in those amino acids. Lateral hypothalamic area neurons respond specifically to lysine ingestion during lysine deficiency indicating a change in appetite for the deficient nutrient. The present study examines the role of the ventromedial hypothalamus (VMH) in such a change to internal signal by applying amino acids iontophoretically in lysine deficient rats. They were painlessly restrained in a modified stereotaxic apparatus and single unit activities were recorded from the VMH by using a glass microelectrode (1.5-3.5 MΩ) glued to a seven barreled glass micropipette (20-80 MΩ). The amino acids, monosodium glutamate, lysine, arginine, threonine, glycine in concentration of 0.5 M or 0.15 M NaCl were applied from one of the seven-barreled glass micropipette using 20-90 nA current. The central barrel was filled with 4M NaCl for current balancing. Some neurons in the VMH responded to applied lysine during lysine deficiency. These neurons which responded to lysine also responded to glutamate. A few neurons responded to lysine, arginine and threonine along with glutamate. These findings suggest a role for the VMH in processing internal signal for regulation of protein nutrition or adaptation to essential nutrient deficiency by induction of neural plasticity.

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The Lateral Hypothalamic Area as a Recognition Site for Lysine Deficiency: Effect of Inhibin or Activin Infusion

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To identify brain mechanisms which mediate specific hunger for amino acid (e.g.L-lysine, Lys) deficiency, rats were trained to bar press (FR30 schedule) to receive 50 mg complete diet. Rats were given lysine deficient (Lys-def) diet ad lib. IP injection of Lys (300mg/rat) 2 hr before testing inhibited bar pressing to the same low rate as when fed complete diet ad lib. Osmotic minipumps containing Lys implanted IP also strongly inhibited bar pressing. When given ad lib. 0.4 M Lys to drink, significantly more Lys solution was drunk in addition to a significant decrease in pressing than when given complete diet. Lys, but not arginine or glutamine, chronically infused by minipump into the lateral hypothalamic area (LHA) also inhibited bar pressing by rats given Lys-def diet. The effective dose range was between 0.1-0.5 nmol/hr. Thus, animals lacking dietary Lys will work to receive complete diet, but replacement of Lys by drinking, IP or LHA infusion inhibits bar pressing. Serum inhibin release has been found when complete diet was available, and activin A level was enhanced when dietary protein was decreased, as previously reported. Inhibin and follistatin may modulate either activin function in the LHA or motivation to work for complete diet, since LHA infusion of inhibin or follistatin, but not activin, was found strongly to inhibit bar pressing. This inhibitory effect of LHA inhibin infusion was replicated, in which concurrent consumption of Lys solution ad lib. was additive with inhibin further to block bar pressing. These results suggest that the LHA is a regulatory site for homeostatic control of specific amino acid deficiency and that inhibin can alter operant behavior to alleviate Lys deficiency.

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Taste-Elicited Responses of Hamster NST Neurons are Blocked by a Glutamate Antagonist. CHENG-SHU LI and DAVID V. SMITH (University of Maryland School of Medicine, Baltimore, MD 21201, USA)

The roles of specific neurotransmitters in the processing of gustatory information are largely unknown. Patch-clamp recording from *in vitro* slice preparations of the medulla show that cells within the rostral portion of the nucleus of the solitary tract (NST) are inhibited by gamma-aminobutyric acid and excited by substance P and by excitatory amino acids (EAAs). Both NMDA and non-NMDA glutamate receptor antagonists *in vitro* reduce the responses of rat NST neurons to electrical stimulation of the solitary tract. These data suggest that glutamate may act as a neurotransmitter in some NST cells, although their responsiveness to gustatory stimuli remains unknown. EAAs appear to be neurotransmitters between primary vagal afferent fibers and second-order cells in the NST. Recent *in vitro* evidence suggests that glutamate may serve as a neurotransmitter in primary olfactory neurons. In the present experiment, we recorded from cells in the hamster NST *in vivo* in order to test directly the hypothesis that EAAs might function as neurotransmitters between primary gustatory afferent fibers and NST cells. Single neurons in the hamster NST were recorded extracellularly and pharmacologically stimulated using a multibarrel pipette assembly. Tips of the injection pipettes were 120 μm from the tip of the recording pipette. The activity of each cell was recorded in response to lingual stimulation with 0.32 M NaCl, 0.32 M sucrose, 0.0032 M citric acid, 0.032 M QHCl, and 25 μA anodal current. Once a cell was identified as a taste-responsive neuron, 30 nl of either 50 mM kynurenic acid, a broad spectrum EAA antagonist, or phosphate-buffered saline was microinjected into the vicinity of the recorded cell. Results to date show that the responses of NST cells to both chemical and anodal current stimulation of the tongue are reversibly blocked by kynurenic acid but not by saline. These data suggest that EAAs may be neurotransmitters between primary gustatory afferent fibers and NST cells. Experiments with specific EAA antagonists are underway to evaluate the role of different glutamate receptors in this process.

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The Effects of NaCl Adaptation and Amiloride Treatment on Taste Responses of Hamster NST Cells. DAVID V. SMITH[1], HONGYANG LIU[2], and MARK B. VOGT[1] (University of Maryland School of Medicine[1], Baltimore, MD 21201 and University of Cincinnati College of Medicine[2], Cincinnati, OH 45267, USA)

Continuous NaCl stimulation produces adaptation of the gustatory response in both human psychophysical and animal neurophysiological studies. The Na^+ transport blocker amiloride partially blocks neural responses to Na^+ salts in animals and has a small effect on their taste in humans. The present study compares the effects of NaCl adaptation and amiloride treatment on the response frequencies of hamster NST neurons to lingual stimulation with Na^+ and non-Na^+ stimuli: 0.032 M NaCl, $NaNO_3$, and Na-gluconate, 0.1 M sucrose and KCl, 1.0 mM HCl, and 3.2 mM QHCl. Responses were recorded from 35 single neurons, each under 3 conditions; in the H_2O-adapted condition the 60-sec pre-stimulus rinse was distilled H_2O, in the NaCl-adapted condition it was 0.032 M NaCl, and in the amiloride condition, 10 μM amiloride was both the rinse and the solvent for the stimuli. Amiloride reduced the spontaneous response frequencies of these NST cells by 57%; this reduction was proportional to the cell's initial spontaneous rate during the H_2O rinse and to it's responsiveness to NaCl. This suggests that a significant proportion of the spontaneous activity of NST cells arises from Na^+-induced activity in the periphery. NaCl adaptation and amiloride treatment produced similar reductions in responses to Na^+ salts. In each case, the magnitude of the reduction was significantly correlated with the magnitude of the NaCl response, both for NaCl-best and non-NaCl-best neurons. In addition, amiloride reduced the response to NaCl to 38% of its value in NaCl-best cells and to 45% in non-NaCl-best neurons, indicating that the amiloride effect was not restricted to NaCl-best cells. Unlike amiloride, which reduced responses only to Na^+ stimuli, NaCl adaptation also significantly reduced responses to KCl, HCl, and QHCl (but not sucrose), predominately in NaCl-best neurons. This cross adaptation is consistent with an overlap of transduction mechanisms for these stimuli in hamster taste receptor cells.

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Effects of adaptation of best and non-best stimuli on evoked taste response in the nucleus of the solitary tract. M.D. KAWAMOTO, C. LEMON, & P.M. DI LORENZO (Dept. of Psychology, Box 6000, SUNY at Binghamton, Binghamton, N.Y. 13902-6000)

Although the investigation of adaptation in gustation has focussed primarily on peripheral mechanisms, there have been several studies that have implied the involvement of the central nervous system in this process. In the present study, the effects of adaptation of individual taste stimuli on taste responses in the nucleus of the solitary tract (NTS) were examined. Electrophysiological responses to sapid solutions of NaCl (.1 M), HCl (.01 M), sucrose (.5 M), and quinine HCl (.01 M) were recorded from single units in urethane anesthetized rats. For each NTS taste-responsive unit, the "best stimulus", i.e. the stimulus that evoked the most robust response, was identified. In the first stimulus adaptation sequence, the best stimulus for a given unit was presented repeatedly without a post-stimulus water rinse until no response was evoked. At that time, one of the other (non-best) stimuli was presented and the evoked response recorded. The best stimulus adaptation sequence was repeated until each non-best stimulus had been presented once. Next, the stimulus which evoked the second highest response became the adapting stimulus and the other stimuli became taste stimuli as described above. Adaptation to the best stimulus of an NTS unit was accompanied by an enhancement or an inhibition below baseline in responses to non-best stimuli. In some cases, inhibitory responses were present following adaptation when no response of any kind was present prior to the adaptation sequence. In general, taste responses to all non-adapting stimuli were attenuated following the non-best stimulus adaptation sequences. These effects of adaptation on response profiles in the NTS may provide insights into the existence of neuron types in this structure.

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Morphology and Location of Neurons in the Rat Rostral NST which Project to the Parabrachial Nucleus and an Investigation of the Chemical Nature of the Projection from the Central Amygdala to the rNST. D.M. MURPHY, M. NYSTROM, E.C. CREWS, K.E. REYNOLDS and M.S. KING (Biology Department, Stetson University, DeLand, FL).

To examine the morphology and location of neurons in the rostral nucleus of the solitary tract (rNST) which project to the medial parabrachial nucleus (mPBN), the fluorescent tracer DiI (Molecular Probes) was injected unilaterally into the mPBN in male Wistar rats (n=3). On average, each injection produced 200 retrogradely labeled cell bodies which could be characterized morphologically. Sixty-three percent of the labeled cells were located ipsilateral to the injection, while the remaining 37% were in the contralateral rNST. These results suggest that the projection from the rNST to the mPBN in rat is bilateral. This is contrary to previous findings (Norgren and Leonard, '73; Norgren, '78) and is currently being re-investigated using DiI as well as other retrograde tracers. Similar to findings in hamster (Whitehead, '90), the DiI labeled neurons were found throughout the rNST, with the highest percentage (33%) being centrally located. The morphology of projection neurons in the rat rNST has been reported to be multipolar and elongate (Lasiter and Kachele, '88) or just elongate (Lasiter, '91). In the current study, all labeled neurons had either multipolar- or elongate-shaped somata with the majority (61%) being classified as elongate. This indicates that both multipolar and elongate neurons project to the mPBN in rat, as previously reported in hamster (Whitehead, '90). Evidence indicates that forebrain autonomic and limbic centers directly project to the rNST (van der Kooy et al., '84). In fact, injection of DiI into the rNST (n=3) resulted in retrogradely labeled cell bodies within the central nucleus of the amygdala. Since substance P (SP) excites most rNST neurons (King et al., '93) and SP-immunoreactive cells and terminals are located within both the rNST and the central amygdala (Ljungdahl et al., '78), SP input to the rNST may originate in this limbic structure. Therefore, we are currently double-labeling amygdala neurons which project to the rNST with DiI and with a SP antibody (Chemicon) visualized with a fluorescein-labeled secondary antibody.

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Characteristics of rat nucleus tractus solitarius neurons that respond to chemical stimulation of the epiglottis. R.D. SWEAZEY and J.A. COOK (Indiana Univ. Sch. Med., Fort Wayne, IN)

Little is known about the properties of rat nucleus tractus solitarius (NTS) neurons that receive information from taste buds located at the laryngeal opening. Therefore, we recorded the extracellular responses of rat NTS neurons to stimulation of laryngeal taste buds with chemical stimuli. Following physiological characterization, biocytin was injected through the electrode to determine the morphological properties of cells at the recording site. Chemically-evoked responses were encountered throughout much of the superior laryngeal nerve termination zone in the intermediate and caudal NTS. Responsive neurons in the intermediate NTS were located in the medial NTS just ventrolateral to the fourth ventricle, while those in the caudal NTS were found in the interstitial and ventrolateral subnuclei. The effectiveness of a chemical stimulus was dependent upon a cell's location. Intermediate NTS neurons responded best to KCl and NH_4Cl while distilled water was the best stimulus for caudal NTS cells. The majority of labeled cells in both intermediate and caudal NTS had fusiform shaped soma and relatively unbranched dendrites. Our data suggest that many morphological properties of NTS laryngeal chemosensory neurons are similar to rostral gustatory neurons. Furthermore, the chemosensory information from laryngeal taste buds is distributed to caudal NTS regions involved in respiration and cardiovascular control as well as to regions of the NTS that receive inputs from taste buds in the caudal oral cavity.

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Acute Isolation of Neurons From the Gustatory Zone of the Rat Nucleus of the Solitary Tract. J. DU and R. M. BRADLEY. (Dept. Biologic & Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078.)

We have used an *in vitro* slice preparation of the rostral (gustatory) nucleus of the solitary tract (rNST) to study the intrinsic properties and neuropharmacology of rNST neurons. In attempting to correlate these properties with morphology we found that neurons with similar morphologies could have different electrophysiological characteristics. Thus, it is possible that the three morphological neuron types in rNST (elongate, multipolar and ovoid) described by us and other laboratories may represent several different biophysical and, therefore, functional groups of neurons. To examine this possibility we have acutely dissociated neurons from the rNST in order to be able to directly examine the morphological, biophysical and pharmacological properties of neurons in rNST. 400 μm horizontal brainstem slices were prepared from rats aged 5-10 days. After incubating the slices for 1 hr in physiological saline the rNST was dissected and placed in HEPES buffer containing 0.6% protease type 23 for 45 min at 37°C. The rNST was then triturated with a series of different diameter, fire-polished Pasteur pipettes to produce a suspension of dissociated neurons which were then placed in a plastic petri dish. Viewed with an inverted microscope elongate, multipolar and ovoid neurons could be easily distinguished. When immunoreacted for GABA, only the ovoid neurons were GABA positive. If the parabrachial taste relay was first injected with DiI, a subpopulation of the dissociated rNST neurons contained the transported label. This new *in vitro* preparation of the rNST will be useful for examining the biophysical properties of visually identified neurons and studying the biophysical properties of neurons with a known projection pattern. When combined with neuropharmacology these experiments will provide new insight into the microcircuitry of the rNST.

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Receptive Field Organization of Gustatory Neurons in the Parabrachial Nucleus of the Rat. CHRISTOPHER B. HALSELL and SUSAN P. TRAVERS (Section of Oral Biology, College of Dentistry, The Ohio State Univ. Columbus OH 43202)

The parabrachial nucleus (PBN) is the second central synapse for the ascending gustatory pathway in non-primate mammals. The purpose of the present study was to map the locations of single neurons responsive to stimulation of individual taste receptor populations (anterior tongue [AT], nasoincisor duct [NID], foliate papillae [FOL], circumvallate papilla [CV], and soft palate [SP]) within the PBN of anesthetized rats. Of the 128 neurons characterized to date, 46 were responsive to gustatory stimulation and 20 of these cells also responded to innocuous mechanical stimulation of defined intraoral regions. Overall, more neurons that responded maximally to posterior oral cavity (POC: FOL, CV, SP) taste stimulation received mechanical input (75%) than anterior oral cavity (AOC: AT and NID) taste neurons (32%). Of the 46 taste neurons, 31 responded to stimulation of only one receptive field: 22 AT, 3 NID, 3 FOL, and 3 CV. No SP specific neurons were recorded. The remaining 15 neurons responded to stimulation of multiple receptive fields. Individual convergent cells responded maximally and secondarily to receptive fields confined to either the AOC or POC (n=10), as well as to receptive fields in both these regions (n=5). Several neurons received convergent taste input from the FOL and CV (n=5). For these cells, the stimulus eliciting the best response from the two fields always matched, the responses were of similar magnitude, and mechanical stimulation of at least one of the fields was also effective. These findings suggest that the FOL and CV supply the same information to a subset of PBN cells. Most of the cells responsive to taste stimulation of only one receptor field, i.e. 19 AT, 1 NID, 2 FOL and 2 CV, were located as a tight cluster just lateral to the 'waist' area, within the central medial and ventral lateral subdivisions and within the superior cerebellar peduncle. Within this cluster of taste neurons, those cells specifically responsive to NaCl (0.3 M) tended to be located lateral to neurons responding more generally to NaCl and other electrolytes (0.03 M HCl, 0.01 M QHCl). The remaining neurons, including those receiving input from multiple taste receptor populations and mechanically responsive cells (n=58), were more scattered throughout the PBN. These findings suggest that there is a differential distribution of neurons within the PBN based on the specificity of their receptive field organization, modality, and chemical responsiveness.

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A Comparison of Structure-Function Relationships in Early Postnatal and Adult Rats. WILLIAM E. RENEHAN[1], ZHIGAO JIN[1], XUEGUO ZHANG[1] AND LAURA SCHWEITZER[2] ([1]Laboratory of Gastrointestinal, Gustatory and Somatic Sensation, Henry Ford Hospital, Detroit, MI; [2]University of Louisville, Louisville, KY)

Previous investigations have clearly established that the neurons in the rostral nucleus of the solitary tract (rNST) of the rodent undergo dramatic changes in their physiology (e.g. Hill et al., J.Neurophysiol. 50:879-895, 1983) and morphology (e.g. Lasiter et al., Brain Res.Bull. 22:313-321, 1989) during postnatal life. We propose that the developmental changes in the response properties exhibited by rNST gustatory neurons are the direct result of specific alterations in the morphology of these cells. We have begun to test this hypothesis by labeling individual physiologically-characterized neurons in young (postnatal [P] day 20-24) and adult (> 60 days old) rats. A total of 32 neurons in young animals and 78 neurons in adult rats were successfully labeled with Neurobiotin and reconstructed in three dimensions using the Eutectic Neuron Tracing System. We were able to demonstrate that P20-24 neurons that responded to NaCl, NH_4Cl and KCl had longer dendrites and greater surface area than cells that did not respond to all three salts. In the adult, however, neurons that were sensitive to all three salts were smaller than the cells that failed to respond. It appeared that this difference was primarily due to an increase in the size of the 'non-responsive' cells during late postnatal development. Our preliminary data indicate that there are indeed relationships between the physiology and morphology of gustatory neurons in the immature rNST. It would appear, however, that these relationships may undergo significant changes during the development of this nucleus.

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The Emergence of Abnormal Dendritic Morphologies in NTS Neurons of Developmentally Sodium Restricted Rats. A. KURT THAW and DAVID L. HILL (University of Virginia, Charlottesville, VA 22903)

Dietary sodium restriction during pre- and postnatal development in rats results in a variety of functional and morphological alterations. One of the most noteworthy effects is that the dendrites of second-order taste neurons in the nucleus of the solitary tract (NTS) of adult rats that were sodium-restricted throughout development expands dramatically, compared to that in normally fed rats. Furthermore, the dietary-induced effects appear specific to multipolar and fusiform neurons (presumptive relay neurons). While these findings show a clear effect, it is not clear whether these neurons show a history of expanded dendrites throughout development, or whether they deviate from normal morphologies at specific times of development (e.g., as peripheral activity emerges). Thus, studying the time course of dendritic expansion in normal and sodium-restricted rats may provide insights into relationships between peripheral activity and central morphologies, as well as between other morphological characteristics (i.e., terminal field development) and central taste neuron dendritic organizations. In order to examine these processes, the dendritic organization of NTS neurons are studied in normal and sodium-restricted rats at three distinct periods. The periods represented are postnatal day 5, day 15 and day 35. Extensive developmental changes in the rostral NTS of normal rats occur between postnatal day 5 and 15, and essentially all morphological characteristics are complete by postnatal day 35. Using a modified Golgi-Cox procedure along with a computer-microscope analysis system, we are able to identify whether the alterations seen in sodium-restricted rats relates to specific hallmarks of peripheral and central gustatory development.

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Gustatory-Evoked Potentials:
GEPs varied according to the concentration

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The authors measured gustatory-evoked potentials(GEPs). It was also confirmed that the amplitude of the GEPs varied according to the concentration of the tastants. In measuring GEPs, the main difficulty is the method with which to stimulate tongues. To elicit GEPs, we separated tastants and pure water with air. The liquids(tastants and pure water) and air flow through a tube. First, the tastants flows through the tube, then the pure water and the air flow in order to separate the tastants and the water. The pressure and temperature added to each liquid were controlled. There is a hole (the size is $15mm^2$) in the tube. The subject bites the tube, and puts his tongue onto the hole. The liquid flows on the tongue. The tastants are presented with a duration of 500 ms, thereafter the pure water flows for 10s, and air flows for 1s. This process to stimulate the tongue is repeated 30 times for averaging. EEGs were recorded from electrodes at Cz(10-20 system). There is a sensor near the hole to measure the time of the tastants' arrival. The electrical resistance was sensed near the hole. The time is used to trigger an averaging computer. Different concentrations were tried(20%,10%,5%,1%). The GEPs appeared about 100 ms after the sensor's resistance became low. The amplitude of the GEPs increased as the concentration of the tastants increased.

