ACHEMS - 1991

ABSTRACTS

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ACHEMS - 1991

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of the

Association for Chemoreception Sciences

ABSTRACTS

This book contains abstracts of the volunteer papers and posters of ACHEMS 1991. 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

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Chemoreception in Marine Larvae. RICHARD K. ZIMMER-FAUST (Department of Biology, University of Alabama, and Marine Environmental Sciences Consortium, Dauphin Island, AL 36528).

Most marine animals reproduce as adults shedding gametes into the seawater where fertilization occurs. Embryos develop into larvae that can remain suspended for weeks or months carried by ocean currents. Many larvae seek food and habitat for colonization before metamorphosing to the adult form. Marine larvae, therefore, provide a unique opportunity for exploring early events in developing chemosensory systems mediating animal behavior of ecological consequence. Given their small size (usually 200 to 300 microns) and sensitivity to handling, marine larvae are difficult subjects for experimental analysis. We overcame many of these difficulties using computer-aided video motion analysis to non-invasively record, then rapidly measure, the locomotory responses of larvae to applied chemical stimuli. The larvae were placed in large experimental chambers (30 to 5000 ml capacity) where they either were bathed in chemical (test) or seawater (control) solutions, or stimulated through injection from a micro-delivery system. Our initial studies have focused on larval crustaceans and molluscs. These larvae respond to algal-food exudates and animal-prey metabolites by decreasing locomotory speed and increasing rate of change in direction, indicating site-restricted search. The behavior displayed probably is effective in exploiting food resources naturally patchy in distribution. We also find larval oysters settling to the seafloor and colonizing substrates in response to water-soluble factors released by adult oysters and by bacteria films growing on adult shell surfaces. Settlement factors exhibit properties suggesting peptides, 500 to 1000 molecular weight. Interestingly, the effects of settlement inducers on oyster larvae are suppressed fully by water-soluble components of mucus produced by snails predatory on oyster adults. Larval competency for response to settlement factors appears highly correlated with distinct swimming patterns expressed in seawater. Our ability to isolate sibling larvae of identical age from culture, based only on differences in swimming, should provide a valuable tool for determining the physiological- and biochemical-basis of developing chemosensory-mediated behavior.

Supported by NSF grants R11-8996152, DIR-8954231 and DIR-9013187.

2

Learning to Learn to Match to Sample

XI-CHUN MAY LU and BURTON M. SLOTNICK (The American University)

In a continuing effort to explore the learning capacity of rats trained with odor cues, rats were tested on a matching to sample problem using 3 olfactometer-generated odors and a single odor-delivery test chamber. On each trial 2 odors were sequentially delivered (e.g., one of 9 different combinations of odors; A-A, A-B, A-C, B-A, B-B, B-C, etc). The rat's task was to sample both odors and respond only if the second odor was identical to the first. The 2 stimulus presentations within a trial were separated by 2 sec and a 5-sec intertrial interval separated trials. Once criterion performance of 80% correct in a block of 15 trials for each odor combinations was achieved, a new set of novel odors was used in the next session.

Each of 3 rats showed rapid improvement in acquisition over 10 3-odor sets; by set 5 or 6 each responded correctly on the first presentation of most pairs of odors and achieved criterion performance with few or no errors. These rats had evidently learned the matching rule and, despite the complexity of the task, their acquisition of a learning-set was similar to that of rats trained on a series of simple 2-odor discriminations (Slotnick and Katz, Science, 1974, 185:796).

The rats were then trained on a 2-odor delayed matching to sample task using 1-10 sec delays between presentations of odor 1 and odor 2 and an intertrial interval of 2x the inter-stimulus delay. While accuracy decreased somewhat with increasing delays, each rat demonstrated excellent performance with a 10 sec delay even under control conditions in which a neutral odor was present during the delay period.

3

Odorant Identification in an Animal Model: An Update

S.L. YOUNGENTOB, L.M. MARKERT, T.W. HILL, E.P. MATYAS and M.M. MOZELL (SUNY Health Science Center at Syracuse)

Youngentob et al. (Physiol. Behav. 47:1053-1059; 1990) have described a new and substantially different type of animal psychophysical procedure in which rats were trained to differentially report (i.e., identify) five different odorants. The results showed for the first time that rats were capable of performing, with a high degree of accuracy, an odorant identification confusion matrix task analogous to humans. Furthermore, using multidimensional scaling techniques, a 2-dimensional perceptual odor space for the animal model was determined. The present study confirms and extends the usefulness of the cross-modal association paradigm as an effective means for developing an extensive nonverbal "vocabulary" with which an animal can communicate multiple changes in sensory stimuli. Given the appropriate nonverbal means of communication, a rat has the channel capacity to differentially report (i.e., identify), at least, ten different odorants with a high degree of accuracy (>90%). Furthermore, the expansion to a ten odorant identification task increases the analytic capabilities of the animal model for the study of olfactory quality perception. For example, increasing the number of alternative odorants for identification increases the ability to study the multidimensional properties of the odorant set in question. An MDS algorithm (ALSCAL) applied to composite data obtained from the ten odorant identification task yielded a 3-dimensional perceptual odor space for the animal model.

This work was supported by NIH grant DC00220.

4

Conditioned dopamine response in the olfactory bulb of young rats.

C.L. KIRSTEIN, F.B. WEIHMULLER, J.F. BRANDT, J.F. MARSHALL and M. LEON. (Dept. of Psychobiology, University of California, Irvine, CA 92717).

Young rat pups come to prefer an odor which has been paired with tactile stimulation. This preference is associated with an increase in the number of olfactory bulb juxtoglomerular cells in glomerular regions of focal 2-DG uptake. Using *in vivo* microdialysis in awake 3 day-old rat pups, we have shown that olfactory preference training potentiates extracellular dopamine (DA) concentrations in the olfactory bulb. We first wanted to determine whether the DA response changes over the course of development. To study developmental changes in DA response between PND 3 and PND 7, four groups of naive pups received either odor, stroking, odor and stroking or air during microdialysis. We then determined whether the learned odor evokes a conditioned increase in DA. To study the DA response to a learned odor, we trained rat pups from postnatal days 1-6 (PND 1-6) with odor and stroking presented in either a paired (forward) or unpaired (backward) fashion. On PND 7, one pup/litter was used for *in vivo* microdialysis and their littermates were given an olfactory preference test. Unlike PND 3 pups which had the greatest increases in DA with combined odor and stroking, DA levels in PND 7 pups were highest when the odor was presented alone. The magnitude of DA increases seen with stroking alone or in combination with odor at PND 3 were diminished by PND 7. Odor presentation on PND 7 in forward-trained pups increased both DA levels and a preference for the odor, neither of which occured with the unpaired stimulus experience. Since DA is present in some juxtoglomerular neurons, this conditioned increase in DA may be related to the increases in glucose utilization and number of juxtoglomerular neurons seen with olfactory preference training.

5

Olfactory Learning is Inhibited after Low-Level Formaldehyde Gas Exposure in the Ferret

R. APFELBACH and M. REIBENSPIES (University of Tübingen, Dept. of Zoology, D-7400 Tübingen).

Ferrets (*Mustela putorius f. foro* L., Carnivora) were exposed to 0.25 ppm formaldehyde gas for six months. Formaledehyde gas was generated by thermal depolymerization of paraformaldehyde; dehumified air was passed over 96% pure paraformaldehyde at room temperature. The formaldehyde gas concentration was monitored by high-pressure liquid chromatography at regular intervals. Olfactory learning was tested in a computerized Y-maze by using operant conditioning. The Y-maze tested for odor dedection, two odor discrimination and threshold evaluation. To distinguish 0.1 vol% methyl acetate from clean air with 75% security, experimental ferrets required on the average 320 trials while control animals needed only 110 trials. To reach the 90% level, controls needed 420 trials, while experimentally treated ferrets did not reach this high criterion. Additional analyses including sequence analytical evaluations of individual behaviors and neuroanatomical methods revealed a high sensitivity of the ferret olfactory system to environmentally induced olfactory manipulations.

Supported by a grant from the BMFT (07INR13).

6

Sexual Pheromone Activity in Lipids and Other Fractions from Urine of Male Mole Rats. R.A. MENZIES[1,2], G. HETH[1,3] and E. NEVO[1], Inst. of Evolution1, Univ. of Haifa, 31999, Israel; Dept. of Psychiatry[2], Univ. South Florida, Tampa, FL, 33613; and Monell Chemical Senses Ctr.[3], Phila., PA, 19104.

Spalax ehrenbergi mole rats are blind, solitary, territorial, aggressive subterranean rodents with a breeding season once a year, peaking in December and January. It was found by Nevo et al., 1976 (Experentia 32:1511), and confirmed here that estral females are attracted to substances present in the urine of adult males. By contrast, we have also found that diestral females show avoidance behavior to the same urine. Our objective was to ascertain the nature of the sexual pheromone(s) activity present in male urine. An active principle, in either two or three choice behavior tests, was found to be extractable from urine with CH_2Cl_2 and mainly found in the neutral lipid fraction. Although a large amount of activity was found in lipids, it only accounted for about 1% of that found in urine. Some activity may have been destroyed or lost during the extraction and some may remain in a lipid insoluble form. As much as 22% could be found in a lyophilized aqueous reconstituted form after volatiles were removed prior to lyophilization. Total lipids were separated by thin layer chromatography on Silica gel G60. The bulk of the activity was found in an intermediate zone bounded by Rfs, 0.2 and 0.7. Cholesterol and other sterols and ethyl esters of fatty acids chromatograph in this zone as determined by standards and staining. Ethyl esters of fatty acids were also detected in the fraction by GC-MS analysis.

7

Effects of Y-Chromosome-Dependent Urinary Chemosignals on Agonistic Behaviors in Mice

EDWARD MONAHAN and STEPHEN C. MAXSON

(The University of Connecticut)

This study examined the differential production of and response to urinary chemosignals in two Y-chromosome-congenic (NPAR) strains (DBA1 and DBA1.C57BL10-Y) for offensive aggression. We have previously shown that DBA1 (testee) males respond more aggressively when encountering intact opponent males of their own genotype (DBA1) vs. males possessing a different Y chromosome (DBA1.C57BL10-Y). However, the latter strain does not make this differential response. In the present study, testee mice of both genotypes were paired against castrated opponent mice from either strain, upon which were daubed one or the other genotype of urine from these intact males. There were significant main effects for strain of testee and strain of urine, significant two-way interactions for urine x opponent and testee x opponent, as well as significant three-way interactions for at least several of the dependent variables measured. Analyses for interactions between testee x opponent as a function of genotype of urine showed that when the genotype of the opponent and the urine daubed upon it were concordant, DBA1 responded more aggressively toward all opponents, whereas DBA1.C57BL10-Y did not show this discrimination toward opponents of its own genotype. It is proposed that for offensive aggression 1) there are chemosignal-dependent and -independent effects of the Y chromosome and, 2) there exist at least two Y-dependent chemosignals which interact to influence aggression in mice.

8

Purification and initial characterization of a pre-ovulatory urinary pheromone from female Asian elephants (Elephas maximus)

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TERRY D. LEE (Beckmann Research Institute of the City of Hope, Duarte, CA 91010)

Urine from female Asian elephants in the pre-ovulatory phase of the ovarian cycle elicits a high frequency of flehmen responses from Asian bull elephants in a non-habituating manner. These flehmen responses are an integral part of the mating sequence and suggest the presence of a sex pheromone. Extraction and partial purification of components with retention of high biological activity was accomplished several years ago (Rasmussen et al., 1982, Science 217:159-162). Subsequently, standard isolation techniques and molecular weight characterization by conventional mass spectrometric methods proved ineffective. The pheromone was not identifiable by gas chromatography/mass spectrometry (both electron impact and chemical ionization) nor by solid probe inlet electron impact mass spectrometry. The pheromone appeared to be a compound of low volatility, of low molecular weight (200-500) and to be thermally labile. It was not a peptide. The purification was hampered by close association of high concentrations of inactive components, often aromatics, which possessed similar chromatographic properties. Purified by an empirically determined series of low pressure and high performance liquid chromatography fractionation sequences, guided at each step and in each preparation, by high frequency positive flehmen responses by Asian bull elephants, the active sex pheromone is apparently a single entity. Recent developments in field desorption mass spectrometric techniques allow molecular weight determinations on several micrograms of thermally labile substances; by this technique the protonated molecular ion was determined to be 249 and a tentative molecular weight of 248 is assigned. Further information is presented on physical and chemical properties of the elephant pheromone including its ultraviolet absorption maximum and its nuclear magnetic resonance spectrum.

supported by NIH grant HD19219-06.

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Application of Enzyme Histochemistry to Studies of Olfactory Mucosal Toxicity in Laboratory Rats. Kevin T. Morgan and Marc Bonnefoi, CIIT, P.O. Box 12137, Research Triangle Park, NC 27709, 919-541-2070.

The olfactory mucosa presents a challenge to toxicologists using morphologic approacnes because of its histologic and biochemical complexity, its susceptibility to fixation artifacts, and the limited data base on 'background' lesions in this region of the nose. Increasing interest in nasal toxicology has resulted in the description of a range of lesions involving the olfactory mucosa, including degenerative, regenerative, hyperplastic, and neoplastic changes. These lesions have been associated with aging, the presence of environmental cage pollutants, and exposure to both inhaled and parenterally administered xenobiotics. Work on a number of nasal toxins has also led to the application of histochemical techniques to determination of mechanisms of toxicity. Cold glycol methacrylate tissue processing has been found to yield improved morpnology and more precise localization of histochemical reactivity for a number of enzyme systems that play key roles in responses to inhaled toxic gases and vapors, including: naphthyl butyrate esterase (esters); aldehyde dehydrogenase (acetaldehyde); formaldehyde dehydrogenase (formaldehyde); and glutathione (formaldehyde, methyl bromide). Correlation of results from histochemical studies with pathology and biochemistry may indicate mechanisms of toxicity in specific target cell populations. Enzyme histochemistry has also been applied to studies of olfactory epithelial regeneration following inhalation exposure to methyl bromide, a chemical that effectively destroys olfactory epithelium following a single inhalation exposure, leaving only the basal cell layer intact, and resulting in a transient loss of olfactory function. This work is shedding light on the origin of olfactory epithelial cell populations, and the possible role of ducts of Bowman's glands in the genesis of olfactory sustentacular cells. It is evident that much remains to be learned about the olfactory system. Morphologic investigations of responses to toxic chemicals using histochemical techniques can provide a valuable approach for studies of the biology of the olfactory mucosa.

10

Nasal Metabolism of Xenobiotics: Potential Role in Olfaction, Tissue Damage, and Protection. JOHNNYE L. LEWIS and ALAN R. DAHL (Inhalation Toxicology Research Institute (ITRI), P.O. Box 5890, Albuquerque, NM 87185)

Olfactory and respiratory nasal epithelial tissues in species ranging from silk worms to humans have high capacities to metabolize inhaled xenobiotics. Enzymes demonstrated in the nasal epithelia include several isozymes of cytochromes P-450, esterases, dehydrogenases, glucuronyl transferases, and the cyanide-metabolizing enzyme, rhodanese. Most enzymes in the olfactory epithelium are primarily localized to acinar cells and ducts of Bowman's glands, and to sustentacular cells. Carboxylesterase has been reported to be secreted into the mucus layer, but the secretion of other xenobiotic metabolizing enzymes has not been demonstrated. The metabolic activity in the olfactory epithelium has been proposed to play a role in olfaction as well as in detoxication or activation of inhaled toxicants. For example, microsomes from nasal tissue metabolize some commonly inhaled compounds (including nasal decongestants and cocaine) to formaldehyde, a nasal carcinogen, while nasal mitochondria metabolize the highly toxic odorant cyanide to the nontoxic, nonodorant thiocyanate. Nasal xenobiotic metabolizing enzymes, especially the P-450s, are generally not as inducible as are the hepatic forms. However, olfactory P-450 IIE1 has recently been reported to be induced by exposure to ethanol. In addition to the olfactory epithelium, enzymes including carboxylesterases and P-450 IIE1 have also been demonstrated in the olfactory bulbs. Recent data from our laboratory suggest that exposure to another IIE1 substrate, pyridine, can induce that enzyme in the olfactory bulbs. This presentation will include a review of enzymes localized to the olfactory epithelium and the olfactory bulbs and a discussion of the potential roles of this metabolic capacity in protecting not only the nasal mucosa, but the CNS as well. The impact on these protective functions and on olfaction as a result of potential alterations in metabolic capacity due to exposure to toxicants such as cigarette smoke or solvents will also be discussed.

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Catalytic Properties of Olfactory Cytochrome P-450 and Possible Relavence to Odor Detection. XINXIN DING and MINOR J. COON (Department of Biological Chemistry, Medical School, The University of Michigan, Ann Arbor, MI 48109-0606).*

Recent biochemical studies in this and other laboratories have revealed that the olfactory mucosa is second only to the liver in the microsomal content of total P-450 and that at least seven different P-450 isozymes are expressed in this tissue. The high level of P-450 is accompanied by a level of NADPH-cytochrome P-450 reductase that is even higher than that of liver or any other tissues studied. These findings are consistent with numerous reports on the extensive metabolic transformation of foreign compounds, including odorants and nasal carcinogens, in the olfactory mucosa. The high metabolic activity of the olfactory microsomes is at least partly due to the tissue-specific expression of two unique P-450 isozymes, P-450NMa and NMb (IIG1), which constitute a major portion of the total microsomal P-450 in this tissue [Ding and Coon (1988), *Biochemistry* 27, 8330-8337]. Studies in a reconstituted system with purified NMa indicate that this enzyme may be the principal catalyst for the biotransformation of several nasal toxicants or carcinogens in olfactory microsomes, including nicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), hexamethylphosphoramide, phenacetin, and N-nitrosodiethylamine. On the other hand, P-450NMb is a major catalyst for the hydroxylation of such endogenous compounds as testosterone and progesterone at substrate concentrations in the μM range. Interestingly, in accord with the proposed role of olfactory biotransformation enzymes as a signal turn-off or modification mechanism for odor detection, both NMa and NMb, as well as other P-450 isozymes that are known to be expressed in the olfactory mucosa, are highly active in the metabolism of a wide variety of odorant compounds in reconstituted systems. The possible relavence of olfactory P-450-catalyzed odorant disposition to odor detection will be discussed.

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Olfactory-specific UDP glucuronosyl transferase: role in odorant signal termination?
DORON LANCET, NAAVA RUBINSTEIN AND DANIEL LAZARD (Department of Membrane Research, the Weizmann Institute of Science, Rehovot 76100, Israel).