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Comparison of voltage-sensitive dyes for optical recording from hamster gustatory cortex. J. D. ZEIGER[1], and J. A. LONDON[1,2] (Center for Neurological Sciences[1] and Department of BioStructure and Function[2], Univ. of Connecticut Health Center, Farmington , CT 06030).

The effectiveness of different dyes were assessed for use with multi-site optical recording in the gustatory cortex of the golden Syrian hamster (Mesocricetus auratus). Comparisons were made between DI-2-ANEPPQ (a gift from Dr. L. Loew) and RH-795 (Molecular Probes). This is the first reported use of DI-2-ANEPPQ (DI-2-Q) on a mammalian cortex. Hamsters were deeply anesthetized, and a craniotomy performed that exposed the gustatory cortex and the surrounding cortical areas. The cortex was stained for 90 minutes with a 500 μM solution of dye, after which the hamster was mounted in the optical recording setup. The cortical surface was imaged onto a 124 element photodiode array by an epifluorescence microscope (2.5 X objective magnification, 0.08 n.a.) employing appropriate excitation and emission filters for each dye. Background, "DC", fluorescence measurements were used to compute $\Delta F/F$. Physiological events were recorded in "AC" mode at 200-4000-fold higher gain than DC. After measuring baseline activity, the bathing solution was changed to one containing 30 μM bicuculline methiodide. Large, widespread, synchronized, epileptiform events were recorded optically within 10 minutes of bicuculline application. To date, two representative experiments have been analyzed. For each trial, the ten detectors displaying the largest signal-to-noise ratio (S/N) were selected to obtain mean maximum signal per captured event; both the S/N (calculated as $(RMS_{signal})^2/(RMS_{noise})^2$-1), and $\Delta F/F$ were used to evaluate signal size. While S/N values were approximately the same for the two dyes, $\Delta F/F$ values were ten-fold higher for DI-2-Q. This difference is primarily due to an approximately eight-fold lower background fluorescence with the new dye. The fact that S/N values were comparable despite very different $\Delta F/F$ values indicates that, for these large signals, photon noise did not dominate. However, small signals, such as those obtained with sensory stimuli, would be expected to show a S/N advantage with DI-2-Q. Supported by NIH Training Grant 5T33DC00025 and NIH Grant 5P50DC00168.

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Facial Lobe Taste Responses to Binary Mixtures of Amino Acids in the Channel Catfish.
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A previous report of taste responses from the facial nerve in the channel catfish, *Ictalurus punctatus*, indicated that binary mixtures composed of amino acids that bind to independent taste receptor sites (i.e. Group I stimuli) resulted in enhanced taste activity, whereas binary mixtures of amino acids that bind to the same or highly cross-reactive receptor sites (i.e. Group II stimuli) did not show enhancement activity (Ogawa and Caprio, *Chem. Senses* abs 18:609, 1993). We now report the results of taste responses to six binary mixtures of amino acids and to their individual components obtained from microelectrode (low impedance metal) recordings of multiunit activity from 33 sites (N=6 fish) in the maxillary barbel lobule of the facial lobe (FL), the primary gustatory nucleus of the medulla in the channel catfish. The independent component index (ICI), defined as the response to the mixture divided by the sum of the responses to the individual component stimuli, was calculated for each binary mixture. Group I binary mixtures (and the mean ICI for each mixture) included L-alanine + L-arginine (0.65), L-alanine + L-proline (0.71), and L-arginine + L-proline (0.65), whereas Group II binary mixtures included one each of the three Group I amino acids and a taste analog of each: L-alanine + L-methionine (0.51), L-arginine + L-α-amino-ß-guanidino propionic acid (0.49), and L-proline + L-azetidine-2-carboxylic acid (0.52). All amino acids were tested at 0.1mM. Statistical analysis (2-way ANOVA) indicated a highly significant ($p<0.0001$) ICI effect. The ICI values for all Group I binary mixtures were significantly greater ($p<0.05$; Tukey) than those for the Group II mixtures.

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The *Soa* taste gene confers sensitivity to bitter alkaloids, including quinine, in mice. BOUGHTER, J.D.[1], HARDER, D.B.[1], and WHITNEY, G.[1] (Florida State University[1], Tallahassee, FL, 32306-1051)

In previous experiments, Whitney and colleagues have shown that among mice the autosomal gene *Soa* has a major effect on aversion to the bitter tastant sucrose octaacetate (SOA), as well as other bitter compounds. To study this phenomenom a congenic quartet was created from SWR/J (SW, SOA-taster) and C57BL/6J (B6, SOA-nontaster) inbred strains. Unfortunately, both of these inbred strains avoid a variety of interesting bitters, obscuring investigation of *Soa* gene effects. In contrast, C3HeB/FeJ (C3, SOA-demitaster) inbred mice are relatively insensitive to many bitter compounds, including quinine. Boughter and Whitney (1994) developed C3.SW-*Soa*^{a} congenics, resulting in animals who contain the *Soa* taster allele transposed onto a 99% C3 genomic background. In several experiments C3.SW, C3, and SW mice were tested (48-h preference tests) with concentration series of SOA, brucine, and quinine. Taster-demitaster variation at the *Soa* locus completely accounted for sensitivity to SOA and brucine, and strongly influenced quinine aversion. Mean quinine preference ratios of C3.SW SOA-tasters (aversion) were significantly different from those of C3 mice (indifference) on at least two concentrations. It appears that *Soa* confers sensitivity to bitter alkaloids, in addition to SOA-like acetylated sugars.

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Taste Preference and Ethanol Consumption in Mice: A Genetic Analysis. A.A.BACHMANOV, M.G.TORDOFF, G.K.BEAUCHAMP (Monell Chemical Senses Center, Philadelphia, PA 19104, USA)

Mice of 129/J (129) and C57BL/6ByJ (B6) strains were offered different concentrations of taste solutions in 48-hr two-bottle choice tests. Ethanol ingestion was higher in B6 mice than in 129 mice. In comparison to the 129 strain, B6 mice had higher preferences for sucrose and citric acid solutions and tended to have a weaker aversion to quinine hydrochloride ($p < 0.08$). As previously reported (Beauchamp and Fisher, Physiol. Behav., 1993, 54: 179), NaCl solutions were preferred by 129 mice and rejected by B6 mice. The strains did not differ in response to capsaicin solutions. These data are consistent with the hypothesis that the higher ethanol intake of B6 mice depends, in part, on higher hedonic attractiveness of its sweet taste component and lower aversiveness of its bitter taste component. They are also consistent with some other studies with rats indicating a reciprocal relationship between sweet and salt preference (e.g. Tordoff et al., Am. J. Physiol., 1990, 259: R411). To further evaluate the genetic basis of the strain differences in 48-hr intakes, the (B6 x 129)F_2 generation has been bred. Correlations among intakes of these sapid fluids will provide insight into the genetic bases for individual differences in preference in mice.

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Phenotypic Variation for the Production of Salivary Proline Rich Proteins Among Inbred Mice: Preliminary Evidence for the Independence of *Soa* and *Prp*. CHRISTOPHER G. CAPELESS and GLAYDE WHITNEY (Psychology Department, Florida State University)*

Isoelectric-focus of microcentrifuge-concentrated whole saliva from six inbred strains of mice (129/J, BALB/cByJ, C3HeB/FeJ, C57BL/6J, DBA/2J, and SWR/J) has indicated substantial variation among the strains for the production of acidic and basic proline rich proteins (PRPs). Strain-specific sexually-dimorphic patterns of PRP production were also observed. Predicted groupings of the inbred strains based upon *Soa* taste phenotypes were not evident. Production of PRP's among the strains of a congenic quartet (C57BL/6J, B6.SW-*Soa*^{a}, SW.B6-*Soa*^{b}, and SWR/J) was discordant with the observed *Soa* phenotypes for these strains. It is suggested that the *Prp* genes and the *Soa* gene may be independent, although closely linked on mouse chromosome six.

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Nutrient-flavor preferences are altered by food restriction in rats. ANTHONY SCLAFANI, and FRANÇOIS LUCAS. (Dept. of Psychology, Brooklyn College of CUNY, Brooklyn, New York)

Previous studies (Sclafani & Ackroff, 1993) show that food restriction alters the rat's preference for carbohydrate and fat. In 30-min, two-bottle tests nondeprived rats preferred a carbohydrate solution (2% sucrose or Polycose) to a fat emulsion (0.9% corn oil), whereas deprived rats preferred the fat emulsion. Given that the nutrient sources were calorically dilute and isocaloric, the deprivation-induced shift in preference appeared to involve a change in the orosensory evaluation of carbohydrate and fat. To test this idea, ad lib and food-restricted rats (85% BW) were given the choice between saccharin (0.2%) and mineral oil (10%), which served as noncaloric sugar and fat substitutes. The ad lib rats reliably preferred the saccharin solution (70%) whereas the food-restricted rats preferred the mineral oil emulsion (70%). This preference profile was not altered by prefeeding the rats with chow 1 hr prior to the choice test. These findings suggest that long-term food restriction and/or the concomitant weight reduction enhances the palatability of the flavor of fat relative to that carbohydrate. Deprivation-induced changes in the relative preference for fat and carbohydrate have been observed with complex foods as well as pure nutrient sources. Studies with low-fat and high-fat food products (cake and milk) show that food deprivation increased the preference for the high-fat food even when it was the less caloric alternative. Changes in the evaluation of fat and carbohydrate flavors may contribute to the altered food preferences observed in these latter studies, although postingestive factors presumably play a role as well.

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Following odor plumes: How behavior is controlled by the interaction of fluid dynamics with sensory systems. N.D. PENTCHEFF, C.M. FINELLI, D.S. WETHEY, AND R.K. ZIMMER-FAUST. University of South Carolina, Columbia, SC.

We are investigating the mechanisms by which animals use an odor plume to navigate to the source of the odor. An odor plume is created when a chemical given off by an odor source is transported downstream by environmental fluid flow. Hence, understanding how animals navigate in an odor plume requires understanding the fluid dynamics of odor transport and dispersal, the sensory systems of the receiving animal, and how the fluid dynamics and sensory systems interact. To date, the best-studied systems where both fluid dynamics and olfaction have been taken into account are moths flying up pheromone plumes and benthic crustaceans walking up prey-odor plumes. In both of these cases, the scaling of the size of the animals relative to the environmental fluid velocities ensures that the odor plumes will be dispersed turbulently rather than by molecular diffusion or laminar flow. These turbulent plumes classically are thought to present an intermittent, highly variable stimulus to a receiving animal downstream. Moths flying in air are thought to use rheotaxis (navigation based on flow direction) to direct both their upwind surges when the odor cue is sensed as well as the direction of crosswind casting (without upwind movement) when the odor cue is lost. We have performed a series of field experiments with blue crabs (*Callinectes sapidus*) navigating up prey-odor plumes in tidal flow. The crabs, like moths, use rheotaxis to direct their movement upstream while the odor cue is sensed. Contrary to the moth observations, however, experiments with crabs in controlled conditions in a laboratory flume indicate that their cross stream navigation upon loss of the odor signal is chemotactic (controlled by measurement of chemical concentrations). The differences in behavioral algorithms follow logically from a comparative consideration of the fluid dynamics that generate odor plumes in air and odor plumes in tidal flow.

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Dimethyl Sulfide is Part of the Olfactory Landscape Detectable to Antarctic Procellariiform Seabirds. G.A. NEVITT, (Institute of Neuroscience, University of Oregon, Eugene, Oregon, 97403)

Many Antarctic Procellariiform seabirds make their living flying over vast expanses of seemingly featureless ocean. The secret of their success is a mystery, but an ability to hunt by smell has long been suspected. Dimethyl sulfide (DMS) is a biogenic aromatic produced by phytoplankton in response to grazing by zooplankton such as Antarctic krill *(Euphausia superba)*, the preferred prey of many Procellariiform seabirds. To examine whether DMS emissions could serve as a foraging cue to seabirds, I conducted field experiments to test whether Procellariiforms could detect DMS. Working at sea, I deployed unscented vegetable oil ("control") slicks paired with slicks scented with either cod liver oil, a well established olfactory attractant, or DMS, and monitored the responses of birds in the area. My aim was to present birds with odor plumes similar to what they would naturally encounter (nanomolar concentrations). A team of "blind" observers then recorded numbers, behaviors, and species identities of new arrivals. Results show that DMS is as attractive an odor as cod liver oil to many Procellariiform species including White-chinned petrels *(Procellaria aequinoctialis)*, Wilson's *(Oceanites oceanicus)* and Black-bellied Storm Petrels *(Fregetta tropica)* (G-test, $P < 0.05$). Interestingly, highly visual preditors including Cape Petrels *(Daption capense)*, Black-browed *(Diomedea melanophrys)*, Wandering *(D. exulans)*, and Grey-headed Albatrosses *(D. chrysostoma)*, responded similarly to both DMS and control slicks (G-test, $P>0.05$). Experiments with aerosol presentations confirmed these results. White-chinned petrels turned 36% more frequently in the presence of DMS aerosol plumes as compared to control water plumes ($N_1= 34$ birds, water plume; $N_2=35$, DMS; $P<0.05$, Mann-Whitney U test). Black-browed albatrosses, however, showed no differences in responses to water or DMS aerosols. ($N_1=20$ birds, water plume; $N_2=30$, DMS). Together, these results suggest that, besides playing a role in regulating global climate, DMS may provide an important orientation cue to foraging Procellariiform seabirds.

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Perception of "Depth" in Arrays of Individual Odors: How Do Animals Determine which Individual's Scent is on Top? ROBERT E.JOHNSTON and ANJALI BHORADE (Dept. of Psychology, Cornell University, Ithaca, NY 14853)

We have previously determined that golden hamsters selectively remember an individual's scent that overlaps or partially overlaps that of another individual, suggesting specialized perceptual abilities to determine which of two scents was deposited most recently. How can they do this? We investigated four hypotheses about the cues that might be used: (1) the quantity or concentration of scent from the two animals, (2) the age of the scent of two individuals, (3) the occlusion of one scent by another (analogous to visual occlusion), and (4) perception of the strength of one scent versus another in a region of overlap. We used behavioral habituation techniques to investigate the perception and memory for individual scents in a variety of spatial arrays. Neither differences in quantity nor in age of scents leads to selective memory of one of two individual scents. Experiments with streaks of scents suggest that animals may determine which scent is most recent by which scent is interrupted and which isn't (olfactory occlusion). Other experiments suggest that they may also be able to determine which scent overlays another in the region of overlap, by some as yet undetermined means. Such mechanisms allow an animal to determine which other individuals have been in an area most recently, which could make the search for a mate or reval much more efficient. Yet other experiments show that the position of a scent mark relative to another (its "depth") does influence its functional significance to a perceiver.

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Olfactory Identification of Binary Mixtures and their Components in Catfish. T. VALENTINČIČ[1,2], V. PIRC[1], M. STENOVEC[1] AND J. CAPRIO[2] (University of Ljubljana, Ljubljana, Slovenia[1] and Louisiana State University, Baton Rouge, LA. 708032[2])

Conditioning experiments indicate that channel catfish, *Ictalurus punctatus* and bullhead catfish, *Ameiurus nebulosus*, recognize binary mixtures of amino acids through olfaction by perceiving the electrophysiologically more stimulatory amino acid as the main component of the mixture. Catfish were conditioned to increase their food search after stimulation with binary mixtures of amino acids. Fish behavior was quantified for 90 secs subsequent to stimulus presentations by counting the number of >90° turns of the catfish from video recordings and by videotracking (Vidmex-V; Columbus Instruments, Columbus, OH). Equimolar solutions of the binary mixtures L-alanine (ALA) + L-arginine (ARG) and L-leucine (LEU) + L-norvaline (NVAL) were tested; based on EOG recordings in the channel catfish, ALA and NVAL are the more stimulatory components of these mixtures. For an equimolar mixture of ALA + ARG, ALA is approximately 2.5 times more stimulatory than ARG based on stimulus concentrations that result in approximately equal EOG response magnitudes. For an equimolar mixture of LEU and NVAL, NVAL is 10 times more stimulatory than LEU. The results indicate that ALA was discriminated from the mixture ALA + ARG in only four of ten test series (N=11), whereas ARG and other amino acids were discriminated from the equimolar mixture in all four test series. Channel catfish failed to discriminate NVAL, the more stimulatory component, from the equimolar mixture of NVAL + LEU in all five test series (N=11), but were able to discriminate the lesser effective LEU component from the the same equimolar mixture in all three test series. In experiments with bullhead catfish, the concentrations of NVAL and LEU were adjusted so that either NVAL ($2x10^{-2}$M NVAL + $3x10^{-3}$M LEU) or LEU ($2x10^{-3}$M NVAL + $3x10^{-2}$M LEU) was the more stimulatory component in the binary mixture. In tests where NVAL was more stimulatory, NVAL was not discriminated from the binary mixture in the 5 test series (N=9), whereas LEU and other amino acids were discriminated. In tests where LEU was more stimulatory, LEU was not discriminated from the binary mixture in the three test series (N=13), whereas NVAL and other amino acids were discriminated.

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Discrimination of familiarity and dominance status by odor cues in the American lobster, *Homarus americanus*.
CHRISTY KARAVANICH and JELLE ATEMA (Boston Univ. Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

Male American lobsters actively compete for high dominance status using stylized fighting behaviors. Once established, hierarchies remain relatively stable. It was hypothesized that they use olfaction in social contexts such as determining familiarity or dominance status in order to maintain their hierarchies. Twenty sets of 3 males established hierarchies in a 2700L tank for 1 week. Subordinates were subsequently run in a Y-flume with a choice of donor odors as follows: 1.) Familiar Dominant vs. Unfamiliar Dominant; 2.) Familiar Dominant vs. Unfamiliar Subordinate; 3.) Familiar Dominant vs. Familiar Subordinate; and 4.) Familiar Dominant vs. No odor. Subordinates avoided Unfamiliar Dominants but not Unfamiliar Subordinates and showed no preference when given a choice of both familiar subjects or between Familiar Dominant and No Odor. The results indicate that lobsters have the ability to distinguish familiarity and dominance status by odor cues alone.

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A Study of Long-Term Odor Memory in Squirrel Monkeys
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It seems reasonable to assume that animals which rely on their sense c smell should be familiar with a large number of different odors and b able to appropriately associate these with specific contexts or meaning Further, such animals should be able to quickly learn new odors, retai them in memory over prolonged periods of time and make use of suc memory content in new encounters with the same odor.
Using a behavioral paradigm designed to simulate olfactory-guide foraging behavior and based on a multiple discrimination c simultaneously presented odor stimuli in a manipulation task, w investigated the efficiency of olfactory long-term memory in a non-huma primate species.
Five squirrel monkeys were trained to discriminate between pairs of odor to a criterion of 80 % correct choices and subsequently tested on the ability to correctly assign them as S+ or S- after retention intervals of 1, 3, 4, 6, 8, 10, 15, 20 and 30 weeks.
We found that : (1) with retention intervals of up to 10 weeks at least fou out of five animals immediately assigned both odors correctly, that i correctly opened the very first manipulation object they inspected whic was scented with the S+ and correctly rejected the very first manipulatio object they sniffed at which was scented with the S- ; (2) with the sam retention intervals of up to 10 weeks the mean performance on the firs one-minute trial (averaging 10 decisions per animal) was above 90 ° correct choices and thus as good as at the end of the acquisition phase ; (3 with retention intervals of 20 and 30 weeks the mean performance of th monkeys on the first one-minute trial declined to 72.6 and 65.8 % correc choices respectively but was nevertheless well above chance level ; an (4) with the same retention intervals of 20 and 30 weeks the pretrainin criterion of 80 % correct decisions was reached within 10 one-minut trials, indicating a relearning process that was much faster than the initi process of acquiring the meaning of odors as S+ and S- during pretraining Thus the results of this study provide evidence of a well-develope olfactory long-term memory in squirrel monkeys and support th assumption that olfaction may play a significant role in this species.

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Response of Cochroach Olfactory Receptor Neurons to Selected Alcohols and their Binary Combinations. W M GETZ[1] and R P AKERS[2] (Dept. Env. Sci., Policy, & Mangement[1], University of California, Berkeley, CA 94720-3112; California Dept. of Pesticide Regulation[2], 1020 N St., Sacremento, CA 95814)

We employed tungsten electrodes to obtain extracellular recordings from two sets of 50 receptor neurons using 1-hexanol, 2-hexanol, 2-E-hexen-1-ol, and their three binary combinations as stimulants for the first set and 1-heptanol, 2-heptanol, 2-E-hepten-1-ol, and their three binary combinations as stimulants for the second set. Every neuron in each set of neurons was stimulated with the same set of stimuli presented at either 3 or 4 different concentrations, ranging from 0.1 to 600 µg/µl, where the components of the binary odor stimuli where each at half the concentration of the corresponding odorant stimuli. The responses were measured in several different ways, including average response rates over the 0.5 sec stimulus intervals and phasic response profiles over ten 0.05 sec bins. We found that each cell had its own individual response profile and were unable to group cells by profile across several concentrations. We did, however, find differences between the largest and second largest spiking cells in each sensillum that had two or more cells respond to one or more of the stimuli in the given set. The largest spiking cells were more specialized and much more sensitive to the hexyl stimuli at low concentrations than the second largest spiking cells. The response to binary stimuli indicated that inhibitory effects were common over a range of concentrations and for almost all combinations of the odorants in question. Finally, the phasic response profiles indicate a rapid response in the first 0.1 secs of stimulation followed by an almost equally rapid decline at high concentration levels to levels that were not strongly concentration dependent.

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Olfactory marker protein (OMP) null mice: Generation and characterization. O. BULAKOVA[1], H. BAKER[2], C. STEWART[1], S. ABBONDANZO[1], L. FRANZEN[2] and F.L. MARGOLIS[1]. (Roche Institute of Molecular Biology[1], Roche Research Center[1] Nutley, NJ and Cornell University College of Medicine[2], New York, NY)

Olfactory marker protein (OMP) is the most abundant protein of mature olfactory neurons. The biological and physical properties of this phylogenetically conserved, 20kDa, protein have been studied extensively. OMP lacks homology with other proteins and its function remains an enigma. To study its function we have used homologous recombination in ES cells to delete the OMP gene and create a line of mice lacking OMP. A targeting vector was constructed containing the neomycin resistance gene (neo) in place of the OMP coding region and the viral thymidine kinase gene (TK) as a selectable marker. ES cells were transfected with the targeting vector by electroporation and neo+/TK- colonies were selected. Southern genomic analysis demonstrated that 10% (9/80) of the initially selected colonies were heterozygous at the OMP locus. Two of these ES cell lines were injected into blastocysts and gave rise to two independent lines of homozygous OMP-null mice. The absence of the OMP gene was confirmed by genomic Southern analysis and by the lack of OMP immunoreactvity in the main and accessory olfactory systems and in ectopic sites previously observed in preoptic para-ventricular neurons. These OMP-null mice appear anatomically normal, breed, deliver and raise pups that appear to suckle normally and are themselves fertile. The time required to find a buried food pellet is the same for wild-type control mice and for the OMP-null mice. Our initial histological and immunocytochemical analysis indicate that except for the absence of OMP the olfactory neuroepithelium is normal. Preliminary analysis indicates that the olfactory bulb is reduced in size by about 15% and that the organization and level of activity of tyrosine hydroxylase is modified in juxtaglomerular neurons. These data suggest that OMP, either directly or indirectly, participates in determining the phenotype of the second order neurons of the olfactory bulb. Current studies to further characterize these alterations may provide insights as to the physiological role of OMP.

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Expression of the EGF family of Receptors in the Olfactory Epithelium

KOUROSH SALEHI-ASHTIANI and ALBERT I. FARBMAN (Northwestern University, Evanston, Illinois)

The growth and differentiation of olfactory sensory neurons are tightly regulated. We had previously shown by immunohistochemistry that transforming growth factor α (TGF-α), and epidermal growth factor (EGF) receptor are present in the olfactory epithelium of untreated adult rat and that TGF-α is a potent mitogen of olfactory epithelium *in vitro*. Expression of EGF-receptor and TGF-α was detected primarily in flat basal cells and supporting cells, but rarely in globose basal cells, which suggested that EGF-receptor is not a likely candidate for the mitotic regulator of sensory neurons. In order to expand the search for candidate regulators, we have now examined another member of the EGF family of receptors. By utilizing reverse transcriptase/Polymerase Chain Reaction (RT-PCR) methodology, we have detected the messenger RNA encoding the *neu* proto-oncogene (also called c-*erbB2* and *HER2*) in the olfactory epithelium. The *neu* proto-oncogene encodes a transmembrane tyrosine kinase receptor polypeptide that may be involved in growth and differentiation of neurons and glial cells of the embryonic brain, as well as adult epithelial, Schwann, and glial cells. The presence of the *neu* transcript in the olfactory epithelium suggests that the *neu* gene product may be involved in the regulated differentiation of the sensory neurons.

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Strategies for the Isolation of Cell Lines Derived from the Olfactory Epithelium DALE D. HUNTER, THOMAS C. BOZZA, and JOHN S. KAUER (Tufts University School of Medicine, Boston, Mass.)

We have been interested in identifying the factors that control neurogenesis in the olfactory epithelium, as well as those that allow for the preservation of olfaction throughout the time that neurons are turning over. Ideally, we would like to address this in a culture system in which olfactory receptor neurons (ORNs) are generated from their progenitor cells, and in which those progenitors continue to proliferate.

We have previously shown that ORNs arise from globose basal cells (GBCs) in the perinatal rat; GBCs, therefore, appear to represent the "stem" cell for ORNs. Unfortunately, we, and several other groups, have not been able to isolate a pure population of GBCs in a way that allows continual proliferation and differentiation into ORNs. Limited success has been achieved in several laboratories with the use of fibroblast growth factors and unknown glial-derived factors; however, neurogenesis in culture never approaches the level found in the animal. This suggests that the culture systems are missing important components that allow for continued neurogenesis, or that the process of dissociation irrevocably alters the potential of the neuronal stem cells.

As an alternative to isolation of the olfactory stem cell, we have begun to construct cell lines derived from the rat nasal epithelium. We have used two approaches, both of which employ a replication-incompetent retrovirus harboring a temperature-sensitive mutant (tsA58) of an oncogene (SV40 large T antigen). In one case, we infected the nasal epithelium *in vivo*, allowed the cells to proliferate and differentiate *in situ*, then dissociated the cells. In the other, we dissociated the epithelium and infected *in vitro*. The advantage of the former is that we should create lines derived from all progeny in the lineage of the originally infected cell; the advantage of the latter is the ease with which the experiments can be performed.

We are currently cloning cells infected with the retrovirus. Preliminary results demonstrate that some of these cells express NCAM, consistent with their derivation from the GBC-ORN lineage. As the lines are cloned, we will characterize the cells for the expression of cell type-specific markers, as well as for their ability to differentiate into ORNs when shifted to the temperature that is not permissive for the oncogene.

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Survival Of Cultured Olfactory Sensory Neurons Is Enhanced By Co-Culture With Target And Non-Target CNS Tissues. GRILL, R.J. and PIXLEY, S.K. (Dept. of Cell Biology, Neurobiology and Anatomy, Univ. of Cincinnati, College of Medicine, Cincinnati, Ohio 45267-0521).

Olfactory sensory neurons (OSNs) send their axons to a central target, the olfactory bulb (OB). In vivo studies suggest that contact with the OB promotes the survival of OSNs, as demonstrated by enhanced survival of mature, olfactory marker protein (OMP)+ OSNs in the olfactory epithelium (OE) (Schwob et al. 1992). In vitro explant studies (Chuah and Farbman, 1983) suggest that this trophic support is contact-dependent and specific to the OB; explants of cerebrum, cerebellum, cervical spinal cord and heart could not increase OMP in explants of OE. We have previously shown that dissociated adult olfactory epithelial (AOE) cells, when cocultured with dissociated newborn olfactory bulb, produce greater numbers of OMP+ OSNs than AOE cultures alone. The goal of the present study was to examine the specificity of the OSNs' trophic dependence on the OB by examining the effects of other CNS and non-CNS tissues on the number of mature OMP+ OSNs in culture. AOE cells were cocultured with dissociated newborn olfactory bulb (OB), newborn cerebellum (CERE), embryonic cerebral cortex (Cort), and embryonic ventral mesencephalon (VM). Cultures were fixed and immunostained for OMP at 5, 9 12 and 15 days. At 9 days, cocultures of AOE/OB, AOE/VM and AOE/CERE produced greater numbers of OMP+ OSNs than AOE alone or AOE/Cort. By 12 days, OB and VM, but not CERE or Cort increased numbers of OMP+ OSNs in AOE cultures. Coculture of AOE with purified OB, cortical glia or rat skin fibroblasts did not promote an increase in OSN survival, suggesting that the trophic support generated in the AOE/OB and AOE/VM cocultures was due to the presence of neurons. Thus 1) the OB does provide trophic support for OMP+ OSNs, 2) Cells from the VM also provide support for OSN survival. These results suggest that CNS tissues other than the target tissue, the OB, can promote the survival of OMP+ OSNs. Further research can now focus on mechanisms of trophic support provided by CNS tissues.

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Response Characteristics of Human Olfactory Neuroblastoma Cells: a Possible Model for the Study of Human Olfaction. GEORGE GOMEZ[1], DIEGO RESTREPO[1] and JAY ROTHSTEIN[2] (Monell Chemical Senses Center[1], 3500 Market St., Philadelphia, PA. Jefferson Cancer Center[2], Thomas Jefferson University, Philadelphia, PA).

Esthesioneuroblastoma or olfactory neuroblastoma (ONB) is a rare malignancy of the olfactory mucosa that is derived from the olfactory epithelium. Unlike normal mature cells of the olfactory epithelium, ONB cells do not contain olfactory marker protein (OMP) but have been shown to express the genetic marker *Hash-1* (the human homolog of the *Drosophila achaete-scute* gene typical of developing neurons), suggesting that ONB arises from olfactory neuronal stem cells. To assess the feasibility of using ONB cells as a tool to study olfactory neuronal cell function, we cultured ONB cells in the presence or absence of growth factors (10 ng/ml TGF α or bFGF) for five days and assessed their responsiveness to known odorants by measuring changes in intracellular calcium (see Restrepo et al. 1993, Biophys. J. 64:1961-1966). ONB cells cultured in the absence of growth factors (n=29) did not respond to odorants known to elicit formation of cAMP (mix A) or IP_3 (mix B) but showed an increase in intracellular calcium when presented with forskolin, a cAMP agonist. Out of 83 cells treated with TGF α 4 cells responded to mix A only, 24 cells responded to mix B only, and 4 cells responded to both A and B. TGF α - treated cells also responded to forskolin (37 out of 44) with an increase in intracellular calcium. These cells showed no morphological differences from the untreated cells but demonstrated an increase in cell proliferation. bFGF -treated cells (n=10) did not respond to mix A, B or forskolin and showed reduced cell proliferation. It appears that TGF α treatment may induce differentiation of ONB cells, providing a readily available source of human cells to study olfactory function and development. Studies including electrophysiological characterization of ONB cells and a comparison with normal human olfactory receptor neurons will be discussed.

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Competence and Specification of the Olfactory Placode in *Xenopus*. CHRISTINE A. BYRD[1], PETER C. BRUNJES[1], and ROBERT M. GRAINGER[2] (Departments of Psychology[1] and Biology[2], University of Virginia, Charlottesville, Virginia 22903)

During development, cells are induced to follow a specific fate based on specific interactions with other cells. For example, in lens formation interaction of the presumptive lens ectoderm with several different tissues (including mesoderm, neural plate, endoderm, and optic vesicle) is necessary; the inductive process begins with lens competence and is followed by specification (Grainger, *Trends in Genetics* 8:349, 1992). Much less is known about how the peripheral olfactory structures are formed. We have begun a systematic analysis of olfactory-placode induction using transplantation and explantation techniques in the frog, *Xenopus laevis*. Our experiments parallel those done for lens induction (*ibid.*). The first step has been defining periods of olfactory placode-forming competence and specification. Competence is the ability of a tissue to respond to an inducing signal; specification is the irreversible commitment of a tissue to a particular fate. The presumptive nasal ectoderm (PNE) from embryos at neural-plate and neural-tube stages was removed and replaced with pieces of ventral ectoderm (tissue that had not been influenced by underlying tissues) from donor embryos of varying stages to determine when ventral ectoderm gains and loses its ability to respond to an inducing signal. Animals were analyzed at late tadpole stages. The donor tissue was labelled with a fluorescent dextran, and the olfactory placode was identified by morphological criteria as well as by antibody labelling. Preliminary results suggest that ventral ectoderm is not competent or only slightly competent before midgastrula stage. Most transplants result in a mosaic placode formed from both host and donor tissue. For analysis of specification, pieces of ectoderm from embryos of varying stages was explanted and cultured until it was evident if the tissue was specified to become olfactory placode independent of other tissue interactions. Explants of PNE from neural-fold stage embryos result in formation of olfactory tissue and brain; explants of PNE from neural-plate stage embryos form cement glands and some brain tissue, but no olfactory tissue. Ongoing analyses will further define competence and specification of olfactory tissue and possible inducer tissues.

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Thyroid Hormone Induces Changes in the Nasal Capsules that Parallel those Observed at Metamorphosis. MARY A. PETTI[1] and GAIL D. BURD[2] (Tucson High School[1] and University of Arizona[2], Tucson, AZ).

In the African clawed frog, *Xenopus laevis*, prior to metamorphosis there are two areas of olfactory sensory epithelium: one in the principal cavity, which is water sensing at this time in development, and the other in the vomeronasal cavity. At the onset of metamorphosis, a third area of sensory epithelium develops in the middle cavity (Key, 1986; Weiss, 1986). This area becomes the water-sensing cavity of the adult *Xenopus*. The principal cavity during metamorphosis is restructured and becomes the air-sensing cavity of the adult (Altner, 1962). Changes in these cavities occur during development with the onset of T^4 secretion (Leloup and Buscaglia, 1977). As a continuation of our work on thyroid hormone effects on olfactory system development, the goal of the current study was to determine whether T^4 is responsible for inducing these changes in the olfactory epithelium. Stage 48, *Xenopus* siblings were placed in groups of 6 per 500 ml rearing solution (controls) or rearing solution containing 5 nM T^4 (experimental group). Animals were processed after 14 or 21 days for immunocytochemistry with a monoclonal antibody, E7 (Matheson and Burd, 1991), that specifically stains olfactory receptor cells. The secondary antibody was goat-anti-mouse-Cy3 (or Texas Red). Volume data and 3D reconstructions were obtained using a Bioquant Image Analysis System (R&M Biometrics, Inc.). Results indicate that T^4 stimulates volume growth in all areas of olfactory epithelia. T^4 also appears to initiate and stimulate the growth of the water-sensing capsule and its olfactory epithelium by 14 days. Nasal capsules under T^4 influence also show a restructuring of both nonsensory and sensory epithelium. Sensory epithelium in the principal cavity of day 21 T^4 treated animals is found primarily in the medial and caudal walls. In addition the principal cavity, the air-sensing cavity of the adult, shows a progressive loss of E7 stain which is characteristic of late metamorphosis in normal animals. This loss of stain may be associated with a change in receptor cell type from larval, water-sensing receptor cells to adult, air-sensing receptor cells. Supported by an NIH grant from Research Resources.

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Do NGF-Positive Mast Cells Participate in Target-derived Neurotrophin Enhancement of Extrinsic Innervation in Chemosensory Organs in NGF-Transgenic Mice? T. V. GETCHELL[1-3], S. TAKAMI[1], A. KULKARNI-NARLA[1], K. M. ALBERS[4] & M. L. GETCHELL[2, 3] ([1]Dept. Physiol., [2]Sanders-Brown Ctr. on Aging, [3]Div. Otolaryngol.-Head and Neck Surg., Dept. Surg., [4]Dept. Pathol., Univ. of Kentucky Coll. of Med., Lexington, KY.)

We examined tissue-resident mast cells and their expression of nerve growth factor (NGF) in the nasal and oral chemosensory organs of transgenic mice in which NGF expression was driven by a keratin (K) 14 promoter. We identified mast cells by staining with pH 1.0 Alcian blue, and localized and quantified their occurrence in the tongue and hard palate of the oral cavity and in the olfactory, respiratory, and vomeronasal mucosae of the nasal cavity. The number of mast cells was 2.0X greater in the tongue ($P<0.05$), and the hard palate ($P<0.001$) of the transgenic mice than in age-matched controls. There were 1.2X more mast cells in the olfactory, 3.1X more in the respiratory and 4.1X more in the vomeronasal nonsensory ($P<0.05$) mucosae of the transgenic mice than in controls. To differentiate between mucosal and connective tissue mast cells (MMC and CTMC, respectively), sections were stained in sequence with pH 0.3 Alcian blue for MMC and 0.5% safranin for CTMC. Mast cells in the tongue and hard palate of control animals exhibited the CTMC phenotype. In contrast, mast cells in these tissues of transgenic animals exhibited phenotypic switching to the MMC phenotype. Mast cells in both the transgenic and control mice were NGF-immunoreactive. The mast cells appeared to be aggregated in the lamina propria in close proximity to NGF-immuno-reactive basal cells. We previously demonstrated that the tongue and hard palate were hyperinnervated by NGF-immunoreactive extrinsic fibers. These results suggest that NGF expressed by the transgene induces phenotypic switching of mast cells and that NGF-immunoreactive mast cells together with K14-immunoreactive basal cells, which also synthesize NGF in the transgenic mice, provide the requisite neurotrophic support for the augmented extrinsic innervation.

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Evidence for a stereotyped and highly organized spatial map of sensory input in the olfactory bulb. SUSAN L. SULLIVAN, KERRY J. RESSLER, AND LINDA B. BUCK (Dept. of Neurobiology, Harvard Medical School, Boston, MA)

The odorant receptor gene family contains ~1000 members, each of which is expressed by only a small fraction of olfactory sensory neurons. Neurons that express the same odorant receptor gene are confined to one of four distinct odorant receptor expression zones in the olfactory epithelium. However, within that zone, those neurons appear to be randomly distributed, such that each zone is a mosaic of sensory neurons expressing different receptor types. Thus there may be an initial broad organization of sensory information in the nose into zones, but information within the zone is still highly distributed. In recent studies, we have found that odorant receptor mRNAs are present in the axons of olfactory sensory neurons. This has allowed us to examine the patterns of synapses formed in the olfactory bulb by neurons that express the same odorant receptor gene. Using in situ hybridization with specific odorant receptor probes, we find high concentrations of receptor mRNAs within a small number of glomeruli in the olfactory bulb. Each odorant receptor probe that we have examined hybridizes to only 2-5 of the ~2000 glomeruli in each mouse olfactory bulb. The glomeruli are located at two distinct sites in each olfactory bulb. Glomeruli that hybridize to different probes are different. Furthermore, they appear to be located at approximately the same place in each olfactory bulb. Our results suggest that in the olfactory bulb, sensory information undergoes a pronounced organization that produces a fine, and perhaps positionally stereotyped, spatial map.

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Spatial Patterns of Olfactory Receptors Expression. BREER, H., BECK, A., WANNER, I., HELFRICH, T., KONZELMANN, S., STROTMANN, J. Institute of Zoophysiology, University Stuttgart-Hohenheim, 70599 Stuttgart, Germany

The topographic compartimentalization of olfactory reactivity has led to the concept that sensory neurons expressing a given receptor type may be spatially distributed within the olfactory epithelium. This notion can now be accessed by in situ hybridization employing receptor-specific probes. Most of the receptor types analysed so far are expressed in one of several rather broad zones. In addition, there is a set of odorant receptors which are expressed in distinct populations of neurons clustered in a small region of the nasal epithelium of rat. This clustered type of expression was also observed in other rodent species but not in rabbit and bovine. The broadly distributed receptor types were found in the appropriate zones in different species. These observations suggest that very strict and conserved mechanisms control the spatial expression of odorant receptors in mammals. Towards an understanding of the principles involved, the onset of receptor expression and pattern formation during development was analysed. The first signs of receptor-expressing cells were observed between embryonic day E12 and E14. During the prenatal stages until birth the number of receptor-expressing cells increased exponentially and the pattern of segregation resembles that of adults.

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Lack of Evidence for an Inherent Spatial Patterning of Olfactory Receptor Sensitivities in the Olfactory Organ of the Channel Catfish. QINHUI CHANG and JOHN CAPRIO (Louisiana State University, Baton Rouge, LA. 70803)

In situ hybridization studies recently suggested that at most, only a few odorant receptor genes are expressed in individual olfactory receptor neurons (ORNs) and that single ORNs which express the same odorant receptor gene(s) are broadly distributed across the olfactory sensory epithelium (Ngai et al., 1993; Ressler et al, 1994). The present *in vivo* electrophysiological recordings support the molecular biological results of a random distribution of ORNs that express specific receptor genes or gene subfamilies across the olfactory organ of the channel catfish, *Ictalurus punctatus*. Each of the paired, bilaterally symmetric olfactory organs is composed of repeating lamellae in which rostral and caudal faces of the lamellae are organized into medial sensory and lateral nonsensory regions (Caprio and Raderman-Little, 1978). Microelectrode (platinum-black) recordings were obtained from different lamellar sensory regions, comprising dorsal and ventral sites, located on rostral, intermediate and caudal lamellae in a total of twenty-eight channel catfish (23-59g). Stimuli included 0.1mM L-Ala, L-Met, L-Arg, ATP, 1.0 mM L-Glu and a mixture of bile acids (Na^+ salts of cholic, taurocholic and taurolithocholic acids, each at 0.1mM), odorants previously reported to bind to independent receptor sites. The peak of the integrated olfactory receptor responses at each recording site was standardized to the response to L-Ala. Statistical analysis indicated that the stimulatory effectiveness of the stimuli were preserved across the ten olfactory lamellae recording sites (Repeated Measures MANOVA, $p>0.05$). These results are consistent with the reported random distribution of expressed olfactory receptor genes across the olfactory epithelium in this species.