Recently we reported the identification and molecular cloning of rat and bovine olfactory UDP glucuronosyl transferase (UGT_{olf}) (1,2). UGT_{olf} is a major glycoprotein (gp56) specific to olfactory epithelial membranes. The inferred protein sequence bears <60% identity to other known UGTs. The olfactory sensory mucosa also contains high levels of several unique cytochrome P-450s. We propose that cytochrome P-450-catalysed hydroxylation, followed by UGT_{olf}-mediated glucuronic acid conjugation, serves as a turn-off mechanism for odorant signals. We have shown that many hydroxyl-containing odorants are glucuronated by UGT_{olf}, and that the conjugates are unable to stimulate the olfactory transduction mechanism, odorant-sensitive adenylyl cyclase. A model for olfactory termination in-vivo is suggested, based on the localization of the biotransformation enzymes in olfactory secretory cells. Following glucuronidation in the endoplasmic reticulum lumen, conjugated odorants are proposed to be secreted and removed by epithelial mucus flow. Preliminary data show that odorant glucuronates may indeed be generated and secreted by odorant-exposed olfactory epithelial explants in organ culture. The proposed role of olfactory biotransformation in inactivating lipophilic agonists may have general implications for signal termination of other cell-surface receptors. In parallel to their role in signal processing, olfactory biotransformation enzymes may protect the sensory neurons (and the immediately adjacent brain) from neurotoxic effects of airborne chemicals.

1) Lazard, D. *et al.*, Biochemistry 29: 7433-7440 (1990).
2) Lazard, D. *et al.*, Nature (Feb. 1991).

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13

Effect of salivary composition on ingestive responses to tannic acid in mice. JOHN I. GLENDINNING (Florida State University)

This study provides direct evidence for a robust effect of saliva composition on ingestive responses to tannic acid. Salivary proline-rich proteins (PRPs) were elevated in mice, using the ß-agonist, isoproterenol (IPR), and the effects of this manipulation on intake of tannic acid solutions was examined. Tannic acid produces both bitter and astringent sensations in humans. In experiment 1, two-bottle preference tests were used to compare the ingestive sensitivity of IPR- and saline-injected mice of three strains of *Mus domesticus* (SW, Balb & B6) and two species of deermice (*Peromyscus melanotis* & *P. aztecus*) to 0.5 mM tannic acid. IPR treatment significantly reduced the tannic acid sensitivity of the Balbs and *P. melanotis*, but not the SWs, B6s or *P. aztecus*. In experiment 2, the effect of IPR treatment on ingestive responses of Balbs to two concentrations of tannic acid (0.5 & 1.0 mM) was examined using an apparatus that analyzed patterns of drinking. Again, IPR treatment produced a significant reduction in sensitivity to 0.5 mM tannic acid. Intake measures (lick rate, burst duration, number of bursts, and overall lick rate) indicated that the IPR-injected Balbs drank the 0.5 mM tannic acid solution as if it was water. Saline-injected Balbs rejected the 0.5 mM tannic acid solution almost immediately. Whereas both the IPR- and saline-injected Balbs rejected the 1.0 mM tannic acid solution, the latter group rejected it more strongly. Because salivary PRPs bind readily with tannic acid, our results are consistent with the hypothesis that salivary PRPs in the Balbs bound to the ingested tannic acid, thereby diminishing its bitterness and/or astringency.

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THE EFFECT OF CAPSAICIN ON TASTE

Lindstrand, K., & Enslen, M.,
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Rats were injected with capsaicin on the second and third days after birth. Directly after weaning and up until 3 months after birth, different groups were subjected to immunohistochemical observation of innervation of the tongue with CGRP fibers. We also observed the influence of capsaicin on taste perception by estimating the consumption of different solutions at 6-7 weeks after birth and 11-14 weeks.

At 6-7 weeks, we observed a decrease in taste sensitivity. But after 11-14 weeks, we noted an even greater rejection of a bitter solution, indicating that the neurotoxicity of capsaicin was reversible. This was corroborated by a denser innervation of taste buds.

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Polygenic determination of quinine aversion among mice. G. WHITNEY, D.B. HARDER, J.D. BOUGHTER, JR., and C.G. CAPELESS (Psychology Dept, Florida State Univ., Tallahassee, FL 32306-1051)

There are substantial differences among inbred strains of mice in the avoidance of quinine solutions (both QS04 and QHCL) in two-bottle preference tests of quinine versus water. A Mendelian cross-breeding experiment was conducted to test the hypothesis that a single segregating locus, dubbed Qui, has a major influence on quinine aversion. Inbred strains C57BL/6J (quinine avoider) and C3HeB/FeJ (quinine indifferent) were the progenitors of two segregating generations. Results from 48 hr, two-bottle preference tests of 100 µM QS04 vs. H_2O (and 30 µM QS04 vs. H_2O) were not consistent with expectations for a single gene. A second experiment was conducted with an outbred genetically heterogeneous stock of mice (CFW) which displays segregation for the Soa locus (sucrose octaacetate (SOA) tasting). Individual CFW mice were tested for both SOA aversion and QS04 aversion. There was no evidence of genetic segregation for quinine aversion. However, regression analysis indicated about 23% common variance between segregation at the Soa locus and quinine aversion. Taken together, our results suggest that quinine aversion is a polygenic phenotype influenced by variation across a number of genetic loci. Although a single gene with a major influence on quinine aversion could exist in other mice, such a single locus was not detected among the mice in these experiments.

Supported in part by grant DC-00150.

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A Behavioral Method for Measuring Sweet Taste in the Laboratory Rat. JAMES C. SMITH AND GWEN B. O'KEEFE (Florida State University) and JOHN D. DAVIS (University of Ilinois, Chicago).

A reliable brief taste test for measuring the behavioral response to sweet tasting substances is described here. The method allows for testing the behavioral response to a variety of concentrations of both nutritive and non-nutritive sweeteners in a single brief daily session lasting approximately five minutes. The method is a variation of the procedure described by Davis (1975). On the outside of the testing chamber there is a line of eight drinking tubes mounted on a platform which rests on linear bearings. By operating a reversible motor, any one of the tubes can be aligned with a drinking port in the side of the rat's chamber. A motor driven shutter can be opened, making any one of the tubes accessible to the rat. Licking is recorded when the rat's tongue breaks an infrared beam. Control of the tube position and measurement of the licking response are controlled by a micro-computer. The rat is made familiar with the configuration of the apparatus during several days of training with .25M sucrose. Once the rat readily drinks from the training tube, the testing session is started. The non-deprived rat is presented with one of a series of 6-8 sucrose concentrations for 30 sec. The shutter is closed for 30 sec. and then a second tube is presented for another 30 sec. This is repeated until the entire series of concentrations have been presented in ascending, descending or random order. The number of licks for 30 seconds is a direct function of the concentration of the solution. We have tested sucrose, glucose, polycose, saccharin and other sweeteners and found excellent reliability.

Supported by USPHS AG04932 and AG06841 and a gift from R. J. Reynolds Tobacco Company.

Sucrose Intake Behavior is Related to Taste Bud Distribution in Fischer-344 Rats. INGLIS J. MILLER, JR. (Wake Forest University) and James C. Smith (Florida State University).

We investigated how variations among rats in sucrose intake behaviors are related to the relative numbers of taste buds that the animals have. Sucrose, water and food intake behaviors were monitored for 24 hours each day (Spector and Smith, 1984). Intake behaviors were quantified for 8 rats at a time in 4 squads for 12 months. Two rats, comprising the most avid and least avid drinkers of sucrose, were sacrificed at six week intervals (N=16, 4/squads of rats) in order to quantify their taste buds. Taste buds were quantified in the fungiform papillae (fungi), nasoincisor ducts (NID), Geschmacksstreiffen (GS), and posterior palatine fields (PPF). Preliminary mean totals (±SD) of taste buds by region follow: fungi: 154±14, N=15; tip: 60±10, N=15; NID: 54±8, N=16; GS: 76±9, N=12; PPF:125±11; Tot. pal. 253±19, N=10; Tot. facial: 409±25, N=10. These taste buds are all innervated by the facial nerve which responds to oropharyngeal stimulation by sweeteners in the rat. Preliminary results show that the concentration of sucrose at which the number of licks/bout at night reaches a maximum is inversely related to the number of taste buds in the NID (for 55 tb or fewer; r^2= .74, F=19.9, df=8, P < .005). The maximum lick rate/bout at night is inversely related to the numbers of taste buds in the GS (r^2=.40, F=6.1, df=11, P < .05). Some features of sucrose intake by rats are related to the number of taste buds they have.

Acknowledgments: Rodwige Desnoyers, Robin Krimm and Amy Sekits assisted in counting taste buds. Rachel Kelley and Kurt Thaw assisted in quantifying intake behavior. Supported by NIH Grant NIA AG 04932.

Taste Bud Distribution and Taste Preference among Mice. INGLIS MILLER, JR., (Wake Forest University) DAVID B. HARDER, and GLAYDE WHITNEY (Florida State University)

Differences in taste preference and taste bud distribution occur among inbred mice. The size of the mouse permits quantification of 3 different taste bud populations (on fungiform, foliate and vallate papillae) comprising the entire complement of lingual taste buds on individual tongues. Since fungiform taste buds respond best to salts and sweeteners, vallates respond best to bitter stimuli, and foliate papillae respond best to acids, we hypothesize that different numbers (or proportions) of taste buds among mice are associated with differences in their taste perceptions and intake preferences for fluids. Adult males (N=8) of the SWR/J, C57BL/6J and Balb/cByJ strain are tested for preference behavior and euthanized by an overdose of ether. Tissue samples containing vallate (val) and foliate (fol) papillae are serially sectioned, and the taste buds are counted with light microscopy. Fungiform (fun) papillae are counted by videomicroscopy of intact tongues, and samples are removed for light microscopy. The average total (±SD) of lingual taste buds is 430±66 /tongue. C57BL mice have the fewest total of 366±20.3 taste buds/ tongue with 40% val, 33% fol and 27% fun. SWR mice are intermediate with an average of 441±68.4 total taste buds of which 45% are val, 36% are foliate and 19% are fungiform. Balb/c mice have the highest avg. total of taste buds with 482±37.4 with 51% val 29% fol and 19% fun. Possible relationships between taste bud distribution and preference are that Balb mice (high vallate) may tend to be particularly sensitive to a variety of bitters, while C57BL mice (high fungiform) are often relatively sensitive to sweets. Subsequent systematic comparisons among mouse strains for individual tastants are necessary to test for a relationship between preference and taste bud distribution.

Acknowledgments. Robin Krimm, Paula Thomas and Mark Oliver counted the mouse taste buds. Supported by NIH Grants DC 00230 and DC 00150.

Intermediate SOA Avoidance Phenotype Suggests Identity of the Mouse Sucrose Octaacetate (*Soa*) and Raffinose Undecaacetate (*Rua*) Taste Loci. D.B. HARDER, C.G. CAPELESS, G. WHITNEY, J.D. BOUGHTER JR. (Dept. of Psychology, Florida State Univ.), and E.A. AZEN (Dept. of Medical Genetics, Univ. of Wisconsin).

Mice have been dichotomized as either Tasters or Nontasters of the bitter compound SOA (sucrose octaacetate). However, 8 of 16 "Nontaster" inbred strains avoided near-saturation 1mM SOA in 48hr, two-bottle preference tests. None avoided 0.1mM SOA. Taster strains avoided both concentrations. Mice displaying the intermediate phenotype were dubbed "Demitasters". Tasters and Demitasters were also found in an outbred line (CFW/Crl) showing monogenic segregation for SOA sensitivity. A consistent dominance order among the phenotypes (Taster > Nontaster > Demitaster) was found. A separate gene for RUA (raffinose undecaacetate) sensitivity has been proposed -- principally because some strains that do not avoid 0.1mM SOA avoid near-saturation 0.4mM RUA. These strains are all SOA Demitasters however (strains that avoid only near-saturated SOA). Relative strain sensitivities thus are maintained across the two compounds. Distinguishing Demitasters from Nontasters obviates a separate *Rua* locus. The *Soa* (= *Rua*) taste locus maps to essentially the same chromosomal location as the two *Prp* (salivary proline-rich protein) loci. *Prp* genomic-DNA rflp haplotypes for 13 inbred strains and the CFW mice were strongly correlated with the three SOA phenotypes. Only one nonconcordance was found (for strain PL/J).

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Concentration-Dependent Changes in Appetitive Responsivity to Sucrose and NaCl in Rats with Parabrachial Nucleus Lesions. ALAN C. SPECTOR (University of Florida), RALPH NORGREN (Pennsylvania State University), and HARVEY J. GRILL (University of Pennsylvania).

The medial parabrachial nucleus (PBN) is the second central relay in the gustatory system of the rat. A complete lesion in this area not only eliminates any processing that occurs in this structure, but also deprives the forebrain of its direct source of gustatory input. We tested whether a rat with lesions in the PBN would be capable of showing normal concentration-dependent changes in its unconditioned appetitive responses to sucrose and NaCl. Nine rats with electrophysiologically-guided bilateral lesions in the PBN (PBNX) and 5 surgical controls were water-deprived and trained in a specially-designed gustometer to lick a drinking spout to obtain 10 sec trials during which they had access to either NaCl (.03 M, .1 M, .3M, 1.0M), sucrose (.01M, .03M, .06M, .1M, .3M, 1.0M) or water. These stimuli were randomly presented during the 30 min sessions. The number of licks elicited during the latter 8 sec of the trial were measured. Rats were tested in this fashion for 4 days. The purpose of this phase was to familiarize rats to the accessibility of these stimuli in the gustometer. They were then given water ad-lib for the remainder of the experiment. Two days later the rats were retested in a non-water-deprived state under the same conditions as described above. This was done across 3 sessions. The data from two rats in the PBNX group were discarded; one due to an inappropriately placed lesion and the other due to failure to initiate at least one trial at each concentration during the hydrated testing phase. In individual rats, the mean number of licks to water across trials during the hydrated testing phase was subtracted from the mean number of licks across trials for each stimulus and served as a standardized stimulus lick score. In the hydrated state, both control and PBNX rats had lick scores which increased monotonically with sucrose concentration and decreased monotonically with NaCl concentration. Sucrose responsivity was significantly depressed in the PBNX rats compared with controls. There was no difference in the NaCl lick scores of PBNX rats compared with controls. PBN lesions "simplify" the gustatory system by leaving only the nucleus of the solitary tract and its local brainstem connections intact. Yet, PBNX rats are still capable of detecting and responding to sucrose and NaCl in a concentration-dependent manner. Although, PBNX rats appear to show a blunted affective response to sucrose.

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Chorda Tympani Nerve Sectioning And Salt Preference Of Preweaning Rats. S.I.SOLLARS & I.L.BERNSTEIN. (Dept of Psychology, University of Washington, Seattle, WA 98195)

The NaCl intake and preference of preweaning rats differs significantly from that displayed by adult rats, particularly with regard to their preference for hypertonic NaCl solutions over water. Recent evidence indicates that the behavioral and electrophysiological responses of the mammalian gustatory system to sodium chloride (NaCl) are dependent upon a sodium transport system which is specifically blocked by lingual application of the sodium transport blocker amiloride. We recently reported that at 10-days of age the strong preference rats display for 2% and 3% NaCl solutions over water was significantly suppressed by amiloride pretreatment. The present studies examined the dependence of the neonatal rat's salt preference and amiloride-sensitivity on the integrity of the chorda tympani nerve (CTn). To do this 10-day-old rats received bilateral CTn transection (CTX) or sham operations (SHAM). Two days later pups were implanted with intraoral cannulae and tested for solution intake. Study 1. CTX and SHAM animals received pretreatment with either distilled water or amiloride hydrochloride (100 uM). They then received test solutions of either 2% NaCl or distilled water and intake was expressed as a percentage of body weight. CTX animals displayed a significant preference for NaCl over water although their intake of NaCl was lower than NaCl intake by SHAM animals. Unlike SHAM operated animals, amiloride pretreatment had no effect on the NaCl intake of CTX animals. These results suggest that the behavioral expression of amiloride-sensitivity at 10-12 days of age is dependent upon the integrity of the CTn. Study 2. CTX and SHAM animals were tested for their preference for NH_4Cl. CTX and SHAM animals both ingested considerably more 2% NH_4Cl than water, and there was no difference between the CTX and SHAM groups. Thus, although NaCl preference of 12-day-old rats is attenuated by CT transection, NH_4Cl preference is unaffected.

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Increases in Salt Taste Preference as Mice Age. GARY K. BEAUCHAMP and AMY S. FISHER (Monell Chemical Senses Center, Philadelphia, PA).

We previously reported that when tested as adults (~ 3 months of age) male C57BL/6 (C57) mice tended to reject moderate concentrations of saline solution relative to water in 48-hr tests. In contrast, male 129J (129) mice preferred the saline solutions. The F1 cross was intermediate in preference, tending to be indifferent across a broad range of concentrations, whereas the F2 generation included some animals that preferred saline, some that were indifferent and some that rejected it at approximately 120 days of age. To examine the stability of the F2 responses, five males exhibiting a substantial preference and five males expressing a rejection were selected for further study. When these males were retested at a standard concentration of 0.075 M NaCl at approximately 230 days of age, it was found that (a) the difference between the two groups was still evident, but (b) that the % preference for all animals had increased substantially: the preference for the "preferers" went from 68% to 81% and the "rejectors" went from 27% to 46%. These data raised the possibility that as mice age their saline preference increases. To further evaluate this, F1 males were tested at approximately 100 days of age and again at approximately 440 days of age. No testing was conducted during the period between tests. A second group of F1 males was tested only once at approximately 350 days of age. Testing consisted of 3 48-hr 2-bottle saline vs. water preference tests with 0.019, 0.075 and 0.300 M NaCl. The order of the tests was counterbalanced and 1-3 days separated each test. As found with the F2 animals, there was a substantial increase in saline preference as a function of age at all 3 concentrations; the overall mean increased from 47% to 61%. The relative preference for the group tested at 350 days of age was intermediate (55%). These data indicate that there was a substantial increase in preference for saline solution as male mice aged. The basis for this change is not known but explanations include changes in peripheral taste sensitivity and changes in physiology of sodium/water balance.

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Increased excretion and intake of NaCl and water by TTR-ANF transgenic mice. R. A. BERNARD, W. R. HARE III and J. D FREEMAN. (Dept. of Physiology, Michigan State University, East Lansing, MI 48824)

Increased preference and/or intake of NaCl is an adaptive behavioral response to sodium depletion. However, salt appetite has also been induced by procedures that do not deplete the body of sodium, such as administration of ACTH, DOCA or aldosterone, and high salt diet. These treatments are characterized by expansion of the extracellular fluid volume and the behavioral response is clearly not adaptive, since it maintains volume expansion. Thus they may be considered models of need-free salt intake. Such behavior cannot result from increased renin or angiotensin levels, which are depressed by these procedures, and the elevated mineralocorticoids cannot by themselves act in the brain to stimulate salt appetite. Since plasma levels of atrial natriuretic factor (ANF) are elevated under conditions of volume expansion and because of ANF's role in renal escape from mineralocorticoid excess, the hypothesis was formulated that ANF (along with an as yet incompletely identified natriuretic factor) may be involved in need-free salt intake. Chronic stimulation of salt and water intake by ANF was investigated by using transgenic mice with elevated hormone levels in the systemic circulation. Transgenic (n=9) and non-transgenic (n=7) mice were studied in individual metabolic cages in which they had continuous access to a powdered standard lab chow containing 1% NaCl. Water was available from two drinking tubes which were presented to the mice for two 2-hr periods, in the morning and at night, respectively. Food and water intake, urine and electrolyte output were measured every 24 hr. Under these conditions the transgenic mice drank significantly more water and excreted significantly ($p<0.01$) more urine and urinary Na and K than the nontransgenic controls. During this period of time there was no difference in food intake and hence in the amount of Na ingested. When placed in a 2-bottle choice situation in standard cages the transgenic mice drank significantly more NaCl solution (.01-.1 M) and total fluid ($p<.05$) than the nontransgenic controls. These results provide support for the idea that a different hormonal system may be involved in need-free salt appetite.