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Cloning and Sequencing of an IP_3 Receptor Partial cDNA from Lobster Olfactory Organ. S. D. MUNGER [*,†], B.W. ACHE [*,†,‡] and R. M. GREENBERG[*] (Whitney Laboratory[*] and Depts. of Neuroscience[†] and Zoology[‡], Univ. of Florida, St. Augustine, FL).

Inositol 1,4,5-trisphosphate (IP_3) has been implicated as an olfactory second messenger (rev. in Breer and Boekhoff, *Curr. Opin. Neurobiol.*, **2**: 439-443). IP_3 may directly gate ion channels (IP_3 receptor, IP_3R) in the plasma membrane of the outer dendrites or cilia in lobster, rat and catfish olfactory receptor neurons (ORNs) (rev. in Ache, *sem. Cell Biol.*, **5**: 55, 1994). Antibodies directed against mammalian cerebellar, endoplasmic reticulum (ER) IP_3Rs recognize membrane proteins of appropriate size in lobster (Fadool & Ache, *Neuron* **9**: 907, 1992) and rat (Cunningham *et al.*, *Neurosci.* **57**: 339, 1993) ORNs. In lobster ORNs, the antibody perturbs the function of the IP_3R. We have exploited the suggested structural similarity between ER and plasma membrane IP_3Rs by amplifying a partial cDNA, homologous to known IP_3Rs, from reverse-transcribed lobster olfactory organ RNA using degenerate primers and touchdown PCR. We extended the clone to the 3'-noncoding region using 3'-RACE (Rapid Amplification of cDNA Ends). We have constructed an IP_3R mini-cDNA library and isolated overlapping clones by plaque hybridization, extending the cDNA 5' of the putative transmembrane regions. The open reading frame of the cDNA isolated to date comprises over 20% of the anticipated coding region. While Northern analysis demonstrates a low level of expression of a 10 kb message in the brain, but none in nose, the more sensitive ribonuclease protection assay shows the message is expressed at low levels in the olfactory organ and, at higher levels, in the brain, consistent with results in the rat (Cunningham et al., ibid.). We are currently working to extend the cDNA clone to the 5'-end and to localize the receptor by *in situ* hybridization and immunohistochemistry.

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Proteins Mediating Phosphoinositide Signaling in Olfactory Rosettes from Channel Catfish. RICHARD C. BRUCH and JIESHENG KANG (Department of Zoology & Physiology, Louisiana State University, Baton Rouge, LA 70803).

The binding of stimulus amino acids to odorant receptors evokes rapid and transient increases in phosphoinositide-derived second messenger levels in isolated cilia from channel catfish [Restrepo et al. Am. J. Physiol. 264, C906 (1993)]. The initial rapid increase in these second messengers is due to the action of an odorant-sensitive phospholipase C (PLC) that catalyzes the hydrolysis of phosphatidylinositiol 4,5-bisphosphate to form inositol 1,4,5-trisphosphate and diacylglycerol. In an effort to identify the odorant-sensitive PLC, we are using degenerate primers in reverse transcription-polymerase chain reaction (RT-PCR) to identify members of the multigene PLC family expressed in olfactory rosettes. Several PCR products have been cloned and are now being characterized by sequence analysis. The results of these analyses will be presented. The rapid decline of stimulus-activated accumulation of phospholipid-derived second messengers (i.e. signal termination) is due to the action of protein kinase C (PKC) [Boekhoff & Breer PNAS 89, 471 (1992)]. Using degenerate primers in the PCR, we have cloned several PCR products that encode members of the PKC multigene family. Thus far, PCR products corresponding to the $\alpha,\beta,\gamma,\delta,\epsilon$, and θPKC isotypes have been identified by sequence analysis. In addition, partial sequence analysis (about 50% of a 1.2 kb product) of another cloned PCR product suggests that it may correspond to a novel PKC isotype. The complete sequence of this clone, together with PCR analysis of its tissue distribution, will be presented.

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Studies of gene expression in single rat olfactory neurons DIEGO RESTREPO[1,2] and NANCY E. RAWSON[1]. [1]Monell Chemical Senses Center, Philadelphia PA 19107 and [2]Dept. Physiology, University of Pennsylvania, Philadelphia PA 19107.

Methods for analyzing gene expression at a single-cell level have been used to correlate gene expression with function in the same cell. Gene expression is characterized by reverse transcription of individual cell mRNA taken up by diffusion into a patch pipette used to study function electrophysiologically. cDNA can then be made and further amplified via synthesis of antisense RNA (aRNA; Eberwine et al., PNAS 89:3010-3014, 1992). We have modified this technique to study gene expression in single, isolated rat olfactory neurons whose odorant responsivity can first be characterized using calcium-imaging techniques (Restrepo et al., J. Gen. Physiol. 102:907-924, 1993). Gene expression is studied using single-cell cDNA directly or with cDNA synthesized after amplification via aRNA. The polymerase chain reaction (PCR) was carried out with primers designed to amplify olfactory-specific (OMP, Gq, olfactory receptor (OR)) and nonspecific (β-actin) cDNA sequences. The presence of contaminating genomic DNA is assessed by primers that will amplify an intron-containing segment of β-actin. Of 28 cells for which cDNA was obtained, 10 produced the appropriately sized β-actin and OMP PCR products. We have also begun to explore the question of whether an individual ON expresses one or more than one type of olfactory receptor (OR). Degenerate primers targeting the DNA regions that encode transmembrane domains II and IV (AYDRYV) and V and VI (HKAFST) of the amino acid sequence were used. These primers produce multiple receptor products when olfactory tissue cDNA is used as template for the PCR reaction. PCR products have been obtained using these primers and cDNA from 5 of the above single ON samples. These products are identical in size to those obtained from olfactory tissue cDNA, and are being cloned and sequenced to assess homology with known OR sequences.

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A Molecular Approach of Odor detection in the Honey Bee *A. Mellifera.*

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Olfactory perception by honey bees includes both specific and general odors. The mechanisms underlying peripheral recognition of odorants imply the molecular stimulation of antennal sensory neurons. On the one hand, odorants have to reach their sensory targets and it is now generally admitted that they are transported and solubilized to pass through the hydrophilic perireceptor lymph by the Odorant-Binding Proteins (OBPs).On the other hand, the specific activation of sensory cells depends on membrane receptors, as well documented in vertebrates. In order to understand such mechanisms in the honey bee *A. mellifera*, we have focused our study on the characterization of both OBPs and olfactory receptors. Five putative Odorant-Binding Proteins have been isolated from antennal soluble fractions by protein purification using non denaturing electrophoresis, chromatography HPLC, and N-terminal sequencing. Partial cDNA clones of four putative olfactory receptors were obtained by nested-PCR amplification with degenerate primers derived from conserved domains of vertebrate odorant receptor genes. The deduced amino acid sequences possess the hydrophobic domains IV to VII of the G protein coupled receptors. These four clones share 30 to 92% amino acid identity and belong to the multigenic familiy of odorant receptors. The microdiversity of OBPs and the evidence of transduction mechanism implying G protein-coupled receptors in the honey bee are discussed in the context of odor discrimination and the evolution of these multigenic families.

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Isolation and Characterization of an Abundant Olfactory Specific Membrane Protein in *A. polyphemus*. MATTHEW E. ROGERS and RICHARD G. VOGT (University of South Carolina, Department of Biological Sciences)

RP11 is the most abundant dendritic membrane protein present in the male antenna of the silk moth *Antheraea polyphemus*. In a previous effort to identify an insect pheromone receptor, a radiolabelled photoaffinity analog of the *A. polyphemus* pheromone was used in binding experiments to successfully visualize a 69 kDa membrane protein present in the olfactory cilia of pheromone specific neurons. This protein was purified and partially sequenced. A cDNA library derived from antennal mRNA was screened with a probe specific to the N-terminus of the protein, yielding a 2.5kb clone (1575 bp coding region) called RP11. No sequence homology was observed for any members of the G-protein coupled receptor family. However, homology searches using the NCBI Blast server yielded several sequences with significant homology with RP11, including two vertebrate derived membrane bound proteins (CD-36 and LIMP II) and two *Drosophila* derived membrane proteins, S43236 and DMEMP-1. Tissue distribution studies by Northern blot and Ribonuclesase Protection Assay (RPA) show that RP11 expression is antenna specific (no expression in CNS), with abundant expression in male antenna and diminutive expression in female antenna. RPAs of total RNA isolated at different stages of adult development, show that RP11 expression initiates very late during development, about one day before adult eclosion. Analysis of the genomic organization of RP11 by Southern blot suggests that RP11 is member of a multigene family containing at least two and possibly three members in *A. polyphemus*; RPA and Northern Blot analysis suggest that at least two forms are expressed as mRNA. The observations that RP11 is antenna specific and expressed in multiple forms late in adult development, suggest that RP11 has an important role in odor detection.

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Determination of Kinetic Rate Constants from Rapid Perfusion Experiments with Olfactory Cyclic Nucleotide-Gated Channels.

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Patch clamp measurements of the response of olfactory cyclic-nucleotide-gated channels to brief (3-33 ms) pulses of cAMP applied using rapid perfusion techniques provide new insights into the kinetics of sensory transduction. The measured time-course of both the current onset and decay in response to a step-pulse of agonist imposes new challenges for kinetic models.

To meet these challenges, we have developed a mathematical procedure for extracting detailed rate information from the results of rapid perfusion experiments - by analyzing the concentration and time dependence of (1) the initial rise of the cAMP induced current, (2) the continued rise in the current after the end of the brief cAMP pulse, and (3) the final current decay. In particular, our analysis of the slow time-dependence of the transient olfactory channel currents induced by pulses of cAMP over a wide range of concentrations (from 1 μM - 10,000 μM) suggests a modified, Monod-Wyman-Changeux type kinetic model in which the unbound closed state of the channel protein undergoes first a slow, $k_{+1} = 8 \times 10^{-5}$ 1/Ms binding step, then a slow conformational step, $\beta = 5$ 1/s, to an open state, followed by additional binding steps to multiply bound open and closed states. Our results provide testable predictions for single channel studies of the channel kinetics. For example, the slow rate limiting step to an open state predicts long (100-200 ms).first latencies preceeding the first opening of the channel following a step application of agonist. In addition, a numerical model of this kinetic scheme provides a good description of the onset, decay and saturated values of the olfactory channel currents and provides the basis for more detailed models of the full G-protein cascade describing olfactory transduction, from the receptor activation by odor molecules to channel opening.

This method of analysis allows us to infer properties of the transient cAMP-pulses from the dependence of CNG-currents. These empirically derived kinetic models serve in turn to motivate new experimental measurements to further elucidate the non-equilibrium dynamics of ligand-gated ion channels.

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Effect of Nitric Oxide Donors on the cyclic nucleotide gated channels of olfactory receptor neurons.

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In vertebrate olfactory neurons the final step of the transduction cascade is mediated by an ion channel activated by cAMP. Nitric oxide (NO) is best known to act as an intercellular messenger via guanylate cyclase and has been reported to have direct effects on some ion channels. To test the direct effect of NO on cyclic nucleotide gated (CNG) channels and to distinguish it from the cAMP pathway, we used excised inside-out patches from olfactory receptor neurons isolated from the nasal epithelium of adult-phase tiger salamanders (*Ambystoma tigrinum*). As a control, cAMP (20-500 μM) was first added to every patch in order to test for the presence of CNG channels. cAMP was then washed out and nitric oxide donors, SIN 1 (200 μM) or S-nitrosocysteine (SNC, 1-1000 μM), were added to the cytosolic solution, resulting in single channel openings and long bursts of openings. The single channel conductance was not affected, but a significant increase in the open probability was observed. To gain insight into the molecular mechanism by which these NO donors activated the CNG channels, we tested the effects of a thioate derivative of cAMP (Rp-cAMPS) known to have an antagonist effect on the CNG channel by binding to the cyclic nucleotide (CN) binding domain of the channel. 500 μM Rp-cAMPS competitively inhibited the action of cAMP (20 μM) but had no apparent effect on channel openings due to SNC (100 μM). These results suggest that NO activation of the channel does not occur via the CN binding site. We also investigated whether the NO effects resulted from a chemical modification of sulfhydryl groups. The application of iodoacetamide (IAA, 2 mM), a sulfhydryl binding reagent, to the patches induced an immediate activation of CNG channels. There was no further activation of these channels when the recordings were made in the presence of both IAA and SNC, suggesting that these two substances might activate the channels in the same way. Our results demonstrate that NO can directly activate the olfactory CNG channels independently from the intracellular regulatory cascade. Its action could be via the chemical modification of SH groups.

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Noise Analysis of cAMP- and Ca^{2+}-gated Channels in Isolated Olfactory Cilia.

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Macroscopic currents activated by cytoplasmic cAMP or Ca^{2+} were studied in single cilia excised from frog olfactory receptor neurons. Ion-channel noise was observed as a fluctuation spectrum in the input admittance of the cilium and analyzed under the assumption that the channels are distributed uniformly along the ciliary membrane. Noise was recorded as a function of cytoplasmic cAMP concentration. Under voltage clamp at -50 mV, ciliary noise was recorded for 60 sec with sampling at 7 kHz and low-pass filtering at 3 kHz. Divalent cations, which block the cAMP-gated channels, were chelated to very low levels on both sides of the membrane. The noise spectrum was fit to a single low-frequency Lorentzian with a corner frequency of approximately 2-5 Hz. Analysis of the variance-to-mean ratio corrected for cable decrement suggests a unit conductance of 9 pS for the cAMP-gated channel. This is close to values determined elsewhere by directly examining single cAMP-gated channels from frog olfactory receptor neurons. Increasing cytoplasmic Ca^{2+} concentration is known to activate a ciliary Cl^- current, but corresponding single channels have not yet been described. Noise analysis of this current at -30 mV indicated the presence of a Ca^{2+}-activated Cl^- channel with a unit conductance of 0.6 pS, inferred from the variance-to-mean corrected for cable decrement. In the absence of cAMP and Ca^{2+}, an order-of-magnitude smaller component of noise was observed, having a spectrum which could be fit by approximately two Lorentzians extending to frequencies of 50-200 Hz. It is known that Ca^{2+} entering the cilium through the cAMP-gated channels can accumulate sufficiently to activate the Ca^{2+}-activated Cl^- channels. In some olfactory neurons this secondary Cl^- current amplifies the initial depolarization caused by opening the cAMP-gated channels. Because the Cl^- current arises from a large number of very small channels, it should faithfully amplify the receptor current while causing little increase in noise.

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Characterization of $InsP_3$-Activated Channels in Reconstituted Membranes and Excised Patches From Rat Olfactory Neurons.

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In olfactory neurons from mammals some odors elicit an increase in the concentration of the second messenger inositol-1,4,5-trisphosphate ($InsP_3$) (Breer H. &. Boekhoff I., 1991). Earlier whole cell patch clamp studies have identified a nonselective cation conductance activated by $InsP_3$ in rat olfactory neurons (Okada Y. et al., 1994). To study the channels that underlie the $InsP_3$-activated conductance(s) in rat olfactory neurons, we have performed single channel measurements employing the patch clamp technique. Membranes isolated from rat olfactory cilia were reconstituted into azolectin bilayers at the tip of a patch pipette. In 18 of 42 high resistance bilayers (>10 GΩ) $InsP_3$ (1.25 to 30 μM) elicited channel openings. When the bath contained 110 mM KCl, 5 mM MOPS pH 7.0 (pseudointracellular solution), and the pipette 55 mM $BaCl_2$, 5 mM MOPS, pH 7.2, $InsP_3$ elicited inward currents at negative holding potentials. In some traces subconductance levels could be noticed. 20 μM ruthenium red blocked the $InsP_3$-activated currents. We also performed measurements in excised plasma membrane patches taken from the cell body of rat olfactory neurons. When currents were measured in the presence of 145 mM NaCl, 1mM $CaCl_2$, 20 mM Hepes, pH 7.2 in the extracellular solution and 145 mM K-aspartate, 10 mM Hepes, pH 7.2 in the intracellular solution, $InsP_3$, added to the cytosolic side, elicited single channel currents whose polarity was *outward* at -60 mV. Some *inward* current fluctuations could also be measured under these circumstances. In contrast, when the $CaCl_2$ in the extracellular solution was substituted by 5 mM $BaCl_2$, $InsP_3$ elicited only *inward* current fluctuations. These experiments provide the first evidence for direct gating of plasma membrane channels by $InsP_3$ in rat olfactory neurons.

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Patch Clamp Analysis of *Necturus* Olfactory Receptor Neurons in a Semi-Intact Slice Preparation. RONA J. DELAY and VINCENT E. DIONNE (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

Most work on olfactory receptor neurons (ORNs) examining their conductances and responses to odor has been done on isolated cells. Cell isolation procedures can be potentially damaging to ORNs, since exposure to degradative enzymes or harsh ionic conditions may affect the proteins which mediate the odor responses and membrane conductances. By using a semi-intact slice preparation it is possible to examine ORNs without subjecting them to such procedures. In addition, the cells can be filled with Lucifer yellow and biocytin during recording, allowing correlation of cell morphology with cell physiology. To prepare the slices, the olfactory epithelium was removed by blunt dissection, cut open along its lateral edges, and glued to a substrate with its apical surface up. Slices 200-300 μm thick were cut using the method of Bigiani and Roper (*Science* (1991) **252**:126-128); the cells remained viable for >24 hr when kept at 4°C in saline. Examination of the slices showed that the ORNs, sustentacular cells and basal cells aggregate in pear-shaped clusters surrounded by ciliated respiratory epithelium. Patch electrodes were readily sealed to each cell type. Spontaneous activity was observed in about one-third of the ORNs in on-cell recordings. Cell morphology was assessed by filling the cells with Lucifer yellow/biocytin from the pipet during whole-cell recording. The structure of ORNs in the slice varied enormously, from cells with long, thin dendritic processes and a cell body located close to the basal lamina to cells with a short, stubby dendrite and a cell body located close to the apical surface. Despite the length of the dendrite or the location of the cell body, all ORNs so far examined have exhibited voltage-activated transient inward currents and sustained outward currents. Studies are now in progress to characterize the voltage-dependent conductances in ORNs in the slice preparation and to evaluate the sensitivity of these cells to odorants.

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Whole-Cell Currents of Isolated Zebrafish Olfactory Receptor Neurons. W. C. MICHEL, F. S. COROTTO, and D. R. PIPER (Department of Physiology, University of Utah School of Medicine, Salt Lake City, UT 84108).

Olfactory receptor neurons (ORNs) were isolated from the zebrafish *Danio rerio* by treating the olfactory rosette with papain (0.25mg/ml in divalent cation-free Ringer's) followed by trituration. Using the whole-cell voltage clamp technique, we found that zebrafish ORNs had an average capacitance of 0.65 pF and an average apparent input resistance of 6.7 GΩ. Cells possessed two inward currents: a fast, transient, TTX-sensitive inward current that was presumably carried by Na^+ and a tiny, sustained, labile, and TTX-insensitive current that was absent when $CaCl_2$ and $MgCl_2$ were replaced with $CoCl_2$ (3.2 mM) in the bath, indicating that it was carried by Ca^{2+}. Steady-state inactivation of I_{Na} was half maximal at -78 mV. At -104 mV, recovery of I_{Na} from inactivation proceeded with two time constants averaging 23 ms and 532 ms. Results from two cells indicate that recovery from inactivation was much faster at -124 mV. Zebrafish ORNs possessed three outward currents all of which were carried by K^+ based on the reversal potentials of tail currents. One was a Ca^{2+}-dependent K^+-current that activated at *ca.* -30 mV, reached a peak at *ca.* 5 mV, and declined at more positive potentials. The other two K^+-currents activated at *ca.* -20 mV and reached peak amplitudes at *ca.* 40 mV. One of these was a transient K^+-current that was largely inactivated at a holding potential of -44 mV thus giving it properties of I_A. The remaining K^+-current had properties of a delayed rectifier. We searched for inward rectification in 15 cells and found it in just one. This was not explored further. Odor mixtures elicited a 20-40 pA current that reversed at *ca.* 0 mV indicating a mixed ionic conductance.