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Transection of Chorda Tympani and Insertion of Stereotaxic Ear Pins produce Equivalent Deficits in NaCl Sensitivity.

HEIKE RENTMEISTER-BRYANT, STACIE SHEELAR, CAROLINE BOLLS and BURTON M. SLOTNICK (The American University)

In pilot studies we found that, relative to unoperated controls, rats with sham and control CNS lesions had moderate deficits in NaCl sensitivity. The possibility that this was due to transecting or crushing the chorda tympani (Spector et al, Amer. J. Physiol., 1990, 258:820) by ear pins used in placing the rat in a stereotaxic machine was investigated. Rats, initially trained to detect 100 mM NaCl and 300 mM sucrose, received bilateral transection of the CT (n=3), bilateral insertion of stereotaxic ear pins (n=4), or sham surgery (n=4). Animals were tested for NaCl and sucrose threshold using a modified descending method of limits procedure (Brosvic & Slotnick, Physiol. & Behav., 1986, 38:711). Six additional rats, trained only on NaCl received unilateral CT transection (n=2) or unilateral ear pin insertion (n=4).

There were no differences between the CT and ear pin groups for either tastant for measures of best performance at each concentration, trials to criterion and absolute threshold: both groups performed as well as controls in the sucrose tests but significantly worse in NaCl detection (mean NaCl thresholds in mM: controls, 1; CT, 21.9; EP, 20). There were no differences between the two unilateral subgroups and these rats performed slightly but significantly worse than controls in detecting low concentrations of NaCl (mean threshold, 2.5 mM). Our results are in good agreement with those of Spector et al. that transection of the CT produces marked deficits in NaCl but not in sucrose detection. In addition, we find that equivalent effects are obtained by the insertion of stereotaxic ear pins and that unilateral treatment produces a small but detectable effect on NaCl sensitivity.

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CONDITIONED SUPPRESSION AS A METHOD OF DETECTING TASTE THRESHOLDS

A. Kurt Thaw and James C. Smith (Florida State University).

Taste thresholds of seven male Sprague-Dawley rats (mean age 10 weeks, mean weight 250 grams) were determined for four basic taste qualities: sweet, sour, salty and bitter. The method of conditioned suppression was employed. An apparatus capable of presenting any one of eight separate drinking tubes during a testing session was designed. Animals were reduced to 80-85% ad lib. body weight. They were then trained to lick a sipper tube through a slot in the back of an experimental chamber for pellet reinforcements. Animals progressed through a series of reinforcement schedules starting with a fixed ratio (FR) schedule of 5 licks for each reinforcement. They advanced to a variable ratio (VR) schedule of reinforcement and finally a variable interval (VI) schedule with a mean of 17.5 seconds was used. While on the VI schedule animals were trained to suppress licking when any tastant other than water was presented. The first lick on any tastant was followed 10 seconds later by a mild electric shock if a rat made more than 20 licks on the tube in the ten second period. Less than 20 licks on a tastant tube resulted in no shock and a 5 second time out before proceeding to the next tube. Threshold was determined using a suppression ratio formula. Threshold was defined as the .33 suppression ratio. Results from this experiment revealed mean thresholds for the seven animals as: sucrose = 2.3 mM, NaCl = .63 mM, Quinine HCl = .005 mM and citric acid = .085 mM. Further testing will continue to determine any changes in threshold due to aging.

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Behavioral Responses of Channel Catfish to Amino Acids. T. VALENTINCIC (University of Ljubljana, Yugoslavia), S. WEGERT and J. CAPRIO (Louisiana State University).

Naive channel catfish (*Ictalurus punctatus*), maintained individually in 20 gallon aquaria, responded to low concentrations of amino acids ($<10^{-5}$M) with orienting responses and occasional food searching behavior. In addition, innate jaw snapping behavior was released at elevated stimulus concentrations. L-arginine and L-proline were chosen as conditioning stimuli since both were indicated from previous electrophysiological experiments to activate relatively independent taste and olfactory receptor sites. The same catfish were conditioned during 40 trials when the amino acid stimulus was paired within 90 seconds with a food reward. Following the application of the conditioned stimulus, the conditioned "food search" response consisted of swims of 40-90 turns (range of medians) greater than 90 degrees in 90 seconds. In contrast, the de-inhibited naive animals which were not conditioned responded to equivalent chemical stimulation with 0-40 turns. Conditioned, intact channel catfish showed behavioral discrimination of L-arginine from L-proline and both from all other non conditioned amino acids. To determine the roles of olfaction and taste in the releasing of different behavior patterns, olfactory organs of amino acid conditioned catfish were surgically removed and the animals were retested following recovery. Catfish previously conditioned to $<10^{-5}$M L-proline, did not respond behaviorally to L-proline at concentrations $<10^{-4}$M, whereas strong snapping behavior was triggered by >1mM L-proline. Thus, the conditioned swimming behavior to low concentrations of L-proline was mediated by olfaction and the snapping behavior by gustation. Anosmic catfish previously conditioned to 10^{-5}M L-arginine searched for food only during brief periods of time after L-arginine stimulation. Following complete recovery and 150 reconditioning trials with L-arginine, the anosmic catfish did not show discrimination between L-arginine and L-alanine. The results suggest that (a) the association of a chemical stimulus with the food reward was a result of olfactory input and (b) the increased food searching behavior of the catfish cannot be conditioned by taste alone. Supported by NSF BNS-8819772 and the Visiting Scientist Program of the College of Basic Sciences, LSU.

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Designer Peptides and Crustacean Responses. D. RITTSCHOF, R. PETTIS,* R.B. FORWARD, JR., C. HAYS, AND B.W. ERICKSON* (Duke University Marine Laboratory and Dept. of Zoology, Duke University; *Chemistry Dept., Univ. of North Carolina-Chapel Hill).

Last year we made predictions of biological potency of tripeptides. The predictions were based upon crab pumping bioassay data on potencies of free amino acids and arginine coboxyl terminal tripeptides. Peptide potencies were predicted based upon the assumption of a relationship that the R group of potent free amino acids would also be potent if they represented the R group of the middle position in the tripeptide. Here we report the results of the tests with the peptides predicted to be most potent. Four peptides were synthesized, purified and tested. Three of the peptides glycyl-methyonyl-arginine (GMR), glycyl-phenyl-arginine (GFR) and glycyl-norleucyl-argine (GNleR) were predicted to be very potent. The fourth peptide glycyl-methoxymethionyl-arginine (GMOR), was predicted to be of low potency due to the presence of the oxygen in the methyonyl R group. As predicted, in the bioassay GMR, GFR, and GNleR were potent inducers of pumping with response thresholds at subattomolar concentrations. Tripeptides were approximately 10 orders of magnitude more potent than free amino acids. GMOR had inhibitory activity and no stimulatory activity. Subsequent to this discovery the same peptides were tested for their ability to induce metamorphosis in larval barnacles. Barnacle metamorphosis is also stimulated by arginine carboxyl terminal peptides. GMR, GFR, an GMR stimulated metamorphosis at approximately attomolar concentrations. Although we have no doubt that native pumping pheromone and barnacle metamorphosis inducer are different molecules than those tested here, the fact that compounds are extremely effective in both systems implies a similarity in the receptors involved. We are in the process of testing hermit crab responses mediated by arginine carboxyl terminal peptides and will provide these data also in the poster.

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Behavioral and Neurophysiological Responses To Taste Stimuli In Hawaiian Fruitflies. CHENGTAO HER and LINDA M. KENNEDY (Dept. of Biology and Neuroscience Program, Clark University, Worcester, MA 01610)

Genetic studies of taste receptor variants in fruitflies could elucidate physiological mechanisms of the sweet taste. We have been studying behavioral and neurophysiological taste responses in the wildtype Hawaiian fruitfly, Drosophila adiastola (Hardy & Kaneshiro, Univ. Texas Publ. 6818, 1968, 171), to provide baseline data for such studies. The large size of these flies (similar to blowfly) can facilitate behavioral and neurophysiological characterizations and eventual genetic molecular analyses. Behavioral tests were an adaptation of the method of Tanimura et al.(J. Comp. Physiol. 147, 1982, 433). After food-deprivation (with water ad lib) for 48 hrs, groups of 10-50 flies were given an opportunity to drink water, and then given a choice between differently-colored (red or blue) solutions (sugars in 1% agar or plain 1% agar) for 4.5 hrs. The flies which had ingested the solutions (those with colored abdomens) were counted and the preference index [PI=(N_R+N_M/2) / N_T], where N_R, N_M and N_T represent the number of red, mixed and total flies, respectively. Tests with sucrose 50mM (red) vs. plain agar (blue) and sucrose 50mM (blue) vs. plain agar (red) yielded similar PI values (0.722 and 0.700) for the sucrose; thus the color of the solutions did not affect the choice. Tests with 2.5 -250mM sugars vs. plain agar gave threshold values (highest concentrations at which PI=0.5) of 10mM sucrose, 5mM fructose and 30mM glucose. The lowest concentrations at which the maximum PI values (0.8) were obtained were 75mM sucrose, 100mM fructose and 100mM glucose. For neurophysiological tests, the action potential responses of receptor cells to taste stimuli were tip-recorded from single sensilla in isolated proboscis preparations. A single cell responded with small-amplitude spikes to "water" (75µM NaCl). While responses to sucrose, fructose and glucose, and those to NaCl, consisted predominantly of large spikes of similar amplitudes, those in the sugar responses appeared different from those in the salt responses in waveform and in a different proportion of frequency components by Fourier analysis.

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Chemosensory Inhibition of Feeding Behavior and Initiation of Post-feeding Responses in Hydra.
W. GROSVENOR, D.E. RHOADS and G. KASS-SIMON. (University of Rhode Island, Kingston, R.I.)

Feeding behavior in Hydra occurs in two phases. 1. The prey-capture and ingestion phase, and 2. The post-ingestion (digestion) phase. The first phase involves the activation of the tentacle organelles that capture and kill the prey. After the prey is captured the tentacles writhe and the mouth opens. This allows prey to be swallowed. In the second phase prey capture and killing cease, the mouth closes and a series of column contractions begin; these continue at a decreasing frequency until the undigested food is regurgitated. Reduced glutathione (GSH), released from the prey, causes the tentacle activity and the mouth opening during the first phase. To analyze the mechanisms controlling cessation of feeding and initiation of column contractions, we prepared crude prey homogenates by rupturing concentrated samples of brine shrimp nauplii (prey) using a syringe with an 18ga. needle and centrifuging the mixture at 1000XG. The water soluble layer was then fractionated by gel filtration chromatography (SEPHADEX G-25-80). The excluded fraction (containing proteins >5000 Da.) from the column was used for all experiments. External application of the fraction causes >80% decrease in prey capture when compared to control animals. It significantly decreases tentacle writhing and mouth opening response of hydra to 10^{-6}M GSH. Competition experiments using [^{35}S]-GSH indicates that the fraction inhibits the binding of the radioligand to its receptor. It also induces a 7X increase in column contractions compared to unfed animals. We propose that substance(s) from the prey, acting on chemoreceptors, cause cessation of feeding by inhibiting prey capture, tentacle writhing, and mouth opening, and also induce column contractions that may be associated with food mixing.

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Monkey and Human Olfactory Epithelium: A Comparative Study
B.R. TALAMO, W.-H. FENG, M. STOCKMAYER, L. CORK AND J.S. KAUER, (Neurosciences Program, Tufts Univ. Med. School).

The distribution of neuronal and glial proteins in olfactory epithelium (o.e.) of the rhesus monkey and human were studied with immunocytochemical techniques and compared with that of human o.e. Thirteen monkeys ranging in age from 8 to 29 years, some with behavioral deficits, were studied. Some neuron-specific antibodies appeared to stain all olfactory receptor neurons (ORN), from dendritic knob to axon. These included antibodies to microtubule associated protein MAP5(1.2,1b; Sigma), neuron-specific enolase, and neuron-specific tubulin (class IIIβ; J1; A. Frankfurter). Other neuronal proteins appeared only in sub-populations of ORNs. In the monkey, olfactory marker protein immunoreactivity (OMP-ir) was seen in a subset of ORNs located in the more superficial levels of the olfactory epithelium, as previously noted by others, while microtubule associated protein tau-ir was distributed unevenly in a subset of receptor cells which overlapped incompletely with the OMP-containing set. Tau-ir was seen in both superficial and deeply-located ORNs, many of which were not OMP-ir, but tau-ir appeared to be uniformly distributed in olfactory axon bundles in the lamina propria. In humans, OMP-ir was often present only in scattered neurons within areas that contained abundant ORNs with MAP5-ir or J1-ir. In both monkey and human o.e., ORNs may be found in very thin epithelial layers that are only 1 or 2 cells thick. No immunoreactivity for neurofilament proteins (light, medium or heavy subunits; phosphorylated or non-phosphorylated) was found in monkey o.e. at any age. Both monkey and human o.e. had interspersed sensory and nonsensory regions, and occasional crypts lined with ORNs. The non-sensory areas in monkeys were sometimes very richly innervated with thick, smooth neurofilament-ir fibers or fine beaded fibers. Some fibers innervating mucosal glands and coursing though the lamina propria were ir for substance P, tyrosine hydroxylase or CGRP. These were seen more rarely in the human (perhaps due to more prolonged post mortem intervals). Unlike the human, where masses of neuritic fibers and occasional receptor cells were stained with various NF or tau antibodies in areas of highly degenerate o.e., monkey tissue never showed such structures.

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Olfactory Mucosal Changes in Experimental Sinusitis of Rabbit
Seung-Kyu Chung, Yutaka Hanamure, Masahiko Egawa, Atsushi Sameshima, Masaru Ohyama(Dept. of Otolaryngology, Faculty of Med., Kagoshima Univ., Kagoshima, Japan)

Major causes of the olfactory disturbance are the nasal/sinus origin, viral infection and head trauma. In Western country nasal/sinus diseases occupies about 30% including allergic rhinitis in the causes. In Japan, 65-80% of the causes is nasal origin, especially most of them is sinusitis. Characteristic of the olfactory deficit by nasal origin is gradual and progressive. For the mechanism of the olfactory deficit in the cause of nasal/sinus origin, mechanical obstruction to odorants is assumed. It is, however, conceivable that the mechanism of olfactory deficit by sinusitis may be different from that by allergic rhinitis. The aim of this study was to observe the changes of the olfactory mucosa in the animal model of sinusitis. We used 15 white rabbits. Animals were sensitized with 2.5% purified ovalbumin 8 times for 2 weeks systemically and 8 times for 2 weeks locally. With the animals in which Arthus reaction was confirmed, endotoxin extracted from Haemophilus influenzae, type b, was injected into the maxillary sinus for 2 times. One week later the olfactory mucosa was obtained and examined with LM and SEM. Light microscopic examination revealed the decrease of the olfactory cell layer, Bowman's glands and olfactory nerve bundles in the experimental animals. SEM observations revealed that the olfactory epithelium was denuded in some part and the other part of epithelium was covered with non-ciliated cells and ciliated cells, which were, however, different from normal respiratory epithelium. No goblet cells were noted. Nearly all olfactory vesicles were disappeared and some atypical vesicles were observed. Therefore the mechanism of the olfactory deficit in the cases of sinusitis was not only the block of the air currents, but also the changes of the olfactory epithelium. So in the aspect of the restoration of olfactory deficit, it is necessary to treat the sinusitis as soon as possible.

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Do Microvillar Cells in the Rat Olfactory Epithelium Contain OMP?
EDWARD W. JOHNSON, PAMELA M. ELLER, DAVID T. MORAN, BRUCE W. JAFEK (Rocky Mountain Taste and Smell Center)

Four cell types have been identified in the rat olfactory epithelium (OE): 1) olfactory receptor neurons (ORNs); 2) support cells; 3) basal cells, and; 4) microvillar cells (MVCs; see Moran et al., J. Neurocytol. 11:721 [1981]). The nuclei of these four cell types are stratified within the OE. Those of both the support cells and the MVCs are located apical to the cell bodies of the ORNs (and the deeper basal cells). Over the past decade, the ORNs have been identified immunocytochemically by their expression of olfactory marker protein (OMP). Yet within the OE, cells whose somata are located apical to the somata of the ORNs label with antiserum directed against OMP (generously supplied by F. Margolis). We suggest that these are MVCs. Although functional characteristics have been documented for three of the cell types in the OE (olfactory receptor neurons, support cells and basal cells), the role of the microvillar cell, which appears to be randomly distributed across the OE (Moran et al., 1981; Johnson et al., *in preparation*), remains elusive. It has been suggested that these cells have "axon-like" processes (see Moran et al., 1981; Iwagana et al., Biomed. Res. 6:329 [1985]; Morrison and Costanzo, J. Comp. Neurol. 297:1 [1990]) that may project to the olfactory bulb (Rowley et al., Brain Res. 502:387 [1989]). Our light microscopic data using the antiserum directed against OMP has demonstrated labeling in randomly spaced cells superficial to the ORN cell layer. We now have preliminary ultrastructural immunocytochemical data that the olfactory MVCs do contain OMP. These studies continue in order to definitively identify the labeled cell type, which is not an ORN.

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A Monoclonal Antibody, 1F4, Specific for Sustentacular Cell Microvilli within the Olfactory Epithelium. S. K. PIXLEY (Univ. of Cincinnati, Cincinnati, OH), and B. Ph. M. MENCO (Northwestern Univ., Evanston, IL).

Cell-type specific antibody markers allow study of unique cell types. Few exist for cells of the olfactory epithelium. To generate new cell-type-specific markers, monoclonal antibodies were made. CB6FI/J mice were immunized against cultured cells from newborn Sprague-Dawley rat nasal tissues. Intraperitoneal injections were followed by an in vitro, direct exposure of mouse spleen cells to live, cultured nasal cells. Antigen-exposed spleen cells were fused with X-63-Ag8.653 myeloma cells and selected following commonly used techniques. Screening of fused cells for antibody binding to subsets of fixed, cultured nasal cells, using immuno-peroxidase staining, showed a positive for the 1F4 well. After dilution cloning, 1F4 culture supernatant immunostained apical borders of both olfactory and respiratory epithelia in tissue sections of adult and newborn rats. To definitively identify the 1F4-positive cell type(s), fresh frozen, freeze-substituted sections of adult epithelia were examined by electron microscopy. Secondary antibodies linked to gold particles localized binding of culture supernatant. Within the olfactory epithelium, 1F4 binding was very specifically restricted to microvilli of sustentacular cells. Microvilli of brush cells (infrequently seen, straight microvilli) and microvillar-like cells were negative. Microvilli in the respiratory epithelium were 1F4-positive. Cilia of olfactory epithelial neurons were completely negative for 1F4 binding, but cilia of the respiratory epithelium showed labelling. 1F4 will be useful for studying sustentacular cells (within the olfactory epithelium), and investigating differences between types of cilia and microvilli.

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P-glycoprotein immunoreactivity in mouse olfactory epithelium. ERIC WALTERS and JOEL A. MARUNIAK (Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211).

This study demonstrates, for the first time, the immunolocalization of the multidrug resistant (MDR) gene product, P-glycoprotein, in the olfactory mucosa of the adult mouse. Monoclonal antibody C219 and rabbit anti-P-glycoprotein (R3) antisera both identified the same distinct, apical pattern of staining in sustentacular (supporting) cells of the mucosa on formaldehyde-fixed, paraffin-embedded tissues. Respiratory regions possessed some degree of immunoreactivity in histological sections, but nothing analogous to the heavy, highly polarized distribution observed in supporting cells. P-glycoprotein was not detected in olfactory receptor cells, olfactory bulbs, Bowman's glands, basal cells or any cell types of the vomeronasal organ. Seromucosal glands showed some reactivity with our antibodies, but not as strongly as that in supporting cells. The apical staining pattern of the supporting cells is consistent with many studies which have reported the presence of P-glycoprotein in epithelial cells facing lumenal compartments. These studies suggest that the presence of P-glycoprotein may contribute to the elimination of xenobiotics, carcinogens, and odorants from the olfactory mucosa and possibly play a role in the normal physiology of the olfactory epithelium. The effects of unilateral naris closure on the distribution of this protein was examined in adult mice.

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Ultrastructural Localization of Antibodies to Olfactory Epithelial-Specific Glycoproteins gp95, Olfactomedin, and 9-OE and 5-OE Antigens in the Frog's Olfactory Epithelium

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Electron-microscopic immunocytochemistry was used to localize olfactory epithelial-specific glycoproteins gp95 , olfactomedin (a 120 kD dimer, *see abstract David Snyder et al., this issue*), 9-OE and 5-OE in the frog's olfactory neuro-epithelium. The first two were thought to play a role in the receptor process (*Chen et al., 1986, J. Biol. Chem., 261: 1299 (1986)*). Monoclonal antibody 9-OE shares properties with neuronal cell-adhesion type proteins in the frog, and the light-microscopic distribution of this antibody and monoclonal antibody 5-OE has been described (*Key and Akeson, J. Cell Biol., 110: 1729 (199); Key and Akeson, Brain Res., In Press*). With rapid-freeze, freeze-substitution post-embedding (embedding in Lowicryl K11M) immunocytochemistry all four antigens localized in secretory granules massively present in apical regions of the frog's olfactory supporting cells. Protein G-gold was used for localizing the polyclonal anti-olfactomedin and IgG/IgM-gold was used to localize the monoclonal antibodies. Antibody 5-OE labeled less dense than the other three. Surprisingly, all four antibodies displayed a different labeling pattern in the mucus layer. The monoclonal antibody to gp95, 18.1 (*Chen et al., Brain Res., 368: 329 (1986)*), labeled the mucus more or less homogeneously; the antiserum to olfactomedin labeled a lower mucus layer surrounding supporting cell microvilli. This layer is smooth in appearance and distinctly different from the granular top mucus layer in which most of the olfactory cilia are found. Antibody 5-OE labeled especially the vicinity of the olfactory knobs. Most curiously, antibody 9-OE labeled characteristic streaks in the mucus layer, suggesting the presence of specific mucus "islands". This study implies 1) that even if the antibodies recognize ciliary membrane components, it are glycoproteins in other epithelial compartments which labeled more pronouncedly. 2). Supporting cell secretory granules play an important role in olfactory transduction in the frog in that they secrete at least four different types of glycoproteins, all surrounding the olfactory receptor apparatus, i.e., dendritic knobs, olfactory cilia and supporting cell microvilli. Hypothetically, these proteins could play a role in the regulation of olfactory reception or could actually be receptors, which interact in turn with components of ciliary membranes. *Drs. Richard Akeson, Robert Anholt, Brian Key, Doron Lancet and David Snyder are thanked for sharing their antibodies. Supported by NSF (BNS-809839).*

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Olfactomedin: A Novel Glycoprotein Expressed in Olfactory Neuroepithelium. DAVID A. SNYDER, ANN M. RIVERS, HIROKO YOKOE (Dept. Neurobiology, Duke Univ. Med. Ctr.), BERT PH. M. MENCO (Dept. Neurobiology & Physiology, Northwestern Univ.) and ROBERT R. H. ANHOLT (Dept. Neurobiology, Duke Univ. Med. Ctr.)*

Previously, we have raised monoclonal antibodies against chemosensory cilia of frog olfactory receptor cells and identified antibodies that recognize olfactory tissue-specific proteins. Further characterization of these antibodies revealed that they all recognize the same protein, which we have named "olfactomedin". Olfactomedin is a glycoprotein with an apparent molecular weight of 57 kD that exists as a monomeric and dimeric form. The 120 kD dimer is formed via the formation of intermolecular disulfide bonds. Olfactomedin binds wheat germ agglutinin and ricinus communis agglutinin I (RCA), indicating the presence of N-acetylglucosamine and galactoside sugar residues. Since olfactomedin is virtually the only protein in our ciliary membrane preparation that reacts with RCA, it can be purified to homogeneity by affinity chromatography on RCA-agarose. Reactivity of the purified protein with the different monoclonal antibodies has been verified and its tissue specificity consolidated by western blots using a monospecific polyclonal antiserum. Immunohistochemical studies on coronal sections through the frog nasal cavity show intense staining of the epithelial surface and non-uniform staining of the apical regions of acinar cells of submucosal glands. Electron micrographs that visualize the binding of antibodies against olfactomedin at the epithelial surface using gold-labeled protein G localize olfactomedin in secretory granules of sustentacular cells and in the lower layer of the mucus. These data suggest that olfactomedin is produced by sustentacular cells and olfactory glands and deposited at the neuroepithelial surface in association with the extracellular matrix that represents the lower mucus layer. The extensive production of this olfactory-tissue specific protein, which results in its massive deposition at the epithelial surface and its association with olfactory cilia, suggests a role for olfactomedin in odorant recognition.

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Glutathione is localized in secretory cells in rainbow trout olfactory lamellae.

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Thiols were localized by fluorescence microscopy following derivatization with the fluorogenic, thiol-specific reagent monobromobimane (MBB). Olfactory lamellae were immersed in MBB, which is membrane soluble. The tissue was subsequently fixed, frozen and sectioned. The thiols were localized in the supranuclear portions of cells in the nonsensory epithelium on secondary folds and on the distal surface of the olfactory lamellae. These locations surrounded shallow grooves that were lined with olfactory epithelium. Examination of these regions by light microscopy of semi-thin epoxy-embedded specimens revealed goblet cells and columnar cells with numerous secretory vesicles. The major thiol in olfactory lamellae was identified as glutathione by reverse phase HPLC. Postulations for the role of the secreted glutathione include participation in a defence mechanism against the effects of lethal chemicals on olfactory receptor cells or the modulation of peri-receptor events.

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Insect Odorant Binding Proteins: Three Classes with Differing Molecular Properties.

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Odorant binding proteins (OBPs) are thought to enhance the aqueous solubility of hydrophobic odorants. Presumably, the result is that odorants partition into the fluid which surrounds the olfactory cilia more readily, and move through this fluid more effectively than they would without OBPs being present. Previously, pheromone binding proteins (PBPs) have been described from several insect species. However, the proposed requirement of pheromone reception for PBPs begged the question of whether general-odorant reception also required OBPs. The answer to this question is apparently yes, as we have identified two additional classes of insect odorant binding protein and these appear to be associated with sensory neurons which detect general odorants. We have called these "general odorant binding proteins" (GOBPs). All three classes of OBP (PBP, GOBP1 and GOBP2) are coexpressed in the same species, but in association with sensory neurons of different specificity. We have compared partial sequences of 13 OBPs isolated from 6 moth species, and have cloned and fully sequenced a GOBP1 and GOBP2 from the moth *Manduca sexta*, in order to compare these with the PBP previously cloned and fully sequenced from this species. Sequence comparisons of these proteins, and their implication for odorant selectivity will be presented and discussed.

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Localization of 5'-Ectonucleotidase and Phosphatase Activity Within Olfactory Sensilla of the Spiny Lobster.

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Electrophysiological studies have shown that the olfactory organ (antennule) of the spiny lobster, Panulirus argus, has chemoreceptors stimulated by adenosine 5'-monophosphate (AMP) in seawater. Biochemical studies revealed that AMP is rapidly dephosphorylated by 5'-ectonucleotidase/phosphatase activity associated with the olfactory sensilla (aesthetascs). In this study the deposition of cerium phosphate was used to examine the distribution of 5'-ectonucleotidase and phosphatase activity in aesthetascs. Utilizing AMP as substrate, dephosphorylating activity was found to be associated with the plasma membranes of both dendrites and auxiliary cells. Moreover, this activity was specifically localized to a narrow band corresponding to the transitional zone where dendrites develop cilia and branch extensively to form the outer dendritic segments. A similar distribution of the cerium phosphate reaction product was found when β-glycerol phosphate was substituted for AMP. The alkaline-phosphatase inhibitor, levamisole, had no apparent effect on the deposition of reaction product from either AMP or β-glycerol phosphate. The occurrence of degradative ectoenzymes in the transitional zone may be important in clearing exogenous chemoexcitants from this region.

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Expression of 5'-Ectonucleotidase and Phosphatase Activity from the Olfactory Organ of the Spiny Lobster in Xenopus Oocytes.

HENRY G. TRAPIDO-ROSENTHAL, MOLLY A. HOLMAN, ROBERT M. GREENBERG, RICHARD A. GLEESON, and WILLIAM E.S. CARR. (Whitney Laboratory, University of Florida, St. Augustine, Florida).

The olfactory organ of the spiny lobster, Panulirus argus, consists of a dense array of aesthetasc sensilla on the lateral filament of the antennule. Each sensillum contains the dendrites of several hundred chemosensory cells, and processes of a number of auxiliary cells. Electrophysiological studies have shown that sensilla include populations of receptor cells that respond to the nucleotide odorant adenosine 5'-monophosphate. Biochemical studies have shown that sensilla also contain a potent extracellular enzymatic activity that rapidly dephosphorylates this odorant. Recent cytochemical studies have shown that this activity is associated with the plasma membranes of both receptor cells and auxiliary cells. In this study, we report that messenger RNA (mRNA) isolated from the lobster's olfactory organ can induce the expression of ectonucleotidase/phosphatase activity in oocytes of the frog Xenopus laevis. Oocytes microinjected with mRNA from lobsters' lateral antennular filaments exhibited up to a 2-fold increase in their ability to dephosphorylate exogenous AMP, relative to non-injected and water-injected control oocytes. The expression of olfactory 5'-ectonucleotidase/phosphatase activity in Xenopus oocytes represents a first step in the study of an important perireceptor phenomenon at the molecular biological level.

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Biochemical Characterization of Taurine and AMP Binding Sites in Olfactory Tissue of the Spiny Lobster. HENRY TRAPIDO-ROSENTHAL (Whitney Lab, University of Florida), CHARLES DERBY and KIRBY OLSON (Dept. of Biology, Georgia State University)

The nature of olfactory receptor systems of the spiny lobster has previously been extensively characterized through electrophysiological, anatomical, and behavioral studies. The present biochemical study has two goals: (1) to characterize biochemically the binding of odorant molecules with their receptors; and (2) to investigate the roles played by these receptor-odorant binding events in the sensory perception of odorant mixtures (e.g., mixture interactions). An olfactory tissue fraction enriched in dendritic membrane from olfactory receptor cells was prepared from aesthetasc sensilla on the lateral filament of the antennules (the olfactory organ). [^{3}H]-Taurine and [^{3}H]-AMP (adenosine-5'-monophosphate), known to be potent olfactory stimulants, were used as radioligands for the olfactory membrane in a filtration assay. Results suggest that specific binding sites for [^{3}H]-taurine and [^{3}H]-AMP exist in this olfactory membrane, with the following characteristics. (1) Steady-state specific binding is reached quickly. (2) Specific binding is completely and quickly reversible. (3) Binding is not Na^+-dependent. (4) K_m values are approximately 1 uM. (5) The activity of the binding sites is retained in frozen olfactory tissue. (6) Binding of radiolabeled odorants can be inhibited by unlabeled compounds. Future studies will characterize the specificity of [^{3}H]-taurine and [^{3}H]-AMP binding to olfactory membranes, and determine whether inhibition is competitive or non-competitively in nature. Correlation of initial results from these biochemical studies with existing behavioral and electrophysiological data suggest that taurine and AMP are binding to olfactory receptor sites, and that the inhibition of receptor binding by components of a mixture may be one mechanism responsible for mixture interactions.

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Partial Sequence of a Ligand-gated Channel Receptor from the Lobster Olfactory Organ. TIMOTHY S. MCCLINTOCK, ANDREW P. BYRNES, and MICHAEL R. LERNER. (Yale Univ. Sch. Med.)

Lobster olfactory receptor cells express a histamine-gated chloride channel, physiologically similar to $GABA_A$ and glycine receptors (McClintock and Ache, PNAS 86:8137, 1989). Because the histamine receptor may play a role in either signal processing or in modulation of receptor cell sensitivity, we are attempting to clone and sequence it. Using the Polymerase Chain Reaction with primer sequences based on conserved regions of an α subunit of the bovine $GABA_A$ receptor, we have obtained and sequenced a 360 bp DNA fragment with a single open reading frame. Northern blots probed with this fragment show a band at approximately 12 kb in olfactory organ and brain. The predicted amino acid sequence is 33 - 37% identical to mammalian $GABA_A$ and glycine receptor subunits, and 45% identical to a snail cDNA sequence which is 65% identical to the β subunit of the bovine $GABA_A$ receptor (Harvey et al., Biochem. Soc. Trans. 18:438, 1990). We are now screening a cDNA library made from lobster olfactory organ in order to obtain a full-length clone for sequencing and for functional expression studies to determine what ligand activates the receptor.

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Evidence for Acetylcholine as a Neurotransmitter in Lobster Olfactory Receptor Cells. E. ORONA and B. W. ACHE. (Whitney Laboratory, and Depts. of Zoology & Neuroscience, University of Florida, St. Augustine, FL 32086)

Although acetylcholine (ACh) is traditionally regarded as the principal sensory neurotransmitter in arthropods, there is little direct evidence for the assertion that ACh serves this role in lobster olfactory receptor cells. Previously we established that the olfactory organ of the spiny lobster (unpublished data), like that of the American lobster [Barker et al., J.Physiol., 226: 205, 1972], is capable of synthesizing ACh from radioactive precursors. Because ACh synthesis could be due to other cell types in the organ, we sought additional evidence that ACh serves as a neurotransmitter in the receptor cells by focusing on the central afferent terminals in the olfactory lobe (OL). Using biotinylated alpha-bungarotoxin (BTX) or fluorescent BTX-FITC binding, putative nicotinic receptor sites were identified in the OL glomeruli, where the primary afferents terminate and synapse with second-order olfactory neurons. Histochemical staining for the degradative enzyme, acetylcholinesterase (AChE), revealed intense staining in the OL, adjacent and complimentary to the region where the axons of the receptor cells terminate. Arterially perfusing ACh into the brain, in an "isolated head" preparation for 30-40 sec at 3 min intervals, altered (increased, decreased) the spontaneous activity of odor-sensitive cells (12 of 15) in various regions of the brain in a dose-dependent and reversible manner. Cells with dendritic arborizations in the OL glomeruli, revealed by filling with biocytin, increased their spontaneous activity in response to 10^{-4} ACh (n=4), mimicking their response to odorants. These results support the hypothesis that ACh is released at the terminals of the primary olfactory afferents. We have previously proposed that HA-containing olfactory interneurons presynaptically inhibit the (cholinergic) afferent terminals in the OL [Orona et al., J. Comp. Neurol., 294: 633, 1990]. It is noteworthy that the HA receptor on lobster olfactory receptor cells has nicotinic cholinergic, as well as histaminergic properties [Bayer et al., J.Exp.Biol., 145: 133, 1989]. Such a mixed receptor would allow subtle regulation at the synapse, but necessitates caution in studies designed to characterize the cholinergic (nicotinic) receptors on presumptive second-order olfactory neurons.

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Biochemical and Behavioral Investigations of the Mechanisms Involved in the Recognition of an Imprinted Odorant by Coho Salmon, (*Oncorhynchus kisutch*)
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Pacific salmon are well known for their ability to learn odors associated with their natal stream as juveniles and later reuse these retained odor memories for orientation during their homing migration as maturing adults. Hasler and his colleagues first demonstrated that coho salmon exposed to artificial odorants, such as morpholine and 2-phenyl ethanol (PEA), could be lured into an unfamiliar stream by metering these odorants into the stream during their spawning migration years later. To investigate the mechanisms involved in the recognition of these imprinted odors, we exposed juvenile coho salmon to PEA, reared them to maturity without further odorant exposure and demonstrated their ability to recognize and respond behaviorally to PEA. These imprinted fish were subsequently used for biochemical studies of odorant recognition and transduction. Purified olfactory cilia from PEA-imprinted and naive salmon were assayed for adenylate and guanylate cyclase activity and phosphoinositide turnover. The *in vitro* responses of these enzyme systems to added PEA and the specificity of these responses to olfactory tissue was examined.

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Cytochrome P-450-like immunoreactivity in normal and naris closure mice. KAY BUCHHEIT, ERIC WALTERS, and JOEL MARUNIAK (University of Missouri-Columbia, Columbia, MO).

This study demonstrates cytochrome P-450-like immunoreactivity in the olfactory epithelium of normal and naris closure mice. Anti-sera generated against purified rabbit nasal microsome (NMb) cytochrome P-450 (gift from Drs.Ding and Coon) identified P-450-like immunoreactivity in the nasal epithelium of paraffin-embedded sections fixed with both Bouin's and formalin fixatives. Control slides were run from the same animals using normal sheep serum. Cytochrome P-450-like immunoreactivity was present in the olfactory epithelium, seromucosal glands, and the supporting cells of the septal organ. In the olfactory epithelium, there was strong immunoreactivity in the apical regions of the supporting cells and in what appeared to be basal cells but not in receptor cells. This type of reactivity was displayed consistently along the rostral to caudal axis. P-450-like immunoreactivity was not present in the vomeronasal organ. The presence of cytochrome P-450 suggests that it may contribute to the normal physiology of the olfactory epithelium by helping to eliminate foreign molecules.