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Dual Effects of Odorants on the Olfactory Receptor Cell

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Olfactory receptor cells show an excitable response to appropriate odor stimuli. We show that some odorants can also suppress this excitation by a mechanism which is distinct from adaptation or inhibition. Whole-cell membrane currents were recorded from a single, dissociated olfactory receptor cells from the newt, *Cynops pyrrhogaster*. Amyl acetate stimulus to a cell caused an inward current expressing a excitation of the cell when the cell was voltage clamped at their resting potential (-50 mV). Additional odor pulse (50 ms) which was timed at the peak of the inward current reduced the current amplitude by 60%. This current reduction was shown to be the reduction in the activities of the ionic channels activated by amyl acetate (very likely to be the mixture of currents through cAMP-gated channels and Ca-activated Cl channels), not by an activation of inhibitory current like K channels. Amyl acetate stimuli also suppressed a current induced by a photolysis of the caged cAMP, indicating that the target of the odorant is present at the downstream to the odorant-receptor proteins. Suppression was also observed in cells which did not show excitable responses, indicating that the expression of receptor proteins is not related to the suppression. This new finding of suppressive effect explains long-standing puzzling phenomena appeared in the olfactory transduction current; a surprisingly long latency (i.e. 500 ms) and the presence of off-response. We also speculate that the suppression may play important roles on the olfactory sensation, such as masking or tuning for odor discriminations.

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Antisense Oligonucleotides To Disrupt Paramecium Chemoresponse. J. YANO, W. E. BELL, and J. VAN HOUTEN, University of Vermont, Burlington, VT USA

Antisense oligonucleotides can be electroporated into paramecia and used to down regulate components of the chemosensory transduction pathways. We have used an anti-calmodulin oligonucleotide to reduce the amount of calmodulin in whole cells and subsequently assayed the ability of these cells to respond to chemical stimuli. The cells are considered to be transformed by the oligonucleotides when a calcium activated Na conductance is reduced making backward swimming in depolarizing conditions much shorter. In order to quantify more directly that the levels of calmodulin after antisense oligonucleotide treatment, we are developing an ELISA.

There are three classes of chemoattractant stimuli for *Paramecium tetraurelia*. One class, represented by NH_4Cl, affects intracellular pH and does not appear to involve a calmodulin regulated pump current or channel. A second class, represented by acetate, appears to affect cell behavior by activating a calmodulin regulated calcium pump. A third class, represented by glutamate, appears to involve not only the pump but also the second messenger cyclic AMP. Therefore, we predicted that the anti-calmodulin antisense treatment would disrupt responses to two of the three classes of stimuli. We measured response to the stimulus by the immediate increase in swimming speed that accompanies the hyperpolarization elicited by these stimuli. Cells treated with sense or anti-sense oligonucleotides increased speed as usual when stimulated with NH_4Cl. However, cells stimulated with acetate did not show the characteristic speed increase when treated with antisense (but not sense oligonucleotides). The effects on the speed of cells in glutamate could not be ascertained because the process of electroporation alone affected this response, regardless of the oligonucleotide used. Currently, we are measuring the membrane potential changes in the transformed and control cells to determine whether the hyperpolarizations in stimuli are reduced by the anti-calmodulin oligonucleotide treatment. We predict that the hyperpolarization will be within normal ranges in NH_4Cl but not in acetate.

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Ionic basis of odor-activated currents in cultured lobster olfactory receptor cells. BARRY W. ACHE AND ASLBEK ZHAINAZAROV (Whitney Laboratory and Depts. Zoology & Neuroscience, Univ. of Florida, St. Augustine, FL 32086)

Odors evoke both inward and outward currents in cultured lobster olfactory receptor neurons held near rest (Fadool et al., *J. Exp. Biol.* 174:215, 1993). We investigated the ionic bases of these currents by pressure-ejecting odors (betaine, adenosine 5'-monophosphate, adenosine 5'-triphosphate, and L-proline, 100μM-1mM pipette concentration) onto the cells. The inward current at rest could be ascribed to activation of a non-selective cation conductance: the current reversed *ca* 10 mv, substituting Li^+ or K^+ for Na^+, changing $[Cl^-]_o$ did not alter the reversal potential, and the current was not blocked by amiloride (100 μM). The outward current at rest could be ascribed to suppression of a background Cl^- conductance: the current reversed *ca* -50 mV, was blocked by Zn^{2+} (2 mM) from the extracellular side, changing $[Cl^-]_o$ from 518 to 60 mM shifted the reversal potential from -50 to -12 mV, and the current was not affected by K^+ channel blockers (TEA, 10 mM; 4-AP, 2 mM; Cs^+, 2 mM) nor by increasing $[K^+]_o$ from 13.4 to 180 mM. All four odors regulated both conductances and in some instances a given odor regulated both conductances in the same cell. In the latter case, blocking the Cl^- conductance with Zn^{2+} left a residual inward current that reversed at 10 mV and could be ascribed to the non-selective cation conductance. The total current evoked by a given odor reversed at potentials ranging from -40 and 60 mV, suggesting that the relative contribution of these two conductances to the receptor current varies across cells. Odors may activate other or additional conductances in some cells. These results reveal a fourth odor-regulated conductance, a background Cl^- conductance, to be implicated in olfactory transduction in the lobster and show that a single odor independently can regulate two different conductances in the same cell.

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Two different G-proteins mediate odor-evoked currents in cultured lobster olfactory neurons. S.J. ESTEY, D.A. FADOOL[1], AND B.W. ACHE (Whitney Laboratory and Depts. of Zoology & Neuroscience, Univ. of Florida, St. Augustine, FL 32086. [1]Present address: Volen Center for Complex Systems, Brandeis University, Waltham, MA 02254).

Odors evoke outward as well as inward currents in the same lobster olfactory receptor neuron maintained in primary culture. Earlier we reported at this meeting that odor-evoked currents of either polarity were (1) increased by perfusing the cells with GTPγS in the patch pipette, (2) decreased by perfusion with GDPβS, and (3) insensitive to pertussis (or pertussis toxin A subunit) and cholera toxins (Fadool, Michel & Ache (1991) Chem. Senses 16:518), although pertussis toxin ADP-ribosylated a 38kD protein in the cells (McClintock, Edwards & Ache (1990) Chem. Senses 15:617). We now report that perfusing the cells with either anti-Goα or antiserum against a common carboxyl terminal sequence of Gq and Gα11 selectively attenuated the odor-evoked inward current. Neither of these antisera, nor antisera to Golfα, Gβ, Gα11, an internal Gq sequence, nor non-immune rabbit IgG perturbed the odor-evoked outward current. Western blots of membranes prepared from lobster olfactory outer dendrites were performed using all these antisera, and the only positive band was one of approximately 40kD labeled by the anti-Gq/α11 antiserum. This band could be eliminated by preincubating the antiserum with the antigenic peptide. Together with our earlier results, these findings suggest that two different G-proteins mediate odor-evoked currents in cultured lobster olfactory receptor neurons, and presumably do so in the same cell. One of the G-proteins may be of the αi or αq family.

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Na^+-activated non-selective cation channels in primary olfactory neurons. ASLBEK ZHAINAZAROV AND BARRY W. ACHE (Whitney Laboratory and Depts. Zoology & Neuroscience. Univ. of Florida, St. Augustine, FL 32086).

Excised inside-out patch recordings were used to describe a novel cation channel from cultured lobster olfactory receptor neurons (ORNs). The channel is reversibly activated by intracellular Na^+ as low as 5 mM. The half-effect concentration of intracellular Na^+ is about 60 mM at -60 mV. The channel is equally permeable to Na^+, K^+, and Li^+. In symmetrical 210 mM Na^+, the open channel current-voltage relationship shows slight inward rectification at positive potentials. The slope conductance of the channel is 107 pS between -90 and 0 mV. Although the channel is not activated by voltage in the absence of intracellular Na^+, the gating of the channel is dependent on voltage as well as $[Na^+]_i$ and $[Na^+]_o$. Both intracellular Ca^{2+} and Mg^{2+} reversibly alter channel activity in a concentration-dependent manner starting at 1 μM. Ca^{2+} decreases both the open probability and the single channel amplitude, while Mg^{2+} decreases the open probability, but has no effect on the single channel amplitude. Five mM Ba^{2+}, but not 20 mM Cs^+ or 100 μM amelioratine reversibly block the channel. We speculate that the channel regulates excitability by accentuating the rate and/or the magnitude of depolarization of the ORN to odors. A "signal boosting" role has also been proposed for Ca^{2+}-activated Cl^- channels in amphibian and mammalian ORNs (e.g., Kurahashi & Yau, 1993) and for Ca^{2+}-activated non-selective cation channels in insect (Zufall & Hatt, 1991) and amphibian (Schild & Bischofberger, 1991) ORNs, suggesting that electrophysiological amplification may be a common component of olfactory transduction that has been addressed by different strategies in different animals.

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L-Glutamate's Excitatory and Inhibitory Effects on Olfactory Receptor Neurons of Spiny Lobsters May Be Mediated by Dissimilar Receptor Types. MICHELE BURGESS and CHARLES DERBY (Georgia State University, Atlanta, GA)

In vertebrates and invertebrates, L-glutamate can be an excitatory and inhibitory neurotransmitter, and a chemosensory feeding attractant. We have characterized the glutamate receptors on olfactory receptor neurons (ORNs) of the spiny lobster *Panulirus argus* and compared them with glutamate receptors in other internal and external systems. To do so, we have used single-unit electrophysiological recordings and biochemical receptor-binding techniques, together with glutamate analogues and food-related odors. Binding studies using olfactory tissue show that glutamate has two affinity sites (Kd values *ca.* 1 μM and 1 nM). The higher affinity site presumably represents receptors involved in transduction; the lower affinity site may also include olfactory receptors, but possibly includes other glutamate-binding sites. Electrophysiological recordings demonstrate that ORNs can be either excited or inhibited by glutamate. For both of these types of glutamate-sensitive ORNs (regardless of whether or not glutamate is the most effective odorant for a given ORN), the activity of glutamate was unlike any of the 7 tested glutamate analogues, but was most similar to NMDA. However, when responses to the entire set of analogues and glutamate is considered, the activity of the glutamate-excited ORNs and glutamate-inhibited ORNs was poorly correlated. These findings suggest dissimilarities in the glutamate receptor sites on the glutamate-excited and glutamate-inhibited ORNs. Based on their response features, these olfactory receptors are unlike other known glutamate receptors.

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Identification of Potential Ligand-Binding Residues in Rat Olfactory Receptors by Correlated Mutation Analysis. MICHAEL S. SINGER[1], GORDON M. SHEPHERD[1], and GERRIT VRIEND[2] (Sections of Neurobiology[1], Yale Medical School, New Haven, CT 06510, and Biocomputing[2], European Molecular Biology Laboratory, Heidelberg, Germany D-69012)

A family of G protein-coupled receptors (GPCRs) in olfactory receptor cells is thought to mediate the recognition of odor signal molecules. We have recently provided evidence from molecular modeling that the ligand-receptor interaction takes place in a binding pocket similar to that found in other GPCRs (Singer and Shepherd, 1994). We report a test of this hypothesis by the independent method of correlated mutation analysis. This method can be applied to a group of homologous proteins, such as the olfactory receptor family, to discern patterns of correlated residues that reflect functional constraints on the proteins. The method has already proven useful in identifying ligand-binding residues in neurotransmitter receptors (Kuipers et al, in press). Analysis of ten receptors (Buck and Axel, 1991) identified several residues in locations consistent with ligand-binding function. Some of these residues have already been implicated in ligand binding by our molecular modeling studies and by site-directed mutagenesis in other superfamily members. Histidine residues were of special interest; properties of the imidazole ring may provide a mechanism for broad affinities for different odor ligands. Higher-order groups of residues may function as modules interacting with single ligand determinants. Combinations of modules could provide a multidimensional basis for recognizing diverse arrays of odor ligands. The results support the presence of a ligand binding domain conserved across olfactory receptors and analogous to that in other superfamily members. They also provide a rational basis for site-directed mutagenesis.

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Determinant-Based Model for Ligand-Receptor Interactions in Olfactory Receptors. GORDON M. SHEPHERD and MICHAEL S. SINGER (Section of Neurobiology, Yale Medical School, New Haven, CT 06510)

Odor discrimination in mammals is probably achieved by differential activation of up to 1,000 distinct G protein-coupled receptors (GPCRs) (e.g. Buck and Axel, 1991). It has been postulated that receptor binding depends on specific ligand determinants, also termed odotopes (Shepherd, 1991; Hildebrand, 1994). By analogy with well-understood mechanisms of ligand recognition (e.g. adrenergic receptors), we propose a complementarity-based model involving a small number of ligand determinants. We define a determinant as a discrete ligand substructure that forms dispersion forces, coloumbic force(s), or hydrogen bond(s) with receptor subsites; in addition, a given interaction must confer receptor specificity for that ligand. An anti-determinant is a substructure that forms a repulsive force. These properties will depend on each ligand-receptor pair. One or more residues may constitute a binding subsite that interacts with a ligand determinant. Each subsite may show graded affinities for a linear array of substructures along a determinant dimension. These affinities may be modelled as a Gaussian function centered on the dominant determinant. Statistical modelling and comparison with other superfamily members suggest that odor receptors contain an average of 2-4 subsites; ligands contain a similar number of determinants. An olfactophore for a given receptor may be defined as the set of odor ligand determinants arranged in a specific (active) geometry. Olfactophore analysis of lyral and lilial and correlated mutation analysis of rat olfactory receptors provide preliminary support for these hypotheses. In addition to offering insight into the information encoded in signal molecules, the determinant-based model provides a practical framework for mapping odor ligand structures and implementing molecular comparison algorithms.

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Tuning specificities to aliphatic odorants in mouse olfactory receptor cells. TAKAAKI SATO[1,2], JUNZO HIRONO[1] and MASAMINE TAKEBAYASHI[1] (Life Electronics Research Center[1], Amagasaki, Hyogo, Japan and Monell Chemical Senses Center[2], Philadelphia, PA)

The tuning specificities of mouse olfactory receptor cells were examined using three homologous series of aliphatic odorants consisting of a straight chain of 3-9 carbons and a terminal functional group (carboxyl, hydroxyl, or amino). Responses were recorded optically by measuring intracellular calcium increases with fura-2. Cells were isolated by the tissue-printing method, which was expected to preserve the relative spatial relationship of the cells in the intact tissue (Hirono et al., 1992, J.Neurosci.Meth.42:185-194). Over fifty cells were sensitive to subsets of series of odorants depending on both the carbon chain length and the functional group. In most cells, the sensitivity was maximal at a single carbon chain length. Some cells could detect only one out of the three functional groups tested within the odorant concentration range examined. Other cells responded to two or three series of odorants with various relative sensitivities among the series. Five cells preferring the carboxyl group to the hydroxyl group responded with less sensitivity to aliphatic amines. In a single cell, the optimal length of the carbon chain was similar for different functional groups. These results suggest that there are a small number of odorants to which a given receptor protein is most sensitive. Based on these data we also suggest that hydrogen bonding and hydrophobic interactions play an important role in determining odorant-receptor binding and receptor specificity.
This study was supported by the grant from the Ministry of International Trade and Industry of Japan.

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Responses of a Population of Olfactory Receptor Cells in the Spiny Lobster to Binary Mixtures are Predictable Using a Noncompetitive Model that Incorporates Multiple Transduction Pathways. PETER C. DANIEL[1] AND CHARLES DERBY[2] (Hofstra University[1], Hempstead, NY and Georgia State University[2], Atlanta, GA)

Coding of quality and intensity of binary mixtures by a population of olfactory receptor neurons (ORNs) in the spiny lobster was examined. Extracellular single-unit responses of 50 ORNs to seven compounds - AMP, betaine, cysteine, glutamate, NH_4Cl, succinate, taurine - and their binary mixtures were recorded. A noncompetitive model (NC model) and a noncompetitive model with a term for binding inhibition (NCBI model) were used to predict responses to mixtures based on responses to their components. Both models assume that different compounds activate different transduction processes in the same neuron leading to excitation or inhibition. Additionally, the NCBI model includes a term quantifying the degree to which binding of an odorant to its receptor sites is inhibited by other compounds noncompetitively as determined from previous studies. The NCBI model usually predicts accurately the responses of the population of ORNs. For seven of 21 binary mixtures, the absolute response magnitude (ARM) was significantly less than the responses to the mixture's more effective component for each ORN. The ARMs for five of 21 mixtures were significantly less than the predictions of the NC model, while there was only one case of mixture suppression and one case of mixture enhancement when the NCBI model was applied. No appreciable differences between the observed and predicted (by NC or NCBI models) across neuron patterns to binary mixtures were observed. These results do not show the emergence of unique qualities of mixtures relative to components of these mixtures. Thus, the NCBI model accurately predicts both response magnitude and spatial pattern of an array of ORNs for almost all binary mixtures tested. Because these results differ considerably from behavioral studies of lobsters showing a much greater number of cases of mixture suppression, it is likely that central rather than peripheral mechanisms must account for the behavioral results.

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In vivo Responses of Single Olfactory Receptor Neurons to Binary Mixtures of Amino Acids in the Channel Catfish.
J. KANG and J. CAPRIO (Louisiana State University, Baton Rouge, LA. 70803)

This investigation is a continuation of studies aimed at determining whether olfactory information concerning a stimulus mixture is processed differently than that for the individual odorants comprising the mixture. *In vivo* microelectrode (platinum-black) recordings were obtained from 54 single ORNs from a total of 23 channel catfish, *Ictalurus punctatus*. The six binary mixtures tested were pairings of 10^{-4} M L-methionine, 10^{-4} M L-alanine, 10^{-4} M L-arginine and 10^{-3} M L-glutamic acid, stimuli previously indicated from cross-adaptation and/or receptor binding studies to bind to relatively independent olfactory receptor sites in this species. The concentrations of the individual components in the mixtures and those tested individually were identical. ORN responses to 128 binary mixtures and to their components were classified as excitatory (E), suppressive (S) and null (N; not significantly different from background activity) based on an analysis (interrupted time-series analysis; Hudson 1977) of the number of action potentials occurring in 200-ms time bins during 5-s periods both prior to and during stimulus application. The tested binary mixtures comprised stimuli that evoked all combinations of the three "response" classifications: E+E, E+S, E+N, S+S, S+N and N+N. Chi-square analyses indicated that the evoked responses to the binary mixtures were highly associated with the response types elicited by the component stimuli ($X^2 = 73.8$, $P < 0.001$, df = 10) and not associated with specific stimulus mixtures ($X^2 = 10.2$, $P > 0.5$, df = 10). Eighty-six percent of the response types to the binary mixtures were classified similarly as that to at least one of the components. Mixture interactions that resulted in a response type different from either component occurred in 14% of the trials. Collectively, these results suggest that profound mixture interactions at the level of the single ORN in the channel catfish are rare.

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Effect of Stimulus Onset on Chemoreceptor Cell Responses in the Lobster.
RAINER VOIGT and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

In nature odors are often distributed in discrete patches which form spatial gradients. In aquatic environments, these gradients contain information which may be used by an animal, such as the lobster, to locate an odor source. Experiments with high-resolution odor measuring devices carried by lobsters during chemotactic orientation have shown reliable distributions of odor concentration patches in aquatic odor plumes. These patches are seen as chaotic series of stimulus pulses by receptor cells. Cells respond best to pulse onset and integrate over 200 ms. In this study we determined cell responses to concentration slopes and amplitudes. We expected that within the 200 ms integration window onset slope and amplitude are directly correlated and give equal responses and that beyond the 200 ms window slope is more important than amplitude. Preliminary results generally confirm our expectation. These properties allow receptor cells to respond best to the edges of arriving odor patches and to classify patches by their onset slopes. These cellular feature extraction capabilities may be the basis of gradient search in turbulent plumes.