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Interaction of Odorants and Endogenous Substrates with Olfactory Monooxygenases: Possible Role of Cytochrome P-450 in the Maintainence of Steroid and Fatty Acid Homeostasis in Rabbit Olfactory Mucosa. XINXIN DING and MINOR J. COON (Department of Biological Chemistry, Medical School, The University of Michigan, Ann Arbor, MI 48109-0606).

Mammalian olfactory mucosa contains high concentrations of cytochrome P-450 monooxygenases. In rabbits, the total P-450 content in olfactory microsomes is second only to the liver; at least seven different P-450 isozymes are expressed in this tissue, one of which, P-450NMb (IIG1), is olfactory-specific. Olfactory P-450 isozymes are involved in the metabolism of a variety of foreign compounds, including some known nasal carcinogens and many odorants. It has been proposed that, in addition to their likely importance in detoxifying xenobiotics, olfactory P-450s may function as a signal turn-off or modification mechanism for odor detection. In the present study, we have examined the capacity and efficiency of olfactory P-450 isozymes in the metabolism of steroids and fatty acids. Olfactory P-450s are active both in biosynthetic pathways, such as the formation of catechol estrogen from estradiol, and in degradative pathways, such as hydroxylation of androgens and retinoids, for several biologically active substances. Of particular interest, marked inhibition of at least some of these reactions, such as testosterone hydroxylation, is effected by odorant compounds such as the boar pheromone, 5α-androst-16-en-3-one. We propose that inhibition by odorants of P-450-catalyzed biotransformation of endogenous compounds, which leads to accumulation of unmetabolized substrates and consequent disturbance of intracellular homeostasis of these biologically active substances, may serve as a mechanism by which odorant molecules regulate cellular processes such as secretory activity of glandular cells and differentiation of basal cells in the olfactory epithelium. The lipophilic nature of these endogenous compounds may also suggest a role for olfactory P-450 in the initial events of olfactory signal transduction and odor discrimination through regulation of the physicochemical properties of biomembranes.

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G-Proteins and Inositol-phospholipid Metabolism Implicated in Odor Response of Cultured Lobster Olfactory Neurons. FADOOL, D.A., W.C. MICHEL, and B.W. ACHE (Whitney Laboratory and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086).

Lobster olfactory receptor cells were sustained in primary organ-culture for up to 23 days in a modified Liebovitz L15 media supplemented with fetal calf serum (FCS), *Panulirus* salts, dextrose, BME vitamins, and L-glutamine. The membrane and odor-evoked properties of the cultured cells mimicked those of receptor cells *in situ:* odors increased membrane conductance; the cells displayed graded, submicromolar sensitivity to odorants; the cells had similar mean breath of tuning (H = 0.257); and odors evoked both inward and outward currents in the same cell. The *in vitro* system was thus used to study the possible involvement of GTP-dependent inositol-phospholipid metabolism in olfactory transduction. Flunarizine (1 μM) consistently and completely blocked odor-evoked inward currents while it increased odor-evoked outward currents (N=16). Including GTPγS in the patch pipette increased outward and inward currents 25-691% over control (same cell without analog) in 7 of 10 cells tested (mean 244.2±90.0% SEM). Including GDPβS in the pipette consistently decreased inward currents by 28-79% (N=4; mean 50.0±10.0% SEM). The latter analog was not tested for its possible effect on outward currents. Thirty-hour incubation with 1 μM pertussis toxin (N=9) or 50 μM cholera toxin (N=12), however, had no significant effect on the odor response, even though the former was found to ribosylate a 38 kDa protein isolated from the olfactory organ. With 2.4×10^{-5} M inositol 1,4,5 triphosphate (IP_3) in the patch pipette, 5 of 16 cells produced a sustained inward current (range 20 to 73 pA) from a holding potential of -60 mV. Outward currents were never evoked by IP_3. When cells (N=9) were incubated for 60 min in 10 mM $LiCl_2$, no inward currents were generated by IP_3. In a double patch experiment, first without IP_3 in the pipette (control) and then with IP_3 included (test), 4 cells tested showed a marked increase in odor-evoked current in test condition over control (174 to 780 percent increase; mean 336.2±113.2% SEM). Externally applied ruthenium red (10 μM) blocked the IP_3-induced increase in the odor-evoked current in 3 out of the 4 cells. These data suggest that odors activate one or more bacterial toxin-insensitive G-proteins in lobster olfactory receptor cells and implicate the phosphoinositol second messenger, IP_3, in at least one transduction pathway.

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Strategies for the Identification and Expression of GTP-Binding Proteins from Olfactory Epithelium. FE C. ABOGADIE and RICHARD C. BRUCH (Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208).

Three members of the family of signal transducing GTP-binding proteins were previously identified in isolated cilia from the channel catfish by immunoblotting and ADP-ribosylation (Bruch & Kalinoski (1987) J. Biol. Chem. 262, 2401). Two cholera toxin substrates (45 & 42 kDa) were identified in these studies. Recently, additional experiments were performed to further characterize the identity and functional expression of these cholera toxin substrates. Immunoblotting using an antibody to rat Golf did not label either of the cholera toxin substrates in catfish. However, the 42 kDa cholera toxin substrate crossreacted with an antiserum to Gs as well as with an antiserum recognizing both Gs and Golf. The latter antiserum also labeled the 45 kDa polypeptide, suggesting that this protein corresponds to Golf. Further confirmation of these results was obtained by hybridization experiments. Plasmid DNA encoding Golf (Jones & Reed (1989) Science 244, 790) was transcribed in vitro using T3 RNA polymerase to synthesize a full-length, digoxigenin-labeled transcript. The transcript hybridized to poly (A)+ RNA isolated from the olfactory epithelium, indicating that Golf mRNA was expressed. Additional studies using unlabeled Golf RNA transcripts in in vitro translation and in Xenopus oocytes have been initiated to study the functional expression of Golf. The results of these studies will also be presented.

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Appearance of the Transduction Proteins Gsα, Golfα and Adenylate Cyclase in the Olfactory Epithelium of Rats Occurs on Different Prenatal Days. BARBARA DAU*, BERT Ph. M. MENCO*, RICHARD C. BRUCH*, W. DANHO** and ALBERT FARBMAN* (*Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, IL 60208; **Hoffmann-La Roche Inc., Nutley, N.J.)

Previous studies using antibodies to $G_s\alpha$ (A569 and 584, Mumby et al., PNAS 83 265, 1986) showed that immunoreactivity appears in olfactory axons on E15 (the 15th embryonic day) and on receptor cell cilia on E16 (Mania-Farnell and Farbman, Dev. Brain Res. 51 103, 1990). It is also known that the first odorant generated action potential occurs on E16 (Gesteland et al., Neurosci. 7 3127, 1982). Recently an olfactory specific $G\alpha$, $G_{olf}\alpha$ (Jones and Reed, Science 244 790, 1989) and an olfactory specific adenylate cyclase (Pfeuffer et al., J. Biol. Chem. 264 18803, 1989) were described. We wished to determine whether the appearance of these two proteins during embryonic development corresponds temporally with the ability of receptor cells to respond to odorants. We used antibodies recognizing (1) a peptide common to both $G_s\alpha$ and $G_{olf}\alpha$ (Dupont, NEN); (2) olfactory adenylate cyclase, type III (Bakalyar and Reed, Science, in press); and (3) a peptide specific to $G_{olf}\alpha$ (Jones and Reed, Science 244 790, 1989). Light microscope examination of immunohistochemically treated tissue sections revealed that at E16 the anti-$G_s\alpha$/$G_{olf}\alpha$ heavily labels axon bundles and a few olfactory cilia. At this age anti-adenylate cyclase distinctly labels the olfactory cilia and very lightly the axon bundles. At E16, we saw no labeling of either cilia or axons with anti-$G_{olf}\alpha$, although the luminal surface of the epithelium was lightly stained. By E18 anti-$G_{olf}\alpha$ distinctly stains occasional cilia and very lightly the axon bundles. The intensity of staining of the olfactory epithelial surface with all three antibodies increases until birth and may diminish slightly in the adult. Labeling of the axon bundles in the adult is very light or absent. Recently, it has been shown that $G_{olf}\alpha$ is the predominant G-protein in the adult rat olfactory epithelium (Jones, Chem. Senses 15 333, 1990), an observation which indicates that our anti-$G_s\alpha$/$G_{olf}\alpha$ is labeling $G_s\alpha$ at E16 and $G_{olf}\alpha$ in the adult. Our studies suggest that although both $G_s\alpha$ and $G_{olf}\alpha$ are present on the olfactory cilia, the expression of $G_{olf}\alpha$ is not temporally linked to that of either $G_s\alpha$ or olfactory adenylate cyclase.

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Characterization and purification of anti-Androstenone Antibodies

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5α-Androst-16-ene-3-one (AEO) is an odorous steroid found in the adipose tissue and salivary glands of boars. Detection of this steroid causes sows in estrus to assume a mating stance. Sows and humans have been shown to detect AEO at concentrations in the nanomolar range. Previous attempts to prove the existence of AEO receptors in olfactory epithelia of sows, using classic membrane-receptor binding assays, have given ambiguous results, most likely due to the lipophilic character of AEO. Our approach is to isolate AEO receptors using an auto-anti-idiotypic strategy by, first, isolating polyclonal Abs to AEO, and then, using the polyclonal Abs to screen for monoclonal anti-ids directed specifically at the binding site of the receptor for AEO, i.e. internal image Abs. We have prepared BSA- and RSA-conjugates of AEO by reacting the steroid with O-(carboxymethyl)hydroxyl amine and then coupling the free carboxyl with the free amino groups of BSA and RSA using the mixed anhydride technique. We have estimated the coupling efficiency to be 10-20 groups substituted per protein. Rabbits were immunized with the BSA-conjugate emulsified in RIBI adjuvant. Sera were tested for anti-AEO using ELISA and a radioimmunoassay (RIA). AEO was shown by competition RIA to inhibit binding of the polyclonal Abs to ^{3}H-AEO at an IC_{50} of 2.5 x 10^{-9}M. Analogs were used in competition RIAs to establish the specificity of Ab binding to AEO. The IC50's (nM) of the analogs were as follows: 5α-androstanone 3.0, 5α-androstan-3,17-dione 5.0, 5α-androstenol 10, 5α-dihydrotestosterone 50, testosterone 90, 5β-dihydrotestosterone 100, progesterone 200, triamcinolone 900, cortisol 1500, corticosterone 3000, dexamethasone >3000, and d-aldosterone >3000. The polyclonal Abs, therefore, recognize the 5α- configuration of the steroid and are sensitive to changes in the planarity of ring C (position 11) and ring D (position 16). We have purified the α-AEO polyclonals on an affinity column made by glutaraldehyde cross-linking of the RSA-AEO conjugate to AH-Sepharose 4B. Active antibodies were eluted with 1M propionic acid and then with PBS. ELISA-active Abs were eluted in the propionic acid peak; RIA-active Abs in the PBS peak. The purified Abs will be digested with papain to create Fabs which will be used to screen mouse-mouse spleen fusions for anti-ids.

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Molecular Cloning of a Human Von Ebner's Gland Protein homologous to Lipophilic-Ligand Carrier Proteins HARTWIG SCHMALE, MICHAEL BLÄKER, KAI KOCK (Institut für Zellbiochemie und klinische Neurobiologie, Universität Hamburg, D-2000 Hamburg 20, FRG)

Von Ebner's glands drain into the clefts of circumvallate and foliate papillae and their secretions may influence taste reception. Previously we have characterized an 18 kDa protein secreted from rat von Ebner's glands (VEG protein) that belongs to a superfamily of lipophilic-ligand carrier proteins such as retinol- and nasal odorant binding proteins [Schmale et al.(1990) Nature 343,366]. Secretions of human von Ebner's glands collected from the trenches of circumvallate papillae contain an 18 kDa protein as major component that crossreacted on immunoblots with antibodies raised against the rat VEG protein. N-terminal sequence analysis of the human VEG protein revealed significant homology to the rat protein. In vitro cell-free translation of poly A^+ RNA isolated from human *post mortem* von Ebner's glands yielded the 18 kDa VEG protein as main product indicating that, like in the rat, mRNA encoding the VEG protein is the most abundant species. The human VEG protein cDNA was isolated from a von Ebner's gland cDNA library after screening with a heterologous probe derived from the corresponding rat clone. Sequence comparison revealed an overall identity of the VEG protein from both species of about 60%. Clusters of sequence identity are most remarkable in the hydrophobic core regions of the proteins. In situ hybridization and immunocytochemical analysis of sections through human tongue confirmed the expression exclusively in von Ebner's gland acini. Studies are underway to express recombinant rat and human VEG proteins in heterologous systems in order to elucidate protein and binding characteristics.

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G-Protein Effectors Alter Binding of Amino Acids to Taste Receptor Binding Sites in Catfish, Ictalurus punctatus. JOSEPH G. BRAND (Monell Chemical Senses Center, Veterans Affairs Medical Center and Univ. of PA, Philadelphia, PA), DOUGLAS L. BAYLEY (Monell Chemical Senses Center) and D.L. KALINOSKI (Monell Chemical Senses Center).

There are at least two high affinity receptor sites for naturally-occurring amino acids in the taste system of the channel catfish, Ictalurus punctatus: one for short-chain neutral amino acids, such as L-alanine; the other for the basic amino acid, L-arginine. Receptor binding studies indicate that the dissociation constants for these sites are in the micro and submicromolar range. Previous studies from this laboratory have indicated that L-alanine is capable of stimulating production of the second messengers, cyclic-AMP and inositol trisphosphate, while L-arginine, in contrast, activates a non-specific cation channel in taste plasma membrane fragments incorporated into lipid bilayers (Teeter et al., Biophys. J. 58: 253). L-Arginine does not stimulate production of second messengers at micromolar concentrations, nor does L-alanine activate ion channels. These fundamentally different transduction mechanisms suggest that the L-alanine receptor is linked via a GTP-binding regulatory protein to second messenger forming enzymes, and that the receptor for L-arginine is coupled to an ion channel. Given these observations, it can be hypothesized that G-protein effectors will decrease the binding of L-alanine to its receptor while having no effect on L-arginine binding to its receptor. A sedimentable partial membrane fraction (P2) from catfish barbel exhibited GTPase activity with a Km of approximately 0.2 mM using GTP as substrate. The G-protein effectors, GTP, GppNHp, GTP[S] and NaF decreased the affinity of L-alanine to its presumed receptor sites in Fraction P2. GDPβS was without effect. In contrast, GTP[S] had no effect on (or slightly enhanced) binding of L-arginine to its presumed receptor sites in Fraction P2. These preliminary results therefore support the hypothesis that the two main high affinity amino acid receptors in the taste system of I. punctatus are linked to fundamentally different transduction systems.

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An Expression System for the Cloning of Taste Receptor Proteins.

G. SMUTZER, E. HONDA, D. RESTREPO, L. KALINOSKI, and J. TEETER (Monell Chemical Senses Center, Philadelphia, PA)

We are utilizing the Xenopus laevis oocyte translation system to study the properties of functionally expressed taste receptor proteins of the channel catfish Ictalurus punctatus, and as a potential method for screening a cDNA library prepared from catfish barbel Poly A^+ RNA. We have characterized total RNA and poly A^+ RNA from catfish barbel. Northern analysis of the poly A^+ RNA yielded a sharp hybridization signal near 2 kilobases when RNA transfered to nylon membranes was probed with heterologous ß-actin. The poly A^+ RNA was essentially intact and thus a good substrate for preparation of full-length cDNA. First-strand cDNA synthesis of catfish barbel poly A^+ RNA utilizing AMV reverse transcriptase yielded cDNAs up to 5 kilobases in length when analyzed on an alkaline-agarose gel. Since little structural information about taste receptor proteins is available we are establishing a strategy for screening a barbel cDNA library using the Xenopus oocyte expression system. In oocytes injected with poly A^+ RNA, but not uninjected or water-injected oocytes, the representative taste stimuli L-alanine and L-arginine (100 μM) elicited membrane depolarization. In addition, L-alanine, which stimulates phosphoinositide turnover in I. punctatus taste homogenates, elicited a large increase in ^{45}Ca efflux. The combination of these approaches will allow for the preparation of a representative cDNA library and the development of a screening strategy for a system where little information of the protein of interest is available.

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Patch-clamp Recordings of Hamster Taste Cells: Effects of Saccharin and cAMP.

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In previous studies we have shown that isolated hamster taste cells are electrically excitable and exhibit a variety of voltage-activated ionic currents, including a TTX-sensitive Na^+ current, a non-inactivating Ca^{++} current, and several K^+ currents. In this study we have examined the effects of the sweetener saccharin on these currents, to elucidate further their role in taste transduction. Taste buds were isolated from hamster fungiform papillae according to the method of Béhé et al. (J. Gen. Physiol. 96:1061-1084). Whole-cell patch clamp recordings were obtained from individual taste cells in the isolated taste buds. Peak Na^+ and K^+ currents were elicited by voltage pulses to -20 mV and +60 mV from a holding potential of -80 mV. In approximately 30% of the cells examined, bath application of Na-saccharin (15 mM) caused a reversible reduction in outward K^+ current, with no reduction in the Na^+ current. The effect of saccharin was variable, ranging from 10% block to over 60% block in some cells. In a small number of cells, the effect of saccharin was transient; i.e., the current recovered in the continued presence of the saccharin. In each cell in which saccharin blocked the K^+ current, bath application of 8-CPT cAMP (0.5 mM) reversibly mimicked the saccharin effect. Saccharin caused no further reduction of the K^+ current in the presence of the nucleotide. In cells which did not respond to saccharin, cAMP had no effect on the K^+ current. In order to investigate further the mechanism of the nucleotide-induced K^+ channel block, we have perfused cAMP (10 μM) intracellularly using a pipet perfusion technique. Sulfa-rhodamine B was included in the pipet perfusion solution, allowing us to verify the arrival of the perfusate by observing fluorescence in the cell; typically, perfusion was achieved in 1-3 min. In pilot studies, cAMP perfused intracellularly has mimicked the effect of bath-applied saccharin. These studies support the hypothesis that the response to sweeteners is mediated by a cAMP-induced closure of K^+ channels.

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Aldosterone Increases Amiloride-Sensitive Sodium Channels In Rat Taste Cells: Implications For Sodium Appetite. **M. SCOTT HERNESS.** (The Rockefeller University, New York, NY 10021)

Aldosterone is known to increase amiloride-sensitive sodium (AS-sodium) channels in many transporting epithelia. Since the tongue is a transporting epithelium that contains these channels experiments were performed to test if aldosterone would increase their number by determining if the amiloride-inhibitable portion of the integrated chorda tympani neural recording was different in control and aldosterone-injected animals. Standard whole-nerve integrated recordings were performed. A sodium stimulus (0.3 M NaCl) was presented for a 30 - 45 minutes to ensure recording stability and was followed by amiloride treatment (10^{-4} M, 4 min). The stimulus was then presented for another 30 minutes to measure the inhibition and recovery of response. Intact rats displayed a mean of 56% inhibition while aldosterone injected animals (40 μgm/subject) were inhibited by 71% inhibition ($p < .0025$; Mann-Whitney U test). These experiments were repeated in 5 to 7 day post-operative adrenalectomized (ADX) animals. In ADX rats the NaCl response was inhibited 52% while ADX animals given aldosterone (10 - 50 μgm/ 100 gm BW) were inhibited 68% ($p << .001$). The greater sensitivity of the neural response to amiloride inhibition by aldosterone treatment is presumed to reflect increased sensitivity of the taste receptor cells via increased channels. Patch clamp analysis is in progress to test this hypothesis.

It has been proposed that the AS-sodium channel is the crucial step in sodium recognition. Since aldosterone levels increase during sodium deprivation, these higher levels may induce formation of AS-sodium channels through either protein synthesis or from cryptic channels thus enhancing the animal's ability to perceive sodium salts.

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The Anion Effect in Na Salt Taste: Evidence For the Shunt Hypothesis. QING YE, GERARD L. HECK, JOHN A. DESIMONE (Virginia Commonwealth University, Richmond VA 23298-0551)

We have recorded the response of the rat chorda tympani (CT) to the taste stimuli sodium chloride (NaCl), sodium acetate (NaAc) and sodium gluconate (NaGlu) with or without the sodium channel blocker amiloride. We have also recorded simultaneously either the lingual transepithelial potential (V) under current clamp or the transepithelial current (I) under voltage clamp conditions. Under current clamp conditions there is an anion-dependent relationship between the lingual transepithelial potential (V) and CT response. The sodium salt with the smallest anion, NaCl, gives the greatest CT response and gives the smallest V response while that with the largest anion, NaGlu, gives the smallest CT response but gives the largest V response. Simultaneous measurements of the transepithelial resistance under zero current clamp show that NaCl stimulation causes the greatest reduction in resistance (shows the highest conductance) among the three test salts. With the inside of the lingual transepithelial voltage clamped at -90mV, the CT response of all the sodium salts becomes nearly identical. With the voltage clamped at +90mV, the differences in the response to different sodium salts become magnified relative to the zero current clamp conditions. The chorda tympani responses to NaCl concentrations below approximately 100 mM can be blocked completely by 10 μM amiloride. Above 100 mM an amiloride-insensitive component that increases with NaCl concentration emerges. With nonchloride sodium salts an amiloride insensitive component is not observed at concentrations as high as 500 mM but is clearly evident at 1 M and 2M for NaGlu. Harper (Annals N.Y. Acad. Sci. 510, 349, 1987) and Elliott and Simon (Brain Research, in press) have proposed that the major part of the anion effect is exerted through paracellular shunt pathways (possibly tight-junctions). Our data affirm this hypothesis. The submucosa-positive sign of the potential and its dependence on the size of the anion indicate that the shunt is weakly cation-selective but anions are permeable in inverse proportion to their size. Clamping the transepithelial potential eliminates or enhances the anion effect, again suggesting the influence of a shunt pathway. The emergence of an amiloride-insensitive part to the NaCl response may be due to Na-channels on the basolateral side accessible to NaCl but not to other Na-salts under a favorable electrochemical gradient across the ion-exchanger shunt.

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A Regional Distribution of Amiloride Sensitivity on the Tongue of Rat. BRADLEY K. FORMAKER (Center for Neurological Sciences, Univ. of Connecticut Health Center) and DAVID L. HILL (Dept. of Psychology, The University of Virginia)

Lingual application of the potassium sparing diuretic amiloride is known to suppress responses of the chorda tympani nerve to NaCl by 60-70% in rat. In order to explore possible functional differences in taste receptors located on the posterior tongue, we recorded electrophysiological taste responses from the glossopharyngeal nerve of spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto rats (WKY). In two-bottle preference tests, SHR tolerates higher concentrations of NaCl than WKY; however, previous research demonstrates that relative multi-fiber taste responses of the chorda tympani to NaCl are equivalent between these strains. In the present study, multi-fiber responses of the glossopharyngeal nerve to a concentration series (0.5 M to 2.0 M) of NaCl, KCl and NH_4Cl were recorded before and after the lingual application of amiloride. Responses to a concentration series (0.0025 M to 0.1 M) of quinine hydrochloride (QHCl) were also recorded. Surprisingly, when expressed relative to the 0.5M NH_4Cl response, responses to NaCl were not suppressed by the sodium transport blocker, amiloride. Relative responses to the monochloride salts were also equivalent between SHR and WKY. In contrast, relative responses to QHCl were greater in SHR than WKY; however, these results do not correspond with previous behavioral QHCl preference data in these animals. More importantly, the lack of amiloride sensitivity in the glossopharyngeal nerve is in direct contrast to the dramatic suppression observed in the chorda tympani. These results indicate that taste receptors innervated by the glossopharyngeal nerve lack amiloride sensitivity, implying a regional distribution of amiloride sensitive sodium transduction mechanisms on the tongue of rat.

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Inhibition of the Hamster Chorda Tympani Neural Response to Sucrose, Fructose and Saccharin by Copper Chloride. WALTER E. MYERS, THOMAS P. HETTINGER, AND MARION E. FRANK (Department of BioStructure and Function, Connecticut Chemosensory Clinical Research Center, and Center for Neurological Sciences, University of Connecticut Health Center, Farmington, CT 06030)

Copper chloride was evaluated as an inhibitor of the chorda tympani whole-nerve response to sucrose, fructose, saccharin (calcium salt) and D-phenylalanine (D-Phe). These stimuli can be considered "sweet" because they are preferred and responses learned to them cross-generalize. They also primarily stimulate hamster chorda tympani S fibers. A solution of $CuCl_2$ was applied to the tongue for 30 seconds and its effects on integrated neural responses to taste stimuli determined. With 0.1 mM $CuCl_2$, sucrose and saccharin responses were partially inhibited but responses to fructose and D-Phe were unaffected. The 20% inhibition of the response to sucrose at low concentration was overcome at high concentration, suggesting competitive inhibition. The 40% inhibition of the saccharin-induced response was not overcome by high saccharin concentration, suggesting non-competitive inhibition. Chelation of the cupric ion by the amino acid may explain the D-Phe results, since $CuCl_2$ did not inhibit the response to a mixture of sucrose plus L-Phe. L-Phe has the same chelating properties as D-Phe but is not an effective S-fiber stimulus. At 1 mM, $CuCl_2$ non-competitively inhibited the response to sucrose, fructose, saccharin, as well as to NaCl, an N-fiber stimulus. However, the response to NH_4Cl, an H-fiber stimulus, was not inhibited. $CuCl_2$ appears to interfere with the transduction of S-fiber stimuli by more than one mechanism and is specific only at low concentration, where its effects are weak. Nevertheless, cupric ions differentially affect transduction of sucrose, fructose and saccharin, suggesting diverse mechanisms of sweet-taste reception.

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ABSTRACT WITHDRAWN

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ON THE QUESTION OF BASIC TASTES (QUALITIES)

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The concepts of the taste qualities sweet, sour, bitter and salty are used as essential tools in taste research and serve as the basis for many physiological and biophysical investigations in the mechanisms of taste. In spite of their extensive use it is not known how closely these conceptual categories correspond to the way the information from the tongue is handled. Thus for example psychophysical data in humans show that most taste buds respond to several qualities.

All taste sensations are transmitted as nerve impulses in taste fibers. It is reasonable to think if these fibers fall in the categories of sweet, salty, bitter or sour, this could serve as the neuroanatomical bases for the concepts of sweet, sour etc. However, single fiber recordings are invasive and can not be obtained in humans. Although this limitation does not apply to animals, single fiber recordings in animals have given results which are inconclusive from this point of view.

Since there are fundamental phylogenetic differences in the sense of taste between species, it is possible that these are responsible for the inability to demonstrate these taste categories in single taste nerve fibers, rather than shortcomings of the hypothesis. As a matter of fact some studies in monkeys suggest this. However, there are differences between the sense of taste in humans and monkeys which may obscure the picture. But we have not found any between humans and chimpanzees.

This study reports results from approximately 25 single taste fibers from 5 chimpanzees to stimulation with 11 different sweeteners, 3 acids, 3 bitter compounds and 5 different salts. One of the most striking findings was how well the single fibers fell into one or the other of the sweet, salty, sour or bitter taste qualities (breath of tuning 0.217). For example, 11 of these fibers were highly specific to the sweet compounds and all but one of these responded to all sweeteners used.

Although our material of single fiber recordings is limited and needs to be extended, it is evident that at least some of the psychophysical taste qualities have their counterparts in the way the peripheral taste information is categorized in the taste fibers of the chimpanzee. In the chimpanzee these categories are more pronounced and delineated than in other species. When one considers the closeness between chimpanzee and homo, these results indicate that sweet, salty, sour, and bitter are not "brain ghosts" but a way in which the peripheral sense of taste categorizes taste on the human tongue.

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CO_2 Sensitive Lingual Nerve Neurons are Differentially Tuned

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CO_2 is a potent trigeminal stimulus. When applied orally at a high enough concentration, it produces a tingling or slight burning sensation. As a first approach to understanding the neural basis of oral CO_2 sensation, we examined single unit responses in the rat to a panel of stimuli: 8, 24 and 53°C H_2O, solutions of CO_2 (5-6,000 ppm = 114-136 mM) at 8, 24 and 28°C, 0.5 M HCl, 2.5 M NH_4Cl, and tactile stimulation. In agreement with previous findings (Kawamura, 1967), units that were sensitive to CO_2 were also sensitive to cold stimuli. However, in the present study, cold units were not always sensitive to CO_2, nor was the converse always true. The pattern of chemical sensitivity was not identical across all units tested; responsiveness to CO_2 was not a good predictor of responsiveness to HCl and/ or NH_4Cl. Several units responded solely to both hot and CO_2 stimuli. In addition, CO_2 (at all temperatures) inhibited spontaneous activity in a cold unit. In most CO_2 sensitive neurons, the excitatory response to CO_2 was delayed by 4-9 sec relative to stimulus onset. Among other possibilities, this latency suggests the importance of a temperature dependent diffusional delay. Carbonic anhydrase (CA) may play a role in trigeminal sensitivity to CO_2. We used several CA inhibitors, acetazolamide and ethoxyzolamide. Preliminary results support a role for CA in trigeminal responsiveness to CO_2.

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Organization of the Rostral Division of the Nucleus of the Solitary Tract in the Golden Hamster: Cytochrome Oxidase, NADH Dehydrogenase, and Acetylcholinesterase Histochemistry.

M.A. BARRY, C.B. HALSELL (Dept. BioStructure and Function, Univ. of Connecticut Health Center), M.C. WHITEHEAD (Dept. Oral Biology, Ohio State Univ. College Dentistry).

The use of histochemical markers has proven beneficial to reveal aspects of the organization of many areas of the brain. Recently the use of the markers cytochrome oxidase (CO), NADH dehydrogenase (NADHd), or acetylcholinesterase (AChe) has been applied to studies of the nucleus of the solitary tract (NST) (Lasiter and Kachele, 1989; Herbert et al., 1990; Davis and Jang, 1986; Halsell and Frank, 1989). CO and NADHd histochemistry reveals the relative capacity for oxidative metabolism. AChe staining reveals the relative levels of this enzyme. In this study, the staining patterns revealed by these markers in the rostral (primarily gustatory) division of the solitary nucleus are related to cytoarchitecture (Whitehead, 1988), and to the pattern of the central terminals of primary afferents of cranial nerves V, VII, and IX. The transganglionic transport of horseradish peroxidase (HRP) was used to reveal these projections. Alternate brain sections were processed to reveal transported HRP and histochemical stains. Elevated levels of NADHd and AChe are found in the neuropil in the rostral lateral and rostral central subdivisions, and are largely coincident with the terminal fields of cranial nerves V, VII, and IX. In addition, large cells located primarily in and just ventral to the ventral subdivision of the rostral NST stain intensely for AChe. These cells have the same form and position of cells retrogradely labeled after IXth nerve fills. Thus, many of these AChe stained cells are probably inferior salivatory neurons. CO levels are relatively high in the rostral lateral subdivision, and lateral parts of the rostral central and ventral subdivisions, but are low in the medial subdivision. In conclusion, these histochemical markers should continue to be useful to understand the organization of the normal NST and to reveal changes in the NST following perturbations of the gustatory system.

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In Vitro Whole Cell Recordings from Neurons in Gustatory Zone of Rat Nucleus Tractus Solitarius.

ROBERT M. BRADLEY AND ROBERT D. SWEAZEY (Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan).

Using conventional intracellular recording techniques we have demonstrated different neuron types in the nucleus tractus solitarius (NTS) (Brain Res. 508;168-171, 1990). However, it is difficult to impale neurons in the rostral NTS and obtain stable intracellular recordings. To improve the quality of the intracellular recordings and increase yield of neurons we are using whole cell recording techniques in rat rostral NTS brainstem slices as described by Blanton et al. (J. Neurosci. Methods 30: 203-210, 1989). With this technique gigaohm seals and stable whole cell recordings can routinely be obtained. Typical resting membrane potentials range from -45 to -55 mV, and input impedances from 200-500 Mohm. As in our previous studies neuron types have been characterized using current injection paradigms and the same three neuron types could be distinguished. A hyperpolarizing pulse delays the first spike initiated by a subsequent depolarizing pulse in Type I and III neurons. A depolarizing current pulse initiates a low frequency of action potentials in Type I neurons, a high frequency in Type II neurons and an irregular discharge pattern in Type III neurons. A number of neurons have been filled with biocytin and later reconstructed. The processes of these cells were finer and extended greater distances into the ventrolateral medulla and spinal trigeminal nucleus than biocytin-filled neurons using conventional intracellular techniques. The reconstructed neurons were either of the elongate or multipolar types. However, within these two groups the neurons vary in the complexity of their dendritic trees. Preliminary results indicate that there is no clear correlation between neuron morphology and intrinsic response characteristics.

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Olfactory Testing in Senior Citizen Centers.

MICHAEL SERBY, M.D.,(Mount Sinai Medical Center,NY,NY). PAMELA LARSON, M.A.

Olfactory deficits have been noted very early in Alzheimer's disease(AD), possibly before the advent of clinically apparent dementia. Relatively "normal" functioning elderly people with subclinical AD could presumably be found in senior centers. We, thus, administered an olfactory battery including measures of odor detection and identification to volunteers in senior centers. We used the University of Penn. Smell Identification Test (UPSIT) to test odor identification and a scratch-and-sniff threshold task using 9 microencapsulated concentrations of geraniol to measure detection. Subjects also were screened cognitively via the Mini-Mental Status Exam (MMS). We tested 90 presumed healthy elderly people in senior centers in NYC. 12 of these 90 had MMS scores of ≤ 25 (mean 24.75), suggesting possible early cognitive impairment. The mean age of these 12 (Group 1) was 82.2 ± 8.8. We compared their olfactory performance to olfactory scores in a group of controls aged 75-85 (Group 2 ; n= 27) with MMS scores > 25 (mean 28.1). The UPSIT score of Group 1 was 23.1 ± 8.3 and in Group 2 was 28.7 ± 6.2. This difference was highly significant at $p=.02$. The ability of Group 1 subjects to detect the presence of an odor (geraniol) also seemed impaired. However, the difference between groups did not reach significance ($p=.06$). These olfactory deficits are interesting in light of those which have been established hyposmia in AD. Olfactory testing may be a useful screening device in elderly populations and in people at risk for the development of degenerative dementia.

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The Syndrome of Atmospheric Pressure Sensitive Paroxysmal Unilateral Phantosmia.

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S.M. LIEBERMAN, M.D. (Rush University.)
S.E. GAY, M.D. (Michael Reese Hospital.)

Two patients presenting with paroxysmal unilateral phantosmia described precipitation or resolution of olfactory symptomatology in response to rapid changes in atmospheric pressure. The phantosmias consisted of the odor of a garbage dump and a dusty moldy odor. At initial presentation, the patients described precipitation of symptoms with diving into water and resolution with flying, forced voluntary Valsalva maneuver and standing on their heads. Kaufman (1) described a similar case which resolved with forced gagging and emesis and confirmed (2) resolution of the phantosmic symptom by forced Valsalva in two additional patients with paroxysmal unilateral phantosmia. Also, both groups of patients report that manual occlusion of the nostril on the symptomatic side with absence of air flow, causing a subsequent change in intranasal air pressure, eliminates the symptomatology. We postulate that rapid atmospheric pressure changes effect the baroreceptors in and around the olfactory receptors, hence altering the dysfunctional olfactory discharge. This is analogous to the effect of proprioceptive discharge in the gate control theory of pain. In the syndrome of atmospheric pressure sensitive paroxysmal unilateral phantosmia, however, the baroreceptors act to modulate the olfactory sensory gate. One may speculate that medically-induced pressure changes (e.g. hyperbaric 02) may help those suffering from the syndrome of atmospheric pressure sensitive paroxysmal unilateral phantosmia.

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Decline in Odor Memory Over Time in Alzheimer's Patients.

ROBBIE RHODES, BETH SHEFFIELD, SAMUEL JINICH, AND CLAIRE MURPHY (San Diego State University and UCSD Medical Center, San Diego, CA)*

Recent advances in our understanding of olfactory system involvement in Alzheimers's Disease have shown that deficits in odor memory (Murphy et al., 1987) occur early in the degenerative process of this disease. Furthermore, significant deficits in odor memory as compared to visual memory in Alzheimer's patients have recently been reported (Jinich et al., 1990). We sought to determine whether odor memory declined over one year's time in a group of well-characterized patients with Probable Alzheimer's Disease and a group of normal elderly persons. The subjects were 22 persons diagnosed as having Probable Alzheimer's disease by two independent neurologists at UCSD Medical Center's Alzheimer's Disease Research Center, using the NINCDS-ADRDA criteria; and 22 age-matched, normal controls. The mean score on the Blessed Dementia Rating Scale for the Alzheimer's patients was 10.64 ± 5.38 in year one and 13.47 ± 5.30 in year two. Thus, using this standardized test of dementia, the patients could be considered mild to moderately impaired. Subjects in this study performed a short-term recognition memory task for common odors. Each subject was tested consecutively for 2 years. We compared memory scores for year 1 and year 2 in Alzheimer's patients and normal elderly. These scores, designated Az scores, take into account both sensitivity and criterion, since hits, misses, correct rejections and false-alarms are used to compute the score. Odor recognition memory was significantly impaired from year 1 to year 2 in the Alzheimer's patients, $p < .05$. The normal elderly persons showed no decrease in their odor recognition memory ability between year 1 and year 2. Results will be discussed in terms of the vulnerability of the olfactory system to the disease.