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Comparison of Peripheral and Bulbar Responses to Sex Pheromones and Amino Acids in the Goldfish.
L. BRESIN[1], P. W. SORENSEN[1], J. KANG[2], and J. CAPRIO[2] (Dept. of Fisheries & Wildlife, Univ. of Minnesota[1], and Dept. of Zoology & Physiology, Louisiana State Univ.[2])

Recent studies have demonstrated that the olfactory epithelium of the mature male goldfish is extremely and specifically sensitive to hormonally-derived sex pheromones, as measured by electro-olfactogram (EOG) recording. The objective of this study was to determine if responses to pheromones could also be measured by Pt/ball electrode recording from the olfactory epithelium and by EEG recording from the olfactory bulb. Two related experiments were performed using male fish, one simultaneously measuring integrated multi-unit peripheral activity and EOG, the other simultaneously measuring olfactory bulb EEG activity and EOG. In both experiments, fish were exposed to an amino acid (10^{-4} M L-Serine), a steroid pheromone (10^{-9} M 17α,20βDihydroxyprogesterone, 17,20βP), and a behavioral spawning pheromone (10^{-7} M 15Keto-ProstaglandinF$_{2\alpha}$, 15KT). Peripheral responsiveness to amino acids and pheromones was very different. Although we were able to obtain amino acid responses at all 11 positions, a detectable response to 17,20βP could only be measured at 1 of 7 positions and a response to 15KT at only 1 of 4 positions. Furthermore, when pheromone responses were measured, they were significantly smaller than amino acid responses. Olfactory bulb EEG activity to pheromones and amino acids was also very different. Amino acids consistently elicited characteristic oscillatory bursts from the bulb at all positions, while pheromone responses could only be detected at 25% of these positions. Pheromone EEG response wave forms were also smaller than those of amino acids even when the EOG response to the pheromone was larger. In conclusion, we were able to measure pheromone responses in both the olfactory bulb and epithelium; however, these responses were different in nature than amino acids responses, strongly suggesting that these olfactory cues are encoded by different neural mechanisms.

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Peripheral Olfactory Sensitivity of Sea Lamprey is Greatest Just Prior to Their Spawning Migration and then Rapidly Deteriorates
P. W. SORENSEN[1], W. LI[1], R. BJERSELIUS[1], B. ZIELINSKI[2], L. BOWDIN[1], and J. SEELYE[3] (Dept. of Fisheries & Wildlife, Univ. of Minnesota, St. Paul, MN[1]; Univ. of Windsor, Canada[2]; National Biological Survey, 2, MI[3])

The sea lamprey, Petromyzon marinus, has a complex and dramatic life history. After hatching in streams, lamprey spend 5+ years as filter-feeding larvae before metamorphosing into a parasitic stage which then enters oceans or lakes. Parasitic lamprey grow rapidly for a year and then enter an adult migratory phase which does not feed. Inland migration is dramatic and brief (4-8 weeks), culminating in spawning and death. Our previous studies have demonstrated that the olfactory system of migratory adults is acutely sensitive to a narrow range of amino acid and bile acid cues, the latter of which likely functions as a migratory signal. In this study we tested the possibility that the olfactory sensitivity of sea lamprey changes in accordance with the different stages of their life. Lamprey were trapped at weekly intervals prior to, during, and after their spawning migration, and their EOG responsiveness to a diverse battery of 22 crude and synthetic odorants (ex. amino acids, bile acids, river water, washings of mature males and females) was measured. Larval and parasitic-phase animals were also tested. Olfactory epithelia from all specimens were examined at both the light and electron microscope level. As measured by EOG, absolute sensitivity to all odorants increased approximately 5-fold between the larval and early-migratory life stages and then declined rapidly during the spawning run ($P<0.05$; $N=88$). Strikingly, by the time animals were caught spawning four weeks after commencing their migration, they were essentially anosmic. Declines in sensitivity were similar between odorants with a possible exception being the odor of mature males which decreased less. Surprisingly, there was no indication of increased cell death in the olfactory epithelium of maturing lamprey, nor were there any measurable changes in receptor cell density or morphology. We hypothesize that this reduction in olfactory acuity is likely attributable to a change at the molecular level and that it may serve to reduce extraneous sensory input during this terminal life phase which is exclusively associated with mating.

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Discovery and Characterization of an Antennal-Specific Protein Associated With Development of Antennal Sensilla, Electrical Responses to Odorants, and Onset of Sexual Behavior in a Hemimetabolous Insect. J. C. DICKENS[1], F. E. CALLAHAN[2], W. P. WERGIN[3] AND E. F. ERBE[3] (USDA, ARS, Boll Weevil Research Unit, Mississippi State, MS[1], USDA, ARS, Crop Science Research Lab, Mississippi State, MS[2], USDA, ARS, Electron Microscopy Lab, Beltsville, MD[3])

Development of sexual behavior in insects coincides with maturation of sexual organs and development of sensory systems to detect mating signals in the adult stage. Insects which undergo incomplete metamorphosis (hemimetabolous) offer an ideal model for investigations of aspects of sexual behavior since immature forms resemble adults thus making them amenable to experimental manipulations. Changes in antennal sensilla, electrical responses to insect and plant odorants, and polypeptide profiles were investigated in final instar nymphs and adults in the hemimetabolous bug, *Lygus lineolaris*. Characteristic olfactory sensilla develop on the second and third antennal segments during the final molt. Concurrent with development of these sensilla in adults, neural responses to a component of green odor (1-hexanol) and an insect-produced volatile [(*E*)-2-hexenyl butyrate] increase dramatically. Antennal extirpation experiments show sensilla responsive to these odorants occur mainly on the second and third antennal segments. A protein with molecular weight of 17,000 is present in the soluble fraction of adult antennae but absent in nymphs. Antisera raised against pheromone-binding protein of the Gypsy moth, *Lymantria dispar*, does not react with the *Lygus* antennal-specific protein (*Lasp*), and the *N*-terminal sequence of *Lasp* shows no significant homology with other known insect protein sequences. Both the lack of reactivity of *Lasp* with antisera raised against an odorant binding protein in a moth, and the lack of homology of its *N*-terminal sequence with other odorant binding proteins indicate that if *Lasp* is an odorant binding protein as we suspect, it is either widely divergent or, more likely, independently derived.

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Capsaicin/Lipid Interactions: Relationship to Biological Activity.

ALEXANDER M. FEIGIN, EVGENEY V. ARONOV, JOHN H. TEETER, JOESEPH G. BRAND (Monell Chem. Senses Ctr., 3500 Market St., Philadelphia, PA 19104)

Current concepts of the mechanism of the irritating effect of capsaicin assume the existance of a specific receptor in the plasma membrane of sensory nerve fibers. Activation of this receptor presumably causes opening of nonspecific cation channels associated with (or part of) the receptor. Our experiments seek to determine the degree to which capsaicin and its analogs affect general membrane properties. We observed that capsaicin (10-50 μM in bathing solution) induced formation of nonselective ion channels with a wide variety of conductances in protein-free lipid bilayers formed from an equimolar mixture of dioleoylphosphatidylethanolamine and dioleoylphosphatidylserine. A similar channel-inducing ability was observed for several analogs of capsaicin. The wide variety of structures of the capsaicin analogs suggests that the channels we observed are a result of a rearrangement of the membrane lipid bilayer and are not formed from the molecule of capsaicin or its analogs. Activity of the analogs towards lipid bilayers was estimated on the basis of concentrations that were necessary to induce channel formation. It was found that the channel-forming activity of capsaicin and its analogs followed the sequence: capsaicin = rezineferatoxin = pellargonic acid vanillyl amide >> methylcapsaicin >> veratryl amine, which reflects the pattern of the biological activity reported for these compounds. This positive correlation between the channel-inducing ability and the biological activity of capsaicin analogs suggests that an interaction with the lipid bilayer (possibly expressed in the formation of ion channels) is important for the biological activity of caspsaicin.

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Characterization of Human Genes Related To Olfactory-Specific P450 2G1. XINXIN DING[1], JIANGJUN SHENG[1], and MINOR J. COON[2]. (Wadsworth Center for Laboratories and Research[1], NYSDH, Albany, NY 12201-0509 and Department of Biological Chemistry[2], Medical School, The University of Michigan, Ann Arbor, MI 48109)*

The goal of the present study was to identify and characterize *CYP2G1*-related genes in the human genome. P450 2G1 is an abundant enzyme expressed specifically in the olfactory mucosa of mammalian species and is believed to have physiological functions important for the olfactory chemosensory system (Ding, X. and Coon, M. J., 1994, *Arch. Biochem. Biophys.* **315**, 454-459). The primary structure of rabbit and rat 2G1 has been determined by cDNA cloning, and the gene structure for rat 2G1 has also been determined. Interestingly, recent immunohistochemical studies with an antibody to rabbit 2G1 did not detect immunoreactivity in human olfactory mucosa (Getchell, M.L., Chen, Y., Ding, X., Sparks, L., and Getchell, T. V., 1993, *Ann Otol. Rhinol. & Laryngol.* **102**, 368-374), raising the question of whether 2G1 gene is present in the human genome and, if so, to what extent it resembles the animal orthologs in structure, activity, and regulation. In this study, two DNA fragments were obtained by PCR amplification of human genomic DNA, with use of primers corresponding to rat and rabbit *CYP2G1* cDNA sequences. One (E7) was 132 bp and the other (E78) was 146 bp in length. E7, which aligned with the nucleic acid sequence of exon 7 of the rat 2G1 gene and contained an open reading frame of 44 amino acids, is highly homologous in deduced amino acid sequence to residues 322-375 in rabbit 2G1 (89% identity) or rat 2G1 (86% identity). On the other hand, E78 aligned with sequences flanking the short intron between exons 7 and 8 in rat 2G1 gene and apparently contained an intron of 111 bp. Southern blot analysis of human genomic DNA fragments generated by digestion with a number of restriction endonucleases with use of cloned E7 as a probe indicates that more than one CYP2G-related genes are present in the human genome. The complete cDNA structure, catalytic activity, and cellular distribution of the encoded protein are under study.

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Entry of Chromium into the CNS via Olfactory Receptor Neurons.

L. HASTINGS, M. MILLER, J. EVANS, E. O'FLAHERTY, and L. OLSON (University of Cincinnati, Cincinnati, OH)

Although it has been known for a number of years that exogenous agents can enter the Central Nervous System (CNS) via the olfactory receptor neurons (ORN) of the olfactory system, whether or not toxic compounds enter the CNS via this route has received little attention. We have shown previously (*Neurotoxicol.*, 12:707-714, 1991; *Fundam. Appl. Toxicol.*, 19:275-278, 1992), using radiolabeled compounds, that cadmium instilled unilaterally into the nasal cavity of the rat is found predominantly in the ipsilateral olfactory bulb. To investigate whether significant transport of compounds occurs when exposure is via inhalation, rats were exposed to airborne chromium. Two forms of chromium were used--Cr VI, which readily crosses the cell membrane, and Cr III, which does not. Furthermore, separate groups of rats were exposed to Cr III or Cr VI via drinking water as a control for blood-borne exposure. Male Sprague-Dawley rats were exposed via drinking water (12.9 mg/l) or inhalation (200 $\mu g/m^3$) to water soluble trivalent and hexavalent chromium compounds, chromic chloride and sodium dichromate, respectively. Rats were sacrificed at 40 days of exposure and total chromium content of the olfactory epithelium and olfactory bulbs as determined using ICP/MS. In a companion study, radiolabled chromium (both III and VI) was instilled intranasally and radioactivity in the olfactory epithelium and bulb determined after 7 days. Inhalation exposure produced no evidence of transport of chromium via the ORN, but chromium was detected in the olfactory bulb after intranasal exposure to Cr VI.

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Changes in Immediate-Early Gene Expression in the Lobster Olfactory Organ Following Osmotic Stress. E. ORONA, A. BARNHART, and R. A. GLEESON. (The Whitney Laboratory, University of Florida, St. Augustine, FL. 32086)

Some euryhaline decapod crustacean species (e.g., the blue crab) can respond to changes in osmotic environments (changing salinities) through adaptive mechanisms in the aesthetasc sensilla of the olfactory organ. As a benchmark for comparison to the blue crab, in this study, we have examined how changes in osmotic conditions affect the "immediate-early" gene (IEG) expression in the olfactory organ of the spiny lobster *Panulirus argus*, a stenohaline marine crustacean. Members of the IEG family, such as c-*fos* and c-*jun*, function as inducible transcription factors in stimulus-response coupling, and their expression has sometimes been observed following exposure to various stimulation conditions, including osmotic stress. Exposure of the lobster's olfactory organs to brief osmotic shock is known, on a short-term basis, to produce a loss of the animal's capacity for chemosensory discrimination. Two hours following the exposure of the olfactory organs to distilled water for 15 min, the tissues from the lumen of the antennules were removed. Expression of IEG family members was investigated using various antisera, against the protein products, applied to Western blots of the tissue extracts. Here we report on the loss of specific Fos-like immunoreactivity, produced by this treatment of the olfactory organs. No changes were observed in Jun-immunoreactivity (c-*jun*, *junB*, nor *junD*). These results show a functional dissociation between *fos* and *jun* expression, paralleling recent studies of similar phenomenon in mammals. The results also suggest that IEG expression may be a useful methodology to investigate adaptive changes to osmotic stress in the olfactory organs of crustaceans.

Supported by NSF (R. G.) and Whitehall Foundation (E. O.).

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A Subpopulation of Cultured Neonatal Rat Trigeminal Neurons Expresses Both CGRP and Sensitivity to Capsaicin. XUE-SONG ZHANG and BRUCE P. BRYANT, Monell Chemical Senses Center, Philadelphia, PA 19104

The cellular matrix in which trigeminal nerve endings are located influences the responses of these neurons to exogenous chemicals. In order to characterize the intrinsic sensitivities of trigeminal neurons, the contribution of extrinsic factors was controlled by culturing neurons from neonatal trigeminal ganglia in the presence of nerve growth factor (0.5 μg/ml). After 2-4 days in culture, the cells were assayed for the presence of neuron specific enolase (NSE), calcitonin gene-related peptide (CGRP), substance P and sensitivity to capsaicin. Cells of 20-30 μm diameter were found to contain NSE. Neurons exhibited capsaicin sensitivity in a dose dependent manner; the percentage of neurons that specifically took up cobalt (visualized by silver enhancement of the precipitated sulfide) in the presence of capsaicin increased to 60% as the concentration of capsaicin increased from 0 to 2 μM. Using immunofluorescence techniques, CGRP was detected in 5.2% of neurons in culture. The application of both techniques indicated that 11.7% of neurons that stained for CGRP immunoreactivity were also sensitive to capsaicin. Substance P immunoreactivity was weak in 2-4 day old cultured cells and not detectable in cells that had been exposed to the conditions of the cobalt uptake assay for capsaicin sensitivity. The finding that CGRP and capsaicin sensitivity are co-expressed in some cultured neurons is consonant with earlier in vivo studies and supports their use in in vitro studies of chemonociception.

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Determinants of Measured Olfactory Thresholds in Normal Persons: KYUNG H. YANG & WILLIAM S. CAIN (Dept. of Surgery, Div. of Otolaryngology, University of California, San Diego, CA 92103-8895)

How one measures thresholds depends on the intended use of the data. If, for example, one wishes to distinguish the clinically normal from the hyposmic-anosmic person, then hyperosmia holds no interest, but speed of testing does, for a clinic always operates under time-pressure. By use of a foreshortened range of concentration, i.e., one that begins only slightly below the threshold of the average normal person, the CCCRC threshold test acknowledges lack of concern with hyperosmia in the interest of speed. The test also acknowledges speed as a priority by its adaptive nature, whereby an error in its two-alternative forced-choice (2AFC) mode of presentation leads to an increase in concentration toward a "correct" threshold. When a subject detects the same level consistently, as defined by five correct answers in a row, testing stops and the concentration reached is designated the threshold. Testing a normal person in the clinic may take only minutes, but testing an anosmic may take a half hour (15 min per nostril). A further disadvantage is that the procedure may underestimate the threshold of the impaired patient who may get five answers correct by chance alone. The longer a string of trials the greater will be the chance of such an error. This problem also exists when one uses the CCCRC test with an extended range of concentrations in studies of normals. Then, the priorities of the clinic do not apply and other procedures may have more justification. Of the adaptive procedures, the up-down procedure has seen considerable use, but also varies in length of testing and often causes the experimenter to average systematically changing results, viz., to compute a threshold from systematically decreasing or increasing reversal points. Another bracketing procedure, the step procedure, uses a computer algorithm to calculate an estimated threshold on every trial and completes the testing in a fixed number of trials. A comparison of the CCCRC test and the step method, both with two-alternative forced-choice presentation, in 100 normals indicated that each had the same variability and reliability. A change from 2AFC to 4AFC testing with the step procedure gave evidence of some gain in reliability, but more importantly afforded the chance to see a role for the size of concentration step in reliability. With an increase in size, reliability increases. With appropriate size, one procedure should serve usefully in both the clinic and laboratory.

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A comparative study of the reliability of 10 olfactory tests and mathematical models of the relationship between reliability and test length. RICHARD L. DOTY, DONALD A. McKEOWN, W. WILLIAM LEE, and PAUL SHAMAN (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania Medical Center, and Department of Statistics, The Wharton School, University of Pennsylvania, Philadelphia, PA USA

Ten tests of olfactory function (including tests of odor identification, detection, discrimination, memory, and suprathreshold odor intensity and pleasantness perception) were administered on two test occasions to 57 subjects ranging in age from 18 to 83 years. The stability of the average test scores was determined across the two test sessions for 14 measures derived from these 10 tests and for subcomponents of the Japanese T&T olfactometer threshold test. In addition, the test-retest reliability (Pearson r) of each test measure was established. With the exception of a response bias measure, the average test scores did not differ significantly across the two test sessions. Statistically, the reliability coefficients of the primary test measures fell into three general classes bound by the following *r* values: 0.43 to 0.51; 0.67 to 0.71; 0.76 to 0.90. Detection threshold values were more reliable than recognition threshold values; those based upon a single ascending presentation series were much less reliable than those based upon a staircase procedure. The relationship between test length and reliability was examined for several of the tests and mathematically modeled. For example, within the staircase series incorporating the odorant phenyl ethyl alcohol, reliability was precisely ($r^2 = 0.9995$) related by a logarithmic formula to the number of reversals included in the threshold estimate [reliability = 0.63 + 0.11*ln (# of reversals)]. Reversal location, per se, had little influence on reliability. Overall, this study suggests that (i) considerable variation is present in the reliability of olfactory tests, (ii) reliability is a function of test length, and (iii) caution is warrented in comparing results from nominally different olfactory tests in applied settings since the findings may, in some instances, reflect the differential reliability of the tests.

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How Shall We Measure Odor Quality?: WILLIAM S. CAIN & MATS J. OLSSON (Dept. of Surgery, Div. of Otolaryngology, University of California, San Diego, CA 92103-8895)

Arguably, the fundamental question in olfaction concerns the relationship between the properties of a molecule and its perceived odor quality. Unfortunately, psychophysicists have yet to derive satisfactory ways to measure differences in quality that one might relate to differences in molecular properties. Direct numerical judgments, such as similarity estimation, applied to arrays of odorants have the disadvantage of poor resolution, subjectivity, and likely context dependence. Multidimensional scaling of similarity judgments has indicated that subjects assess principally pleasantness when asked to judge similarity. The judgments therefore seem degenerate. Their use has led to no archivally useful data, which means that the field has accumulated no storehouse of information to build upon. We see resolution of the problem in labor-intensive investigations of discrimination that essentially define the boundaries between the qualities of any two odorants. Hence, we ask: How well can subjects discriminate differences in quality between one component and a two-component mixture that varies from all of one component (100:0) through mid-range proportions (...60:40; 50:50; 40:60...) to all of the other (0:100)? Subjects studied intensively (almost 500 judgments each) showed considerable uniformity in discriminative performance, with a coefficient of variation of just 35% in quality discrimination thresholds. Confidence ratings given in conjunction with judgments of differences illustrated the considerable insecurity of subjects regarding judgments of odor quality. Such insecurity reflected itself markedly in judgments of the "amount" of a component in a mixture. Subjects found it very difficult to commit themselves either to identity when it existed, i.e., judgment of 100% of component A in a stimulus of just component A, or to the absence of a component when none existed. The insecurity has undoubtedly played a role in the degenerate nature of direct judgments of similarity among groups of odorants. The measurement of discriminative boundaries between stimuli eliminates such degeneracy, greatly enhances resolution, and eliminates subjectivity and context dependency. The study of discriminative boundaries should yield archival data upon which one could build a stable psychological space for odor quality.

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Early temporal events in odor identification. MATS J. OLSSON & WILLIAM S. CAIN (Dept. of Surgery, Div. of Otolaryngology, University of California, San Diego, CA 92103-8895).