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Odor Familiarity and Odor Recognition Memory in Alzheimer's Patients with Mild, Moderate and Severe Dementia*

SAMUEL JINICH (SDSU and UCSD Joint Doctoral Program in Psychology) and CLAIRE MURPHY (San Diego State University and UCSD Medical Center, San Diego, CA)

Recent reports make it clear that the olfactory system is particularly vulnerable to the neuropathological changes seen in patients with Alzheimer's disease. Psychophysical studies indicate impairment in a variety of olfactory-mediated tasks: olfactory threshold, odor identification, odor discrimination, and odor memory. The present study was designed to investigate the degree to which odor familiarity judgments were related to recognition memory for odors in the Alzheimer's patients. Familiarity with an odor is indication that the subject has smelled the stimulus before and may even know its identity. Hits and correct rejections in a recognition memory paradigm require no knowledge regarding the identity of the stimulus. However, familiarity with an odor may facilitate its recognition. We sought thereby to investigate whether familiarity with odors predicted better performance at recognition. Participants were 20 patients with Alzheimer's disease and 20 normal controls. All had been either been diagnosed as Probable Alzheimer's patients or as normal by two different neurologists at the UCSD Alzheimer's Disease Research Center who applied the NINCDS-ADRDA criteria for diagnosis. The Alzheimer's patients were also tested for olfactory threshold. All were hyposmic, but not anosmic. Results suggest a strong relationship between familiarity and recognition, particularly in the moderately affected individuals. The relationship breaks down in severely demented individuals. Results will be interpreted within the framework of verbal facilitation of encoding.

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Olfactory Deficits in Patient's Infected with the Human Immunodeficiency Virus.

D.E. HORNUNG, D.A. LEOPOLD, D.C. BLAIR, E.C. CLARK, S.L. YOUNGENTOB (SUNY Health Science Center, Syracuse, New York)

The 40 item University of Pennsylvania Smell Identification Test (UPSIT) was administered to 38 HIV positive patients seen in the SUNY Medical Immunodeficiency Clinic. Twenty-three (61%) of those tested showed a reduced olfactory ability (55th percentile or less) as compared to that expected for the patient's sex and age group. The observed olfactory losses were examined with respect to various epidemiologic and clinical factors associated with AIDS. Age, sex, and the method of contracting the disease appeared to have no association with olfactory ability. Similarly there was no evidence to suggest that the presence of AIDS symptoms or disease severity was associated with olfactory loss. On the suspicion that the immune status would explain the decreased olfactory ability, the absolute T_4 lymphocyte level was evaluated, and likewise found not to demonstrate an association with olfactory ability. Many of these patients are being treated with multiple medications, most notably AZT. Here again, we could find no evidence to suggest an association with olfactory loss. Therefore, although the olfactory loss described in this study cannot now be ascribed to any underlying cause(s), an association between HIV positivity and olfactory dysfunction has been demonstrated to a sufficient degree so as to warrant further investigation.

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Differences Among Patients with Smell Impairment Resulting From Head Trauma, Nasal Disease, or Prior Upper Respiratory Infection. HEATHER J. DUNCAN, ALLEN M. SEIDEN, SEOG I. PAIK, and DAVID V. SMITH (University of Cincinnati College of Medicine)

Three common etiologies -- Head Trauma (HT), Nasal/Sinus Disease (NSD) and Prior Upper Respiratory Infection (URI) -- account for 66% of patients presenting to the University of Cincinnati Taste and Smell Center with impairment of olfactory function. These patients all had a smell deficit, measured by either the University of Pennsylvania Smell Identification Test (UPSIT) or University of Connecticut smell battery (butanol threshold, odor identification). Patients with smell loss following HT were more often anosmic than hyposmic ($p < .001$), although anosmia and hyposmia were equally likely in NSD and URI patients. However, UPSIT scores for URI patients ($\bar{x}$ = 23.6 out of 40) were significantly higher than for NSD patients ($\bar{x}$ = 19.0; $p < .05$) or HT patients ($\bar{x}$ = 15.4; $p < .001$). Dysosmias, either distorted odors (parosmia) or phantom odors (phantosmia), were reported by patients in each of the three etiologies. The proportion of URI patients experiencing dysosmia (63%) was significantly higher ($p < .05$) than the proportion seen in either HT (34%) or NSD (34%). On the other hand, for HT patients UPSIT scores were significantly higher ($p < .05$) for those with dysosmia ($\bar{x}$ = 18.6) than for those without ($\bar{x}$ = 13.7). This greater degree of olfactory functioning in HT patients with dysosmia was also reflected in their butanol threshold scores. The average butanol score for dysosmic HT patients ($\bar{x}$ = 3.36 out of 7.0) was significantly greater ($p < .01$) than for those without dysosmia ($\bar{x}$ = 1.24). In the NSD and URI groups, however, patients with dysosmia were not different from those without dysosmia on any test measure. Leigh (1943) reported that for 72 head trauma victims with anosmia, 25% of those who experienced dysosmia eventually recovered smell function, while only 5% of those without dysosmia recovered. Followup studies on our patients examine the relationships among dysosmias, olfactory scores and recovery from smell loss.

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Olfactory Mucosal Biopsy In Patients With Congenital Anosmia. SEOG I. PAIK, ALLEN M. SEIDEN, HEATHER J. DUNCAN, and DAVID V.SMITH (University of Cincinnati College of Medicine)

Preliminary studies indicate histopathologic changes in the olfactory mucosa in traumatic and viral-induced anosmia. The morphology of the neuroepithelium in congenital anosmia, however, remains poorly understood. It is not clear whether the olfactory mucosa is totally absent in cases of congenital anosmia, although mucosal biopsies of the neuroepithelium have been obtained by several investigators. Among them, only one previous report has demonstrated the presence of olfactory epithelium, which contained cells similar to early fetal olfactory receptors (Douek *et al.*, 1975). Other investigators, however, have shown only respiratory epithelium (Igarashi *et al.*, 1986; Jafek *et al.*, 1990). These authors suggested that there might be no olfactory epithelium due to an absence of olfactory bulbs. Since 1986, 7 patients have been diagnosed with congenital anosmia at the University of Cincinnati Taste and Smell Center. In two recent congenital patients, nasal tissue has been obtained using the olfactory biopsy procedure in order to evaluate the ultrastructural characteristics of the mucosa. In both cases, olfactory epithelium was demonstrated on light microscopic examination of semithin sections (1 μm) and ultrastructural examination with transmission electron microscopy. Morphologic changes included decreased numbers of olfactory receptor cells and the presence of microvillar cells with disintegration of cellular organelles. In the first case (37-yr-old female), a few ciliary projections from olfactory vesicles were present but no definite basal bodies were found. Instead, multiple electron dense particles, which are considered to be immature or degraded basal bodies, were seen sometimes near the ciliary rootlets. Except for these few abnormal cells, no other receptor neurons were found. In the second case (23-yr-old female), there were more receptor cells present, several of which had normal basal bodies. These and earlier findings imply that there may be different morphologic manifestations in congenital anosmia, depending upon its cause.

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Effects of Unilateral Medial Temporal Lobe Resection on Olfactory Functioning: Pre- vs Postsurgical Comparison. B.A. MARTINEZ, W.S. CAIN, R.A. DE WIJK (John B. Pierce Laboratory and Yale University, New Haven, Connecticut), D.D. SPENCER (Yale School of Medicine), R. NOVELLY (West Haven V A Medical Center and Yale School of Medicine), K.J. SASS (Yale School of Medicine)

A comprehensive olfactory test battery was administered monorhinically to 21 epilepsy patients just before and 6 mo after unilateral medial temporal lobe resection for treatment of intractable seizures. Ten patients received left-lobe resections and 11 right resections. Thirty-seven normal control subjects closely matched for age, education, and gender were also tested twice. Presurgically, normal controls and patients exhibited normal and essentially equivalent absolute sensitivity for 1-butanol. Higher-order olfactory tests administered presurgically did reveal significant differences between groups. Patients performed at levels significantly below controls on odor discrimination and odor recognition memory, but the two groups of patients did not differ significantly from one another on any olfactory test. All three groups tended to perform more accurately when odors were delivered to the right nostril. Following surgery, left-resected patients showed a mild bilateral decline in sensitivity, whereas right-resected patients demonstrated improvement with the left nostril (contralateral to the resection), and a decline with the right nostril (ipsilateral to the resection). Surgery also brought about significant changes in olfactory functioning as measured by combined performance on tests of discrimination, odor recognition memory, and odor naming. Controls and left-resected patients improved somewhat on their second olfactory testing, demonstrating a probable learning effect for the test battery. Right-resected patients, however, demonstrated again an improvement with the left nostril (contralateral to the resection), and a decline with the right nostril (ipsilateral to the resection). These findings imply that right medial temporal lobe structures play a greater role in olfactory processing than do corresponding structures in the left hemisphere.

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The Odorant Confusion Matrix as an Aid to Diagnosis H.N. WRIGHT, P.R. SHEEHE, D.A. LEOPOLD (SUNY Health Science Center @ Syracuse)

The Odorant Confusion Matrix is a test of olfactory ability where a group of 10 odorants is repeatedly presented to patients presenting with complaints of olfactory dysfunction. In addition, a blank (VEX) stimulus is presented to determine the possible occurrence of a response bias. The results from the main diagonal of the OCM, or percent correct identifications, correlate highly with the results from the UPSIT; forming a predictive relation between one and the other. There is no apparent relation, however, between a patient's diagnosis (eg. trauma, aging, airway obstruction, etc.) and percent correct identifications. In an effort to assist in classifying a patients performance, additional statistical measures are available from the OCM, which are not available from the UPSIT; response bias being a salient feature. Other capabilities include measurements of the tendency to consistently substitute the identification of one odorant for another (for any one odorant or for all odorants) as well as the patient's tendency to substitute the identification of the same odorant for all odorants. These measures are derived from the off diagonal responses. The OCM also lends itself to multivariate techniques, such as multidimensional scaling and cluster analysis, to evaluate presumptive differences among clinical classifications in the processing of olfactory information. The implications of these procedures as an aid to patient diagnosis will be presented.

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Compatibility of Odor Discrimination and Odor Identification
RENÉ A. DE WIJK and WILLIAM. S. CAIN (John B. Pierce Laboratory and Yale University, New Haven, CT 06519)

This investigation comprised background research aimed towards construction of a rational clinical test of odor identification. The work dealt principally with the association between odor quality discrimination and odor identification on the premise that, in such a clinical test, errors of identification should be relatable to failures of the more fundamental process of discrimination. Forty-seven young normal subjects performed a same/different discrimination task for 120 combinations of 6 common odors represented at two matched suprathreshold levels. Half the subjects performed the discrimination task with the veridical labels of the items available and half without the labels available. Availability of the labels reduced discrimination errors. A subsequent test of identification given to subjects who had no labels available correlated significantly with discrimination performance. Tests of multiple-choice recognition given to both groups also correlated significantly with discrimination performance. The best correlate of discrimination performance proved to be the consistency of subject generated labels from one concentration to another. When asked to identify 19 everyday odors from outside the original test set, subjects still performed consistently with their discrimination scores. Hence, it appears that even in the restricted range available in the performance of young, normal subjects, odor quality discrimination and odor identification will indeed prove relatable one to the other.

Supported by NIH Grant DC 00284.

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Perceived Intensity and Similarity in Odor Mixtures.
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WILLIAM S. CAIN and RENÉ DE WIJK (John B. Pierce Foundation and Yale University, New Haven, CT 06519)

In the current experiment three series of binary odorant mixtures were investigated regarding overall odor intensity and similarity. Linalyl acetate was present in each mixture series, whereas the second odorant was cineole, geraniol or hexyl salicylate. Each mixture series consisted of a high and a low concentration range of binary mixtures whereby the concentration of each odorant increased inversely with respect to the other component; a high and low concentration range for the single components was also included. Each subject was asked to make 44 assessments for each mixture series (33 Ss participated in the study). The first task was to rate the odor intensity of each stimulus with respect to a standard stimulus (either linalyl acetate or the second odorant). Then, the stimulus was rated for similarity against an exemplar. The exemplar was one of the components of the binary mixture, or in the case of the single odorants, either the same or the complimentary odorant. Both odor intensity and odor similarity were rated on a line segment scale. Hypo-additivity prevailed for each mixture series and the higher concentration range showed a larger degree of hypo-additivity than the lower concentration range. The highest degree of hypoadditivity coincided with the lowest degree of discriminability of the odorants in the binary mixtures.

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Association of Age and Memory Demands with Odor Intensity Discrimination. JOSEPH A. PAOLILLO, JR., JOSEPH C. STEVENS, AND WILLIAM S. CAIN (John B. Pierce Laboratory, New Haven, CT 06519)

Studies commonly find diminished olfactory performance in the elderly. When do procedural demands of testing contribute to measured age-related differences? The chance of procedure-based contribution would seem more likely for tasks that make obvious demands on memory. The n-alternative forced-choice procedure (NAFC) provides an example. The 2AFC procedure would seem to make minor demands on memory, the 3AFC greater demands, the 5AFC, still greater demands. We tested the influence of number of choices, from 2 to 5, on odor intensity discrimination in young (av. age 21 yr) and elderly (av. age 76 yr). Ss attempted repeated discriminations of two pairs of concentrations of 1-butanol: 80 vs 240 ppm (easier discrimination) and 160 vs 240 ppm (harder). The S had to choose which bottle, from among 2, or 3, or 5, contained the stronger concentrations. Each S made 15 discriminations of 6 conditions, given in random order from trial to trial: 2, 3, and 5 AFC on each of the easier and harder pairs (90 discriminations per S). Outcome (ANOVA): Ss performed better on the easier than on the harder discrimination, and above chance on both, but ease of discrimination interacted with age in a curious way; elderly performed worse than young on the easier discrimination, but about the same on the harder discrimination. Number of alternatives produced a similarly intriguing outcome: number mattered more for easier than for harder discrimination. For harder discrimination, number had no discernable influence on elderly's performance. Such level performance seemed unlikely to arise from a "basement" effect. We tentatively conclude that motivational or strategical factors may account for the findings. An analogous touch experiment, underway, may help clarify the difference between easy and difficult discrimination and decide whether it is modality specific. Supported by NIH Grant AG04287.

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Memory For Odors Over The Course Of One Hundred Seconds.
FRANK R. SCHAB (GM Research Labs)
RENE DE WIJK (John B. Pierce Foundation)
WILLIAM S. CAIN (John B. Pierce Foundation)

Previous work on odor memory has examined separately short- (less than 30 secs) and long-term recognition of odors. Short-term odor memory experiments with retention intervals of about 30 secs showed little evidence of forgetting. Believing that the process of encoding (and therefore the manifestation of forgetting) may require more time in olfaction than in vision or audition, we performed two experiments in which subjects remembered a single odor over retention intervals of up to 100 seconds. On each trial in Experiment 1, subjects smelled a single, common odor (target odor) and then performed one of three distractor tasks during the retention interval. One group of subjects received another odor during the interval and was asked to remember it. A second group was given the name of an odor and told to imagine it. A final group was excused from performing a distractor task. After a retention interval of 6, 20, 40, or 80 seconds, subjects were given an odor and asked to decide whether it was the same as the target odor or different. The type of distractor had no effect on odor memory. Recognition memory was affected by the length of the retention interval, despite high performance overall. Experiment 2 examined memory for familiar and unfamiliar odors over retention intervals of 8, 20, 40, and 100 seconds. The retention interval was filled with a demanding semantic task. The results showed better memory for familiar odors than unfamiliar ones, especially at the longer retention intervals. Both experiments showed that recognition memory improved as retention interval increased from 6 or 8 secs to 20 secs and then declined with further increases. The "spike" at 20 secs was greater for familiar than unfamiliar odors. We suggest that this "spike" in the forgetting curve is the result of a sluggish coding or elaboration process in memory for odors, one which may be more semantic than perceptual, because it affected familiar odors more than unfamiliar ones. Reaction times (RTs) for making the "same/different" judgments paralleled the accuracy data and showed little difference between familiar and unfamiliar odors at the shortest retention interval. However, as retention interval increased, RTs increased for both familiar and unfamiliar odors, but particularly for the unfamiliar ones.

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On the Encoding of Odors: Is There a Visual and/or Semantic Component? MAGDALENA M. GILMORE (Brown University).

The present study investigated whether odors are encoded and stored strictly as olfactory sensations or whether the memory trace consists of a verbal or visual component. Various distractor tasks were used to test the nature of the encoding process under the premise that similar codes interfere with the storage process more than dissimilar codes. The experiment consisted of three phases: acquisition, retention, and recognition. Sixty persons, 18-30 years of age (Mean age = 20.8) participated. During acquisition, the subjects were presented a set of five odors (e.g., fruits, spices, herbs, etc.) at a rate of approximately one every five seconds. Subjects were randomly assigned to one of the following tasks which was performed during the retention intervals (30 seconds and 2 minutes): (1) No distractor task-the control condition, (2) Odor distractor task-smelled four additional odors for the 30s condition and 7 additional odors for the 2m condition, (3) Verbal distractor task-repeated words rapidly presented and indicated whether the words were nouns, verbs, or adjectives, or (4) Visual distractor task-rapidly searched for hidden pictures embedded in complex scenes. For the recognition portion of the test, subjects were presented with either one of the original odors or a distractor odor, and they indicated whether the odor was "old" or "new". Subjects recognized fewer odors after the 2-minute retention intervals than after the 30-second intervals. Unexpectedly, and contrary to what was predicted, with an interval of 30s, subjects who performed the verbal interference task performed significantly better than subjects who were in the control condition. However, after 2 minutes of performing the verbal interference task, the ability to recognize the odors greatly decreased. The implications of these results will be discussed.

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Odor and Emotional Memory: The Role of Sex Differences and Social Factors.

HERZ, RACHEL S. (University of Toronto).
CUPCHIK, GERALD C. (University of Toronto).

An exploratory study investigating the relationship between the experience of odors and the nature of odor-evoked memories was conducted on 20 male and 20 female subjects. Subjects received either an objective (analytical) or a subjective (emotional) instructional set to follow. Each subject rated 20 different odors on 4 rating scales (pleasantness, familiarity, interest, calm-stimulating), attempted to name each odor, and then indicated whether or not the odor evoked a personal memory. Several interesting results were obtained. First, it was shown that the most familiar odors evoked the greatest number of personal memories. Second, while odor-evoked memories occurred most often after a subject had correctly named that odor, memories were also evoked without an odor name. This suggests that the semantic identification of an odor is not a necessary precondition for an odor to evoke a memory. These findings were obtained regardless of subject sex or instructional set, implying that the evocation of a memory by an odor is a fundamental process, prior to the influence of social or individual difference variables. However, females used *more emotion words* in their written descriptions of odor-evoked memories and reported *clearer memories* than did males. In addition, females in the subjective set reported the most *vivid memories* of any other group overall. An interaction between subject sex and instructional set was also observed for odor-naming; females named the most odors in the subjective set and males named the most odors in the objective set. These results are consistent with earlier work from this laboratory and relate to a large body of research demonstrating sex differences in emotional-cognitive processing. It is suggested that these effects may be due to a greater integration of emotional and cognitive aspects of memory and experience for females than for males.