Two experiments were performed to investigate response latency and correctness for identification of odors presented monorhinically on two occasions. Principal objectives were to investigate: 1) any difference in response latency for correctly and incorrectly identified odors, 2) any facilitatory effect of previous exposure on odor identification, and 3) performance per nostril, assuming that any difference would reflect laterality in hemispheric processing. In Experiment I, 20 normal subjects (ten males and ten females, 19-28 years old) were asked to identify 48 common odors, 24 presented to the left nostril and 24 to the right. Response latency was timed. One week later the subjects returned for the same procedure, only this time odors previously presented to the left nostril were presented to the right and vice versa. As anticipated, subjects responded faster to items they identified correctly. Latency discriminated correct from incorrect responses with a d' of 1.0. Subjects responded faster on the second occasion ($p=0.02$), with the increment in speed due specifically to an increase for odors presented to the left nostril in session 1 and then to the right nostril in session 2. In Experiment II, 32 subjects (16 males and 16 females, 18-41 years old) were divided into two groups, one with subjects who used only the left nostril throughout the experiment and another with subjects who used only the right nostril. All subjects were first presented with six odors and asked when they had smelled them last. After some practice, they were presented with 12 test odors and asked to press a button when they "realized" what they were smelling but were asked not to give names for the stimuli. Six of these 12 odors were those presented previously and six were new. Reaction time was considerably shorter than in Experiment 1 and significantly shorter ($p=0.04$) for odors presented previously. When this effect was analyzed per nostril, there was a strong tendency ($p=0.06$) for the subjects who used the left nostril to account for the gain. The results are relevant to repetition priming and hemispheric processing. Only the left hemisphere appeared to mediate priming. This seemed to be true both for vocal identification and for a presumably pre-lexical form of identification and for both short and long retention intervals.

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Blindsmell: An olfactory analogue of Blindsight?
GARY E. SCHWARTZ, JOHN P. KLINE, MERCEDES FERNANDEZ, ZIYA V. DIKMAN, LYNN TURKSTRA and ERNEST H. POLAK (University of Arizona)

Patients who have sustained lesions in areas of primary visual cortex can be trained to behaviorally detect the presence of a visual stimulus despite the fact that they do not report seeing the stimulus. Discrimination performance in the absence of reported perception has been documented for the visual system (termed blindsight) and the tactile system (termed blindtouch). The present findings unexpectedly provide evidence for an analogue in olfaction. In a double-blind experiment examining EEG registration of a subthreshold concentration of isoamyl acetate using a two bottle discrimination paradigm in 86 college student (chance performance would be 50%), we divided the sample into three performance subgroups (1) high (68% correct, n=32), (2) medium 52% correct, n=29) and (3) low (36% correct, n=25). Subjects did not differ on ratings of perceived intensity (1.8, 2.2, 1.7) or confidence of guesses (3.2, 3.3, 3.3) using a 0 to 10 scale. EEG theta (4-8 Hz) showed registration of the odorant in all three subgroups, EEG alpha (8-12 Hz) showed registration of the odorant primarily in the high performance group. Hence, the high performance group showed both behavioral and EEG registration of the subthreshold odorant in the absence of self-reported awareness (blindsmell). In an on going study of EEG in total anosmics, a patient who scored completely anosmic on an odor identification test performed 95% on a two bottle discrimination task in the absence of self-reported awareness. His Topographic EEG revealed a pattern that was similar to the subthreshold odor pattern found in normals. Functional and neurologic blindsmell may exist in some subjects and patients.

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The Effects of Long-Term Exposure to Odorants on Olfactory Thresholds and Perceived Odor Intensity
PAMELA DALTON and CHARLES J. WYSOCKI (Monell Chemical Senses Center, Philadelphia, PA)

Individuals who live or work in an odorous environment often report that, with continued exposure, the perceptual intensity of the odor is greatly reduced. We attempted to determine whether this reduction in perceived intensity is due to olfactory adaptation. However, most experimental descriptions of olfactory adaptation are from studies employing single, and often brief, odorant exposures that do not typify the real-world situation.

In two studies, we examined the expression of olfactory adaptation during two weeks of extended odorant-exposure and recovery of function following this period to determine whether any persistent changes in odor perception were revealed. Both studies used a design that combined psychophysical testing in the laboratory with odorant-exposure in the home, which mimicked the conditions of real-world odor adaptation. In Experiment 1, we tested eight subjects at weekly intervals for five weeks to determine their detection thresholds and intensity ratings for two odorants (citralva and iso bornyl acetate). During weeks 2 and 3 they were exposed to one of these odorants in their home. A minimum of 6 hr of daily exposure produced a significant loss of sensitivity to that odorant, but not to the control odorant ($F=3.53$, $p<.01$) and recovery to pre-exposure sensitivity levels did not occur for some individuals until two weeks following odorant removal. A positive correlation also was found between changes in threshold sensitivity to the adapting odorant and ratings of the perceived intensity of the odorant in the home. Experiment 2 replicated these results using a third odorant (benzyl acetate) and, by extending the period of recovery testing, showed that the loss of sensitivity that characterizes long-term olfactory adaptation can persist for up to four weeks.

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Odorant Dissimilarity as a Measure of Changes in the Human Olfactory System: Implications for the Evaluation of Dysosmia DANIEL KURTZ, DAVID HORNUNG, PAUL KENT, PRECHA EMKO, THERESA WHITE, PAUL SHEEHE (Smell and Taste Disorders Clinic at the SUNY Health Science Center at Syracuse, NY)

Psychophysical tests provide valuable information about the etiology of olfactory dysfunction. Unique patterns of odorant confusions, as measured by the Odorant Confusion Matrix (OCM), are seen in patients with head trauma, polyposis, and colds. Direct scaling of odorant similarity allows for estimates of odorant confusability / dissimilarity while eliminating the need for odorant identification. A Labeled Magnitude Scale (LMS) was created to allow direct psychophysical ratings of odorant dissimilarity.

As an initial evaluation of the potential of the LMS to distinguish between disease processes, we explored the consistency of LMS ratings and the ability of the LMS to distinguish between different experimental manipulations. All of the manipulations - prior adaptation to mint, reduced concentrations of rose and cinnamon, decreased concentration of all odorants, and partial anesthesia - produced patterns of response which were different from each other and from normal controls. Thus, like the OCM, the LMS is able to reflect changes in odorant confusability or similarity. As in the OCM, these confusions may reflect changes in odorant perception which may be associated with specific etiologies of olfactory loss. In addition, the LMS may allow odorants to be chosen on the basis of their physiochemical and psychophysical properties, rather than ease of identification, which could aid in the differential diagnosis of dysosmia.

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Absence of Olfactory Event-related Potentials to Amyl Acetate in Idiopathic Congenital Anosmia. W. JAMES EVANS and LIYING CUI (University of California, Irvine).

Olfactory function was evaluated by olfactory event-related potentials in nine subjects with idiopathic congenital anosmia and nine age- and gender-matched controls. The anosmic subjects had no other congenital anomalies and were in good health. Anosmia was confirmed by the Smell Identification Test and odor detection thresholds for phenylethyl alcohol and isoamyl acetate. CA-phenone detection thresholds did not differ between the two groups. Evoked potentials were recorded from Fz, Cz, and Pz, referenced to A1, in response to amyl acetate and air control stimuli presented to the right nostril at a volume flow rate of 5 L/min, stimulus duration of 40 ms, and randomized interstimulus intervals of 6-30 s. In the control subjects, evoked potentials to amyl acetate were characterized by four reproducible components (P1, N1, P2, and N2). In the subjects with congenital anosmia, no reproducible evoked potential components were identified in response to amyl acetate. No reproducible evoked potential components were seen in response to the air control stimulus in either the anosmic or control group. To the extent that these subjects represent selective lesions of primary olfactory neurons, the results of the current study indicate that olfactory event-related potentials to amyl acetate yield information specific to the functioning of the olfactory system.

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Very Early Decline in Recognition Memory for Odors in Alzheimer's Disease. STEVEN NORDIN and CLAIRE MURPHY (UCSD Medical Center, San Diego and San Diego State University)

The objective of this study was to determine when in the progression of Alzheimer's Disease (AD) a decline begins in detection sensitivity (butanol) and short-term recognition memory for odors (common household odors). This was performed by studying 78 patients with probable AD and 78 controls, comparable in gender distribution, age, and education, diagnosed by two senior staff neurologists from the UCSD Alzheimer's Disease Research Center. By means of the Dementia Rating Scale, the patients were categorized as very mild ($\geq$ 136), mild (115-135), or moderate (95-114) in degree of dementia. Detection sensitivity for taste (sucrose) and recognition memory for visual stimuli (faces of US presidents and vice presidents and engineering symbols) was studied for comparison. For the recognition task, ten stimuli of each modality were initially inspected by the subjects, followed by five old and five new stimuli to be evaluated as either old or new. The results showed that whereas the earliest decline in odor sensitivity and visual-recognition memory was found in patients with mild dementia, a decline in odor memory was found in patients with mild dementia. Similar taste thresholds in the four subgroups suggest that the poor odor detectability in AD was sensory rather than cognitive in origin. The findings imply that memory-based olfactory tests may contribute to early diagnosis of AD.

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Olfactory Tasks Differentiate between Patients with Alzheimer's Disease and Huntington's Disease. CLAIRE MURPHY, JILL RAZANI, STEVEN NORDIN, ANNA BACON and JOANNE HAMILTON (San Diego State University and UCSD Medical Center, San Diego, CA)

Deficits in olfactory function have been reported in patients with various cortical and subcortical dementias. The emergence of differential performance on tests requiring different cognitive and olfactory skills would enhance the potential for olfactory testing to contribute to differential diagnosis of dementia. To determine their ability to correctly classify patients, performance on a series of olfactory tasks that tap both sensory and cognitive ability was assessed in a group of patients with Probable Alzheimer's Disease (AD), a cortical dementia, and a group of patients with Huntington's Disease (HD), a subcortical dementia. The participants were 17 persons with Probable Alzheimer's Disease and 11 patients with Huntington's Disease, diagnosed by two independent neurologists at UCSD Medical Center. These patients had undergone extensive batteries of neuropsychological and neurological tests as well as clinical examinations to rule out other causes for dementia. Scores on the Dementia Rating Scale (DRS) for Alzheimer's patients (117, SD = 10) and the Huntington's patients (123, SD=12) indicated that the two groups were mildly impaired and equated for dementia. Subjects in this study performed 1) an olfactory detection threshold using a two-alternative, forced-choice procedure, 2) odor identification using the UPSIT, 3) odor identification using a non-lexically-based test, and 4) a recognition memory task for odors and visual stimuli. Scores for each test were subjected to logistic regression to determine the ability of the various tests to correctly classify the patients into the two diagnostic groups. In addition, the sensitivity and specificity of each test was determined using ROC curves. Results suggest that the combination of all tests produces the highest predictive power, but that the more cognitively-based tests are particularly sensitive and specific. The results will be discussed within the context of recent advances in our understanding of olfactory system involvement in Alzheimer's Disease.

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Olfactory Function in Schizophrenia: Relationship to Clinical, Neuropsychological, and MRI Volumetric Measures. PAUL J. MOBERG, RICHARD L. DOTY, DONALD A. McKEOWN, BRUCE I. TURETSKY, RAQUEL E. GUR, and RUBEN C. GUR (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, and Department of Psychiatry, University of Pennsylvania Medical Center, Philadelphia, PA USA)

Olfactory identification and sensitivity were measured in 21 patients with schizophrenia and in 16 healthy controls using the University of Pennsylvania Smell Identification Test (UPSIT) and a detection threshold test for the odorant phenyl ethyl alcohol (PEA). Clinical, neuropsychological, and magnetic resonance imaging (MRI) measures were also obtained. Relative to controls, patients exhibited statistically significant deficits on the UPSIT. In patients, lower UPSIT scores were associated with longer duration of illness, and increased severity of negative symptoms. By contrast, there was no correlation between duration of illness or negative symptoms with other neuropsychological tests. Decrease odor detection threshold was specifically associated with first-rank or productive symptoms (i.e., hallucinations, delusions). Higher left temporal lobe volume was associated with better UPSIT performance in controls but not in patients. With the exception of a modest association between UPSIT performance and scores on a non-verbal free recall task in the schizophrenia group, no significant correlations were observed between olfactory measures and neuropsychological tests. The results suggest that olfactory function may be sensitive to the progression of illness in schizophrenia and related differentially to clinical symptomatology.

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Olfactory Function in Multiple Sclerosis: Correlation with Plaque Numbers in Olfactory Cortex. CHENG LI, RICHARD L. DOTY, DAVID M. YOUSEM, and WILLIAM LEE (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA)

While there has been controversy concerning loss of olfaction in multiple sclerosis (MS), it is now well-documented that a number of individuals with MS evidence olfactory deficits. In this study we determined, using high resolution magnetic resonance imaging (MRI), whether a relationship is present between the number of MS-related plaques and olfactory function. Strong associations between plaque numbers and scores on the University of Pennsylvania Smell Identification Test (UPSIT) were observed within olfactory-related structures in the 14 MS patients we examined. Thus, a -0.92 Spearman correlation ($p < 0.001$) was found between bilateral UPSIT scores and the number of plaques within subcallosal brain regions associated with olfactory function (i.e., the subfrontal and subtemporal lobe regions). In contrast, no relationship was observed between UPSIT scores and the number of plaques in other brain regions (Spearman $r = 0.007$). Unilateral testing revealed no evidence for unique left:right influences on UPSIT scores, although high correlations between left and right UPSIT scores, as well as between the number of plaques on the left and right sides of the brain, precluded more specific assessment of this issue. This study suggests that the basis for olfactory deficits in patients with MS lies in the primary process of demyelination of central nervous system axons in central olfactory pathways.

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Localization Of Pheromones And Peptides That Induce Attachment Of Balanus Amphitrite (Darwin). MARION MCCLARY, JR. and DAN RITTSCHOF (Duke Marine Laboratory, Beaufort, N.C.)

Settlement by barnacle larvae is gregarious. Gregarious settlement is the result of physical contact between the cyprids and pheromones adsorbed on a surface. Alternative mechanisms of pheromone detection have been hypothesized. They are that effects are due to changes in the tenacity of temporary adhesive, or that effects are mediated through chemoreceptors. Peptides with neutral amino acids immediately preceeding arginine or lysine at the carboxyl terminus mimic pheromones. Bradykinin, a nanopeptide, has the sequence of a pheromone mimic. Cyprids, less then a day old, were exposed to 10^{-8} M bradykinin in a flow of 6.8 ml/min for 10 minutes, and under static conditions at 10^{-8} and 10^{-9} M for 22 hours at room temperature. Bradykinin induced larval settlement behavior and initiated metamorphosis. Studies with bradykinin antibodies suggest that: (1) binding sites for exogenous bradykinin may be located in the cuticle at the edges of the carapace valves and on the thoracic appendages; (2) cyprids may contain compounds that react with bradykinin antibodies; and (3) barnacle pheromones and other peptide mimics of this pheromone compete with bradykinin for binding sites.

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Chemical Ecology of the Seastar, *Asterias forbesi*: The Role of Chemical Signals in Foraging Behavior DEBORAH M. E. LEPPER and PAUL A. MOORE (Department of Biological Sciences, Bowling Green State University, Bowling Green, OH 43403)

The Forbes seastar, *Asterias forbesi*, is known as a voracious predator, decimating oyster beds in the wild. While it is assumed that the seastars locate these beds through chemical signals, it is unclear what role these signals actually play in the foraging behavior. Although this has been clearly established for many brittlestars, the use of olfaction during foraging has yet to be clearly demonstrated for the Forbes seastar. The experiments presented here were designed to answer three fundamental questions concerning the chemical ecology of the Forbes seastar. We will determine attraction or avoidance by the seastar to natural prey items, prey extracts and an amino acid mixture, what are the behavioral thresholds to these chemical signals, and if there is any behavioral discrimination between prey items by *Asterias forbesi*. Behavioral assays have been performed using extracts of natural prey items, which included mussels, squid, and cod, and a mixture of amino acids. These results indicate a functioning distance-mediated chemosensory system. Experiments are in progress that will determine both threshold and dose response functions for these amino acids and extracts. These experiments are designed to provide us insight into the chemical ecology and the role of chemoreception in the foraging behavior of *Asterias forbesi*.

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Behavioural responses of prolarval and larval sea lamprey *(Petromyzon marinus)* to L-arginine. B.S. ZIELINSKI[1], S. ISRAEL[1], M. MASTELLOTTO[1], E. WONG[1], T.J. HARA[2] (University of Windsor[1], Windsor, ON Canada. Freshwater Institute[2], Canada Department of Fisheries and Oceans, Winnipeg, MB, Canada

During the life cycle of the sea lamprey, a primitive jawless fish, prolarvae hatch from eggs in nests in gravel stream beds where they stay before moving downstream to pass the larval stage in feeding areas. Chemostimulation of larvae with L-arginine elicits strong electro-olfactogram responses. In this study we have investigated behavioural responses of prolarvae and larvae to chemostimulation with L-arginine. Following the application of 10 and 100 μM L-arginine, prolarvae increased swimming activity and moved away from the site with the chemostimulant. When larval behaviour was investigated in a preference/avoidance apparatus, (Tongiorgi et al., 1986 J. Fish Biol. 28, 501-510), preference to 100 μM L-arginine was observed. The preference choice was lost following olfactory nerve lesion, indicating that the larval L-arginine preference was mediated by the olfactory system. The results imply that during development, the behavioural response to L-arginine changes from avoidance by prolarvae to preference by larvae.

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A Behavioral Assay to Characterize the Ontogeny of Chemosensory Behavior in the Zebrafish *Danio rerio*.

SARA M. LINDSAY, RICHARD K. ZIMMER-FAUST & RICHARD G. VOGT (University of South Carolina, Department of Biological Sciences)

Development of the olfactory system during embryogenesis requires the coordination of a multitude of processes. The result is a sensory interface between organism and environment allowing the appropriate expression of an assortment of chemosensory based behaviors. The zebrafish *Danio rerio* is an amenable model for examining the molecular mechanisms regulating olfactory development within the context of the development of behavior. The species is easily kept in the laboratory; embryos are readily obtained and raised to reproductive age. Embryogenesis is rapid (3 days), and the initial period of juvenile life requires dynamic changes in feeding and predator avoidance strategies with presumed accompanying changes in chemosensory behavioral functions. We have recently isolated clones of several zebrafish olfactory receptor genes and described the dynamics of receptor expression during embryogenesis and through juvenile development to the adult (see presentation of Vogt, Byrd & Rogers). In complementary studies, we have developed an assay to examine behavioral changes to chemosensory stimulants throughout juvenile development beginning at hatching (3 days post fertilization). Fish are acclimated in small dishes and presented odors (single or in mixture) either in a homogeneous exposure (which assesses changes in activity) or in an asymmetric exposure (which assesses orientation). Fish are video recorded before, during and after stimulus, and recordings are analyzed through a Motion Analysis VP-110 Video Processor coupled to a SPARC station. Processed data are available for individual paths or groups of paths, and include change in velocity as a function of time, number of turns, turning angles, distance traveled, etc. Studies have demonstrated that while embryos begin hatching at 72 hrs post fertilization, they do not acquire an ability to respond to chemosensory stimulants until 96 hrs post fertilization. We are using this system to identify behaviorally relevant ligands to test against cloned-expressed receptors, as well as to probe olfactory plasticity by evaluating embryonic imprinting of novel odorants.

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Discrimination of Related Complex Mixtures and Their Components: Analysis of Mixture Perception Using the Spiny Lobster in a Generalization Assay. CHARLES DERBY, MICHELLE HUTSON, WILLERT LYNN (Georgia State University)

We examined the ability of spiny lobsters (Panulirus argus) to discriminate between a 7-compound mixture and its parts using a generalization assay. Animals were conditioned by pairing a single compound or a 7-compound mixture with an unconditioned aversive stimulus ('pseudopredator'). Prior to and during conditioning, animals were not exposed to any of the non-conditioned test stimuli. Following conditioning, we examined generalization of the conditioned response to test stimuli that included single compounds and mixtures containing from 2 to 7 compounds. The 7 compounds (5'AMP, betaine, cysteine, glutamate, succinate, taurine, ammonium) were tested at response-matched concentrations. Responses (number of searches) to these stimuli were compared for animals that were either unconditioned, conditioned to a single compound, or conditioned to a 7-compound mixture. Degree of generalization of the conditioned response to any stimulus was evaluated by comparing the response of conditioned animals with the response of unconditioned animals for that stimulus. We found that animals conditioned to a single compound usually showed little generalization to mixtures (even binary mixtures) containing that compound. Animals conditioned to a 7-compound mixture generalized poorly to mixtures containing fewer than 4 components but showed some generalization to larger mixtures. Thus lobsters can discriminate well between mixtures and their components, even closely related mixtures.

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High Resolution Odor Measurements from Freely Moving Lobsters in Turbulent Odor Plumes. JENNIFER BASIL, FRANK GRASSO, AND JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

Lobsters, *Homarus americanus*, are capable of navigating to distant odor sources, relying on their lateral antennules to make directional choices in turbulent odor plumes. The search patterns of lobsters orienting towards an odor source indicate that they are capable of extracting both directional and distance information from cues contained within the plume: their headings improve as they near the source (Moore et al., 1988). Measurements of the fine scale three dimensional structure of turbulent odor plumes show spatial gradients of features such as pulse height, onset slope, and frequency which could provide directional cues to orienting animals (Moore and Atema, 1991). In the following study, we simultaneously measured the orientation behavior of a freely moving lobster moving in a turbulent odor plume, while measuring the fine scale features of the plume itself with microelectrodes mounted on, and positioned directly over the lateral antennules of the orienting lobster. The simultaneous video record of the lobster's path was digitized and analyzed to determine heading angle, turning angle, and walking speed. The microelectrode trace from the orientation trial was synchronized with the video record of the orienting lobster so that instantaneous bilateral odor plume characteristics and the behavior of the orienting lobster could be correlated. Lobsters with electrode backpacks oriented accurately to distant odor sources. In addition, the fine scale structure of the odor plume measured from a moving lobster in its bilateral olfactory sampling areas shows a strong resemblance to those measured by stationary electrodes. Thus, the spatial features present in an odor plume are similar to those encountered by a freely moving lobster. We use the information gathered from a freely moving animal to test the predictions of our olfactory signal processing model. This model 1) extracts 'detectable' peaks from an odor plume, based on electrophysiological data from lobster olfactory receptor cells, 2) categorizes 'peak types', and 3) predicts turning decisions based upon the premise that lobsters compare information (peak types, number, dual peak events) at the left and right antennules over a given integration time.

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Spatial Features of Turbulent Odor Plumes: Potential Information in Phase-portrait Reconstruction KUN SHAO and PAUL A. MOORE (Department of Biology, Bowling Green State University, Bowling Green, OH 43403)

Many animals derive distant and directional information from the spatial-temporal patterns of odors in turbulent plumes. Chaos theory can provide both physical and mathematical insights into the turbulence that governs the dispersal of chemical signals. Reconstruction of phase portraits is a new technique designed to analyze chaotic phenomenon and provide a simple graphical representation of highly complex physical systems. Phase portraits and subsequent analysis from them can classify dynamical systems into physically meaningful groups. We have applied this approach to reconstruct the phase-portrait graphs of odor signals measured at various downstream sites in a simulated benthic boundary layer. Dramatic changes in the 3-dimensional structure of the phase portraits occur both with direction and distance from the odor source. This analysis suggests that spatial information may be present within the phase portraits and that this information may be detected by simple neural networks. This new approach is suitable to the study of turbulent odor dispersal and may provide insight into neural mechanisms used to extract information from environmental odor signals.

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Inhibition of Upwind Flight in a Pheromone Plume Tainted with a Behavioral Antagonist is Correlated with Deformed Responses to Single Filaments

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The presence of small amounts of Z11-16:Acetate in an otherwise attractive blend of pheromone components reduced upwind flight and source location in Heliothis virescens males. The suppression of upwind flight in a plume tainted with this novel behavioral antagonist was investigated by presenting casting males with single filaments similarly tainted with the Z11-16:Ac. The upwind surge of males evoked by these poor quality filaments was deformed compared to the response to normal, control filaments. As the proportion of Z11-16:Ac in the filaments increased the surge response became progressively more compressed. Males fail to make as much upwind progress because they do not generate the same sustained high levels of airspeed compared to the control situation. Furthermore, males return to casting flight more quickly when presented with a sub-optimal filament. Thus when flying in a tainted plume comprised of many such single encounters with tainted filaments the males spend less time making upwind progress toward the source and more time in crosswind, casting flight. Their ability to bridge the clean air gaps in between filaments and reiteratively surge upwind is therefore hampered reducing the levels of source location. The neurophysiological underpinnings of the surge/cast response with respect to the effects of the antagonist will be discussed.

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Relationship Between Individual HS(a) Neuron Spike Output, Electroantennograms, and Behavior of the Cabbage Looper, Trichoplusia ni.

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Behavioral responses of moths to their sex pheromones are known to be a function of the response of ensembles of antennal specialist neurons. The question examined is whether or not the central nervous system processing of peripheral receptor neuron response and the amount of upwind flight behavior is simple or complex. All behavioral responses, electroantennograms and single cell spike output measures were obtained with (Z)-7-dodecenyl acetate (Z7-12:Ac), the most active of the sex pheromone components. The use of airborne concentration as the common measure for the abscissa enabled the comparison. A $\log_{10}$-$\log_{10}$ relationship closely fit all three measures of activity. The data suggest that Z7-12:Ac processing in the central nervous system is simple. Data from other laboratories are comparable.

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The sublingual plicae (anterior processes) are not necessary for garter snake vomeronasal function. MIMI HALPERN and SAI HAN. (Department of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, Brooklyn, NY.)

It has been suggested (Gillingham and Clark,1981) that the anterior processes (AP), connective tissue masses located in the lower jaw just below the orifice of the vomeronasal (VN) duct of snakes provides the mechanism of transfer of odorants from the tongue to the vomeronasal organ. To test whether the AP were required for a behavior known to depend on a functional VN system, we tested 8 garter snakes (Thamnophis sirtalis) preoperatively with artificial earthworms covered with earthworm wash (EWW) or distilled water. The snakes attacked the EWW-covered artificial worms but not the controls. Following cauterization of the AP or a control cauterization, snakes continued to attack the EWW-covered artificial worms and not the controls. To determine whether the AP are needed to deliver odorants to the VN organ, four snakes with AP cauterization and four snakes with control cauterization tongue flicked radioactive proline. The radioautographs of the VN organs of snakes with and without anterior processes were indistinguishable. Experimental and control animals had reduced silver grains over the VN sensory epithelium as had been reported earlier from this laboratory with intact animals (Halpern and Kubie, 1980) and there was no grossly observable difference between the two groups of animals; some animals in each group had many silver grains over the VN epithelium and some had few. These findings indicate that for a behavior known to require a functional VN system and for delivery of odorants to the VN organ, AP are unnecessary.

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Seasonal Changes in Olfactory Preference of Male Ferrets towards Female Odors. RAIMUND APFELBACH and ELKE WEILER, University of Tübingen, Institute of Zoology/Animal Physiology, Auf der Morgenstelle 28, 72076 Tübingen, Germany

In the seasonally breeding ferret olfaction is the dominant sensory system for many behavioral aspects including prey recognition and mating. During the non-breeding season in males testes are small and testosterone levels are very low. No sexual behavior is shown. In spring, the mating season, the testes grow and testosterone levels are high; males search for their females. The aim of the present study was to analyze whether males distinguish between non-estrous and estrous females and whether the behavioral reaction to the changing sexual status of the female is correlated to the changing testosterone levels in males. To test for olfactory preferences we used a Y-maze. At the two ends of the "Y" the odors of estrous and non-estrous females were supplied. Males were tested in spring and in autumn. Searching time, choice of the odor (odor preference) and sniffing duration at the odor inlets were recorded.

Results show that in spring male ferrets prefer estrous odor (80%) versus non-estrous odor. In autumn, estrous was experimentally induced in females by hormone (estradiol) application. In the choice situation males still preferred estrous odor versus non-estrous odor, however, to a significant lesser extend (60%). That indicates that the preference for estrous odor is dependent on the testosterone levels in males. Whether there are morphological differences in the olfactory epithelia between spring and autumn, see abstract Weiler.

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Isolation of the MHC-associated odor-active substances that promote outbreeding in mice: A new approach. IGOR A. MEZINE, KUNIO YAMAZAKI, GARY K. BEAUCHAMP and ALAN SINGER (Monell Chemical Senses Center, Philadelphia, PA 19104)

MHC-regulated individual odors are apparently involved in promoting outbreeding in mice (Yamazaki et. al., 1976; Potts et. al., 1989). The chemical nature, however, has remained elusive. To identify the compounds in urine which permit discrimination between MHC congenic animals (C57BL/6 *vs* C57BL/6-H-2^k) we have tried to develop a new approach. The active substances can be extracted by precipitation with $HgCl_2$. We also have developed a new procedure to recover active substances from precipitates under mild conditions. For this purpose we have investigated use of several complex-forming or $Hg^{(II)}$-binding reagents (EDTA, KI, $(NH_2)_2CS$, Na_2S) and also the influence of pH. The most efficient recovery reagents were: solutions of KI (4-8 M) or Na_2S (0.3-0.6 M). The percentage of the correct recognition of odor in the obtained recovered solutions was $>75\%$ and $>70\%$ respectively. (The bioassay used to locate active compounds was the response of trained mice in a Y-maze.) Then the active substances were isolated from the recovered solutions by solid-phase extraction using reversed-phase cartridges and the obtained samples were analyzed by HPLC and GC. We have found that these samples have a low concentration of the substances, suggesting a high degree of purification. From this and also from other model experiments, we can make preliminary conclusion that this procedure selectively isolates amino-substances and possibly sulfur-substances, but not mercaptans. It appears that we are closer to a final solution of this important problem.

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Learning of Old Aged Rats in an Olfactory Skinner Box. SIMONE KRÄMER and ELKE WEILER, University of Tübingen, Institute of Zoology/Animal Physiology, Auf der Morgenstelle 28, 72076 Tübingen, Germany

Learning in Wistar rats is well known. Yet, data about learning in very old rats are scarce since such animals are often difficult to handle and they are generally regarded as slow or even unable to learn. Therefore, most investigators prefer to work with young active animals. To test whether there are age related changes in the olfactory threshold or sensory deficits due to old age, we trained 30-months old rats (N=8) in an olfactory skinner box. The system used is similar to that described by Slotnick & Schoonover (1984).

Before establishing the olfactory threshold it is important to familiarize an animal with the experimental procedure and to condition it to the task. In a first series of experiments each rat had to learn to put its nose in a testing tube with the test odor before getting a reinforcement. The required time to keep the nose in the testing tube was increased from 0.05 s to 0.3 s. To reach learning criterion, 65 trials had to be performed within a 60 min period. If an animal did not reach the criterion in one session a new session was started from the beginning the following day. Times of all sessions were added. As a result the times needed varied from a minimum of 107 min to a maximum of more than 600 min (10 sessions). Analysis the individual data revealed that six animals (75%) absolved the task in less than 10 sessions; two animals (25%) did not reach criterion.

We, therefore, conclude that even very old rats can be conditioned to respond to experimental stimuli in an experimental setup that is adequate for the animal. Establishing of olfactory thresholds are therefore possible.

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Evidence for Long-term Chemical Memory in Elephants

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Asian elephants may have a mechanism to avoid close inbreeding that is mediated through the chemical senses. During the year-long postnatal, nursing period and for a number of years afterwards, young elephants live in intimate association with their mothers and closely related females. Although receptor cells were not detected in the vomeronasal organ (VNO) of newborn elephants (Rasmussen et al., 1993. *Chemical Senses* 18:618), the VNO is presumed to mature within 4-17 weeks postnatally to coincide with the first recognizable flehmen responses by young elephants. Over the past decade we have recorded the chemosensory-oriented behaviors of five calves during their first year. These young elephants, living within their natal group, were observed to flehmen to the urine from their mother twice as frequently as to urine from other females, both relatives and non-relatives. In the wild, by gradual exclusion, and in captivity, by actual removal for management reasons, prepubertal male elephants are physically separated from their natal group. As adults, these males, prior to mating, cue into pheromones in preovulatory urine with a high frequency of flehmen responses (Rasmussen et al., 1993. *J. Chem. Ecol.* 19:2115). However, individual cues in maternal urine may override estrous cues. Adult males did not respond with high frequency to estrous urine from their mothers. However, chemical extracts of estrous maternal urine elicited high responses by male offspring, suggesting that the individual identity cues have been removed. We hypothesized that young elephant calves imprint on maternal urine and that they retain a chemical memory of this maternal urine over years. We have tested elephants who have been physically distant from their mothers for two to twenty-seven years. These offspring demonstrated a significantly higher response to maternal urine, whether recently collected or stored frozen since the test elephant's postnatal period, than to all other controls including long-time-ago-familiar, unrelated or non-maternally related urine, recently familiar urine, and non-maternal, lactating urine. Our data suggest chemical memory, via maternal urine, may allow filial-to-maternal recognition over time and space separations.

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Chemosensory Directional Tracking in Dogs: Enhancing the Track's Polarity. EVAN MILLER, REBECCA HOUGHTON, AND WILLIAM CARR Beaver College, Glenside, PA

When tested on grass or asphalt highly trained police dogs intersecting a person's track of footprints (FPs) at its midpoint detect the track's polarity and travel in the same direction as the tracklayer (TL) at 90-100% accuracy (Steen & Wilsson, 1990; Thesen et al. 1993). After brief training in a gym 4 dogs also exhibited ($p < .05$) chemosensory directional tracking (CDT), but at only 68% accuracy (Carr et al. 1993). CDT is mediated by an intensity-gradient, the scent from each FP being stronger than preceding ones since these dissipated longer (Steen & Wilsson, 1990). Therefore, to improve CDT we enhanced the track's polarity by increasing the temporal gap between FPs. We trained and tested a Cairn Terrier in a gym, rewarding CDT with food and praise. Before each trial a clean strip (0.4 X 13 m) of newsprint (NP) was laid between 2 doors facing each other across the gym and exiting the building. When laying a track the TL held his trailing shoe above the NP for 3-4 sec before taking the next step. [Carr et al's (1993) TL walked normally at 1 step/sec.] Also, as the TL neared a track's midpoint he left the NP, walked 11 m away and returned 80 sec later to complete the track, thus producing a large temporal gap between 2 FPs at the midpoint where the dog met the track at the outset of the trial. (Dogs detect a track's polarity by sniffing 2-5 FPs; Thesen et al. 1993). The direction taken by the TL was varied randomly and other controls insured that tracking was mediated only by the TL's scent. The dog left the building by the same door as the TL on 80% of 20 trials ($p < .02$) and on 90% of 10 more trials ($p < .05$) when the TL walked backward. The latter finding refutes Johnson's (1914) heel-to-toe explanation of CDT, as do Steen & Wilsson (1990). Our findings are congruent with the intensity-gradient explanation, but qualitative changes in a TL's scent after tracks are laid may also be involved (Droscher, 1969). Enhanced track-polarity may hasten the acquisition of CDT under more complex field conditions. If so, the enhancement could then be gradually phased-out.

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Odorant Threshold Following Methyl Bromide Induced Lesions of the Olfactory Epithelium.
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Several investigators have described in detail the early anatomical consequences of experimentally induced lesions of the olfactory epithelium as well as its remarkable capacity to recover. However, there is a paucity of information regarding both the degree of functional compromise immediately after lesion and the capacity for functional recovery. Therefore, the present study was undertaken to assess the functional consequences of peripheral degeneration on the minimum detectable levels of stimulation, as defined by psychophysical techniques, for the odorants 2-propanol, d-limonene and ethylacetoacetate. Long-Evans rats (3 per odorant) were trained to criterion (>90% correct) on an air vs. odor discrimination task. Odorant threshold was then determined on ten consecutive testing sessions, using a computer automated olfactometer (operating range $1x10^{-1}$-$3.13x10^{-11}$ of vapor saturation) and a tracking procedure. Following the last testing the rats were lesioned by exposing them to 330 ppm MeBr gas for 6 hrs. For each lesioned animal the anatomical state of the olfactory epithelium was evaluated relative to behavioral performance on the odorant threshold task at three days post-lesion. A comparison of pre- and post-lesion performance demonstrated that odorant threshold was not altered by lesions which destroy roughly 95-98% of the epithelium. These data will be discussed relative to performance recovery on a five odorant identification task.

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The Characteristic Female Axillary Odors and their Precursor Proteins: Qualitative Comparison to Males. G. PRETI[1,2], A.I. SPIELMAN[1,3], X-N. ZENG[1] and J.J. LEYDEN[2].
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Odors produced in the female axillae are of both biologic and commercial importance. Several studies have suggested that extracts from female underarm secretions can alter the length and timing of the female menstrual cycle. In addition, more than 1 billion dollars/year are spent on products to eliminate or mask the axillary odors. Our recent studies have determined that the characteristic axillary odors consist of C_6-C_{11}, saturated, unsaturated and branched acids, with (E)-3-methyl-2-hexenoic (3M2H) acid being the major compound in this mix. The 3M2H appears to be carried to the skin surface bound to 2 proteins in the axillary secretions. Data reported here show that the same mixture of odorous compounds is found in female axillary secretions, with several minor qualitative differences. Separation of the female apocrine secretions into aqueous and organic soluble fractions demonstrated that 3M2H, and several other members of the acids in the characteristic odor, are released by hydrolysis with base. Further, Western blotting experiments and data from immunohistochemical studies, using antibodies raised against male apocrine secretions odor binding proteins, demonstrated that the same proteins, which carry 3M2H, appear to be present in the female apocrine secretions.

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Evidence suggesting that the odortypes of pregnant women are a compound of maternal and fetal odortypes.
KIYOAKI KATAHIRA[1,3], GARY K. BEAUCHAMP[1], KUNIO YAMAZAKI[1], JULIE A. MENNELLA[1], JUDITH BARD[2] and EDWARD A. BOYSE[2] ([1]Monell Chemical Senses Center, Phila. PA 19104, [2]University of Arizona, Tucson, AZ 85724, [3]Fukushima Medical College, Fukushima, 960-12, Japan)

Odortypes, namely body odors that distinguish one individual from another on the basis of genetic polymorphism at the major histocompatibility complex (MHC) and other loci, are of fundamental importance in the social life and reproductive behavior of the mouse. Odortypes are strongly represented in urine. During mouse pregnancy, an outcrossed mother's urine acquires fetal MHC odortypes of paternal origin, an observation which we took as the focus of a search for odortypes in humans, using a fully automated computer-programmed olfactometer in which trained rats are known to distinguish precisely the odortypes of another species. Five women provided urine samples before and after birth, which in each case appropriately trained rats were found to distinguish in the olfactometer. Whether this olfactory distinction of mother's urine before and after birth reflects in part the odortype and hence genotype of the fetus, and not only the state of pregnancy per se, was tested in a second study in which each mother's postpartum urine was mixed either with urine from her own infant or with urine of a different, same-aged infant. Responses of trained rats were more positive with respect to the former (congruous) mixtures than to the latter (incongruous) mixtures, implying that, as in the mouse, human fetal odortypes of paternal genomic origin are represented in the odortype of the mother, doubtless by circulatory transfer of the pertinent odorants. The presence of the fetal odortype as a component of the pregnant womans overall scent could provide an early aid to parental olfactory binding with the infant.

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Garlic Ingestion by Pregnant Women Alters the Odor of Amniotic Fluid. JULIE A. MENNELLA,[1] ANTHONY JOHNSON,[2] CAROL STALEY[1] and GARY K. BEAUCHAMP[1] (Monell Chemical Senses Center,[1] Philadelphia, PA and Jefferson Medial College of Thomas Jefferson University,[2] Philadelphia, PA)

Amniotic fluid samples were obtained from ten, pregnant women undergoing routine amniocentesis procedure. Approximately forty-five minutes prior to the procedure, five of the women ingested placebo capsules, whereas the remaining five ingested capsules containing the essential oil of garlic. Randomly selected pairs of samples, one from a woman who ingested garlic and the other from a women who ingested placebo capsules, were then evaluated by a sensory panel of adults. The odor of the amniotic fluid obtained from four of the five women who had ingested the garlic capsules was judged to be stronger or more like garlic than the paired samples collected from the women consuming placebo capsules. Thus garlic ingestion by pregnant women significantly alters the odor of their amniotic fluid.

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Exploratory Sniffing in Humans. BRYAN RAUDENBUSH and ROBERT A. FRANK (University of Cincinnati, Department of Psychology, Cincinnati, OH 45221).

We have developed the capacity to measure sniffing behavior as the first step in elucidating the influence of individual differences in sniffing on olfactory judgments. Studies have been carried out with a piezoelectric pressure transducer connected to a nasal cannula used to measure air pressure changes associated with sniffing behavior. During tests, subjects wore a nasal cannula connected to the transducer. Subjects rated the intensity of 13 stimuli using a category rating scale. Samples of common food products were presented in opaque glass bottles which concealed their identity. They were told that the cannula must be worn to measure the concentration of the odors. In reality, the cannula was used to measure the characteristics of their sniffing patterns. The sum of pressure values recorded over the duration of a sniff (calculated by summing the pressure readings, collected every 10 msec) was the best measure of both the magnitude and duration of the sniff. Given the rapid sampling time, this measure, which will be referred to as sniff magnitude, provides a good approximation of the area under the sniff curve. Data analyses revealed that people who sniff vigorously for one stimulus also sniff vigorously for others. These differences in sniffing magnitude were observed even when subjects were matched for their maximum sniffs. This suggests that subjects exercise substantial control over the vigor of their sniffs. Taken together, the results demonstrate that reliable individual differences in sniffing behavior exist, and that we can measure these differences using the procedures and apparatus we have developed.

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Future Meetings

April 17-21, 1996

July 7-12, 1997 (ISOT, San Diego)

April 21-25, 1998