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Simple and Complex Task Performance During Exposure to Intermittent Odors.

SUSAN C. KNASKO (Monell Chemical Senses Center).

Ninety subjects (45 women and 45 men) between the ages of 18-35 participated individually in the study. During the first hour subjects worked on four tasks, each for 15 minutes. The tasks included simple math (addition of 2 two-digit numbers), complex math (multiplication of 2 three-digit numbers), simple verbal (choice of odd-word from a list of four), and complex verbal (proofreading). The order of the tasks was randomized. Following the tasks subjects completed questionnaires concerning their mood, perceived health, and perceptions of the environment. While subjects worked on the tasks and questionnaires the testing room was scented intermittently with either pleasant odors (lemon and ylang), unpleasant odors (skatole and isovaleric acid) or no odors, depending on the group to which the subject had been randomly assigned. After completing the questionnaires subjects where taken to another room where they were given the Stroop Color-Word test to check for aftereffects. They also completed a questionnaire rating the degree to which they felt the odor of the testing room had affected their performance, mood and health. Following this, they completed two personality tests (Level of Extraversion and the Arousal Seeking Tendency scale). Finally, they were given four squeeze bottles containing the odors used in the study and were asked to rate the pleasantness of each.

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Effects of Instruction to Image Odors on Reaction Time CLAIRE MURPHY (San Diego State University, San Diego, CA 92182-0551)

Experiments on odor recognition memory suggest that verbal encoding accounts for a good proportion of the variance in this task, but that other factors are also significant (Murphy, Cain, Gilmore and Skinner, Amer. J. Psych., in press). One potential aid to odor recognition memory is the process of odor imagery. There is some debate about whether the process of mental imagery operates in the olfactory realm. The present experiment sought to detect the possible influences of odor imagery, age, and difficulty on a simple olfactory task. Sixteen subjects were presented with word pairs, i.e., odor names, on a computer screen and asked to choose as rapidly and as accurately as possible which member of each pair possessed more of a particular odor quality, e.g., mustiness. Half the subjects were instructed to image the odors while performing the reaction time (RT) task. Half the subjects in each condition were young college students and half were active, community living, normal elderly subjects. ANOVA on RT yielded significant effects of age, difficulty level and the interaction of imagery instruction and difficulty level. Older persons had longer average RTs, but the pattern of effects of other variables was similar in the two age groups. Odor comparisons rated as increasingly difficult yielded RTs which were progressively slower. Most interestingly, the instruction to image the odors while performing the task had no significant effect on the speed with which subjects performed comparisons rated as possessing low and medium difficulty levels; however, the instruction to image the odors resulted in significantly faster RTs for those rated as most difficult. Hence, odor imagery appears to facilitate making more difficult judgements in this olfactory task.

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Olfactory Event-Related Potentials to 1-Butanol: Intensity effects and correlation with odor threshold.

TYLER S. LORIG (Washington & Lee University)
AMY C. SAPP (Washington & Lee University)
JAMIE T. CAMPBELL (Washington & Lee University)

Fifteen subjects participated in a study designed to record event-related brain potentials to differing concentrations of the odor 1-butanol. Odor administration was based on subjects' inhalation of the odors and averaging of the brain responses was synchronized with each inhalation. EEG data were collected from nine electrode sites during administration of each odor concentration. Subjects pressed a response key to indicate detection of the odor. Results of the study indicated a consistent, yet slow, increase in negativity followed by a concentration dependent increase in positivity which peaked at 430 msec following the stimulus ($F(3,42)4.52$, $p=.021$). Amplitude of this positive peak increased as a function of odor intensity and was maximal at Pz. Wave shape and topography of the positive peak suggest its similarity to the commonly observed P300 wave found during stimulation of other modalities. Peak amplitude of the waves occurring over frontal electrode sites were found to be significantly correlated with subjects' thresholds for 1-butanol. The correlation indicated that a lower threshold was associated with greater frontal negativity. These findings suggest that event-related potentials to inhaled odors produce a long-lasting frontal negativity which over-laps later, and more posterior positive event-related potential components.

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THE EFFECTS OF DIFFERENT ODORANT ON THE OLFACTORY EVOKED POTENTIAL James D. Prah U.S. Environmental Protection Agency and the University of North Carolina, Chapel Hill, NC, USA.

Objective: The goal of this experiment was to examine the effects of two different odorants on the olfactory evoked potential (OEP).

Methods: Fourteen subjects (age) participated in this experiment and received on a random basis 50 or 55 trials of each stimulus, butanol (5000 ppm) or toluene (16000 ppm). Order of odor presentation was counterbalanced and delivered by an olfactormeter. The stimulus duration was 500 msec. Electrode placement was over the frontal cortex at F7, Fz, and F8. Subjects were given a 5-min break after about half the stimuli were delivered and a 5-min break between odorants. Subjects were asked to count the number of times they smelled the odorant. This verified attention to the task and stimulus detection. Stimuli were delivered intranasally on a pseudorandom basis (ITI=10-15 sec) to the left naris. Data were amplified by a Beckman Dynagraph with a bandpass of 0.333 to 22 Hz and collected for 2 sec at 125 Hz on a PC. Subject were also requested to magnitude estimate the intensity of the two stimuli.

Results Though, the odorants were not matched for intensity, amplitude comparisions indicated that the OEP of the butanol was smaller in amplitude than that for toluene. This is consistent with previously obtained data in olfaction and with other sensory systems. Because of the variability in latency for the individual subjects, the data were peak shifted prior to averaging in order to examine the waveform. Examination of the plots of the data revealed that there is little difference between the plots. The waveform for each odorant presents a large P1 and a much smaller P2. The latency for the first positive peak was 867.3 for toluene and 694.67 for butanol. Thus, while the waveforms were identical the latency to response for butanol preceded that of the toluene. This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

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Development of a Microencapsulated Odor Detection Test

MICHAEL SERBY, M.D. (Mount Sinai Medical School, NY,NY).
DAVINA KALKSTEIN (NYU Medical Center) & PAMELA LARSON (Mount Sinai).

Strips containing microencapsulated geraniol in 9 different concentrations were developed exclusively for our laboratory by 3M Company. The concentrations are equal to 6.3, 9.97, 15.8, 25, 39.6, 62.8, 99.6, 157.9, and 250cc of geraniol dissolved in one liter of odorless diethyl phthalate. Strips of microencapsulated odorless mineral oil were also produced to be used as blanks; these strips match the appearance of the geraniol ones. A forced-choice ascending method of limits procedure was administered to subjects. Three strips were presented in each of nine trials; two of the strips in each set contained mineral oil while the third strip contained geraniol. The concentration of geraniol increased in each successive triad of strips to minimize adaptation effects. The position of the geraniol strip in each set was randomized. We had previously performed the exactly analogous test using liquid geraniol and solvent in bottles. We noted that geraniol vapors can linger for some time after a bottle is opened. Strips are also more portable and neater than bottles. Therefore, we compared threshold tests utilizing bottles to threshold tests using the strips. Subjects ($n=36$) performed both tasks on the same day and under similar conditions. Subjects were randomly drawn from different cohorts (various ages and diagnoses). The nine concentrations of geraniol in bottles and strips were identical. A threshold value was designated as the lowest of three consecutive concentrations which were correctly identified. The threshold score was designated from 1 (lowest concentration) through 9 (highest); a score of 10 signifies no perception of odorant. We found an excellent correlation in performance on the two methods (Pearson's $r=.85$; $p<.0001$). We have also tested 14 normal subjects on two occasions, one week apart, and find highly consistent task performance with these strips. The microencapsulated geraniol strips need not be limited to a pure threshold study but may be used in other paradigms, e.g. signal detection testing.

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Effects of (-) Menthol on Nasal Patency, Perception of Nasal Patency and Respiratory Behavior.

DONALD W. WARREN; AMELIA F. DRAKE; HUI LIU (University of North Carolina at Chapel Hill) and JAMES C. WALKER (R.J. Reynolds Tobacco Company, Winston-Salem, NC).

A previously developed technique (Walker et al., 1990; Warren, 1984), which combines psychophysical measurements with continuous recording of nasal cross-sectional area and respiratory behavior, was used to assess the responses of 10 normal adult subjects (6 females, 4 males, ages 33-55) to four concentrations (.35, 1.12, 3.5, 11.2 ppm) of (-) menthol. All four concentrations were well above the odor detection threshold. In most subjects nasal cross-sectional area increased slightly during exposure to (-) menthol, but this increase was not statistically significant when compared to the effect of breathing clean air. Similarly, breath volumes were not significantly changed by (-) menthol. However, ratings of perceived nasal airway openness were significantly elevated, in a concentration-dependent manner, by all but the lowest (-) menthol concentration. These findings, obtained using continuous recording of respiratory responses to well-controlled (-) menthol stimulation, agree with previous reports by Eccles et al. (1983) that (-) menthol can increase the perceived openness of the nasal airway in the absence of actual changes in nasal resistance.

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Decrease in Human Olfactory Sensitivity to Some Odors Due to Substitution of Air in the Nasal Cavity by Helium

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The content of oxygen(O_2)-dependent oxidases of foreign substances in the olfactory epithelium is comparable to that found in the liver, but their full function remains unknown. We present data suggesting that O_2 may be required in the initial events in olfaction. We limited O_2 by substitution of air with helium (He). Air or He was used as a carrier gas to present odors to subjects via a mask that surrounded the nose but did not obstruct breathing by mouth. Four odors were used: phenol, toluene, benzaldehyde and camphor -- known substrates of different oxidases. A fifth odor, amyl acetate, also was employed. A non-oxidative enzyme (carboxylesterase) is responsible for degradation of amyl acetate. The substitution of air by He increased (2 -32 times) the thresholds for phenol (4 cases of 6), camphor (3 cases of 7), and benzaldehyde (4 cases of 6), but not for amyl acetate or toluene. In no case, however, was a threshold lower in He than in air. The addition of O_2 (10 -20%) to the stream of He returned thresholds to the level obtained in air, which suggests that the shortage of oxygen, not the presence of He, was responsible for the decrease in olfactory sensitivity to some odors in He. Importantly, we noted (i) differences among people in their apparent requirement for O_2 to perceive a particular odor and (ii) differences between the O_2 requirements of individuals to smell different odorant. These observations suggest that changes in odor sensitivity in He may not be the result of a general diminution of olfactory cell activity due to a decrease in O_2 (O_2 continues to be available for the olfactory cell via the blood supply). If this was true, we should have observed decreases in the ability to smell all of the odorants. Hence, these results suggest that O_2 plays a specific, yet little understood, role in olfaction. We propose that the differences between odors in apparent requirement for O_2 in the perceptual process may reflect differences in chemical modification of odors in the olfactory epithelium. Furthermore, individuals may differ in the battery of oxidases present in the olfactory epithelium.

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Stimulus-response functions in the peri-threshold region for homologous series of alcohols in humans.

SHIGERU FURUTA[1,2] AND RICHARD L. DOTY[1] (Dept.of Otorhinolargngology, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima, 890, Japan[1], and Smell and Taste Center, University of Pennsylvania, Street, Philadelphia, PA 19104[2])

Stimulus-response functions for four homologous alcohols (ethanol, proponal, butanol, and hexanol) were obtained from three subjects using a forced-choice method of constant stimuli procedure. Light mineral oil served as the dilutent. Seventeen quarter-log steps of each alcohol were utilized. The 100 trial test sessions lasted approximately 50 min. each. A total of 6,800 test trials were administered to each subject. Although considerable intra- and inter-subject variability was present, several of the psychometric functions evidenced "notches" or reversals in slopes. No consistent relationship was observed between the location of such notches and their position within the homologous series.

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Smell and Taste Function in the Visually Impaired: Comparison to Trained and Untrained Sighted Subjects.

RICHARD S. SMITH, RICHARD L. DOTY and GARY A. BURLINGAME (Smell and Taste Center, Department of Otorhinolaryngology and Human Communication, University of Pennsylvania, and City of Philadelphia Water Department, Philadelphia, PA)

Twenty seven blind and 33 sighted subjects were administered a battery of olfactory and gustatory tests, including the University of Pennsylvania Smell Identification Test (UPSIT), a phenyl ethyl alcohol odor detection threshold test, a 16-item odor discrimination test, a taste quality identification test, and a suprathreshold taste test of the perceived intensity and pleasantness of sucrose, citric acid, sodium chloride, and caffeine. Thirteen of the sighted subjects had completed a training program designed to optimize performance on a water quality evaluation panel. Although no significant differences were noted between the blind and untrained sighted subjects on any of the test measures, the trained sighted subjects outperformed the blind subjects on the taste quality identification test and both the blind and untrained sighted subjects on the odor discrimination test. Relative to the two other groups, the trained sighted subjects found the taste of citric acid to be less unpleasant. These findings suggest that training alters performance on a number of chemosensory tasks and that untrained blind persons do not differ from untrained sighted ones in their ability to perceive chemosensory stimuli.

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The Effect of Ambiant Olfactory Stimuli on the Evaluation of a Common Consumer Product.

ALAN R. HIRSCH, M.D. (Smell and Taste Treatment and Research Foundation.)
S.E. GAY, M.D. (Michael Reese Hospital)

Thirty-Five volunteer subjects were recruited to participate in a study evaluating sensory impact on common consumer goods. Subjects were not told prior to the study that the sensory modality being tested was that of olfaction. The patients were alternately assigned int two identical rooms to examine a pair of Nike shoes. The rooms differed in regards to one variable: ambient air room was in one room whereas another room was odorized with a mixed floral smell; which in prior studies has been shown to have positive hedonic impact upon subjects. Patients were asked to examine the consumer product present in the room for the duration of 30 seconds. At that point in time, the subjects were presented with an evaluation form discussing the hedonics, likelihood of purchase and monetary value of the consumer product (shoes). Between each period of examination, the subjects were placed in a neutral, odor-free room for three minutes to eliminate any impact of adaptation. The odor affected the desirability of the shoes in a positive way in 84 percent of the subjects. Sixteen percent of the subjects were affected adversely in regards to the desirability of the shoe by the odorant. The likelihood to purchase the shoe and hedonic value for the shoe were influenced by odor independent of the following: 1) Odor strength, 2) Hedonics for odor, 3) Olfactory ability, 4) Hedonic rating of the shoes, 5) Purchasability of the shoes, 6) Age. The one group that had a negative hedonic response to the shoes in the presence of the odorant were cigarette smokers.

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A light microscopic survey of the development of the olfactory mucosa in *Macaca nemestrina*.
CHERYL SINKEVITCH and BARBARA S. ZIELINSKI . (Department of Biological Sciences, University of Windsor, Windsor, ON Canada N9B 3P4).

Prenatal and postnatal changes in the structure of the olfactory mucosa were studied by light microscopy of semi-thin sections stained with a modified Richardson's stain. At gestational day (F)121 (birth is at F165) the olfactory receptor cells (ORC) stained darkly compared to the surrounding sustentacular cells (SC). Cilia that were up to 5 um long extended from olfactory knobs and cell bodies were located below the SC nuclei. The microvilli of SC reached a length of 6 um. Blood vessels (BV) were crowded at the interface of the lamina propria with the olfactory epithelium(OE). Bowman's glands (BG) contained secretory granules and ducts opened into the mucociliary matrix. At F156 ORC staining varied from pale to dark. A population of ciliated darkly stained ORC with vacuoles contained nuclei that were located superficially in the olfactory epithelium. The microvilli on SC surfaces were not as obvious as in F121 and in the lamina propria, the blood vessels appeared numerous. On postnatal day (P)7days and P12 weeks, the cells of the OE stained uniformly and were closely packed. The BG appeared more abundant and larger. In the adult the arrangement of OE cells was very regular, with SC nuclei distal to ORC nuclei. Olfactory knobs protruded above the flat SC surface.
During the prenatal period the organization of the OE was established and ORC may have begun to turnover. Postnatal development was characterized by an increase in OE cell density and BG expansion, followed by larger and more clearly defined cells in the OE of the adult.
Supported by NSERC.

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Number and Location of Mitotic Cells in the Olfactory Epithelium of Postnatal Rabbits. ROYA ZARRABY AND ESMAIL MEISAMI (Physiol. Dept., Univ. Illinois, Urbana, IL 61801, U.S.A.). ROBYN HUDSON AND HANS DISTEL (Inst. Mediz. Psychol., Ludwig-Maximilians-Univ., Munchen, Germany)

We recently reported (Meisami/Louie/Hudson/Distel, Cell Tissue Res. 262:89-97,'90) that surface area and total number of different cell types in the olfactory epithelium (OE) of postnatal rabbits increases markedly from birth to weaning (basal cells, 4x; olfactory receptor neurons, 6x; supporting cells, 3x). It is not known whether the expansion occurs at the edges of the growing OE or all across it, although on morphometric grounds we proposed the latter. Also the localization of mitotic activity underlying cell proliferation is not clear. We therefore used complete 10u serial sections of newborn and weanling (30d) rabbits' entire OE and determined densities of mitoses in the basal cell zone (bcz), receptor cell zone (rcz) and supporting cell nuclei zone (scz). Mitotic nuclei were found throughout OE (septum and conchae) and in all three OE cell zones of both newborn and weanling. Mitoses densities in all cell zones were higher (50-100%) in the newborn than the weanling. In each age, highest densities of mitoses (#/mm OE) were found in the bcz and the lowest in the rcz. The frequency of bcz mitotic nuclei did not vary rostrocaudally or mediolaterally. The cell type of the dividing nuclei in the rcz is unclear; they may belong to the "globose" basal cells type of Graziadei or undifferentiated "blastema" cells of Andres. Based on these data and total numbers of each OE cell type, we estimate the following average rates of cell divisions in the OE cell zones, for the newborn vs. weanling rabbit: bc 3% vs. 2%; sc 1% vs. 0.5%; rc 0.1% vs. 0.02% (data are % of total cell population).

(Supp: UI Res. Board & Deutsche Forschungsgemeinschaft)

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[^{3}H]Thymidine Incorporation Results Supports Reduction in the Rate of Basal Cell Proliferation in the Olfactory Epithelium of Developing Hypothyroid Rats. MARK PATERNOSTRO AND ESMAIL MEISAMI (Dept. of Physiol., Univ. of Illinois, Urbana, IL 61801)

We have previously shown that the marked increase in surface area and total neuronal number occurring in the olfactory epithelium (OE) of growing rats is severely reduced in hypothyroid (HT) animals. We proposed that the reduction (40% in days 1-25) was caused by a decline in the rate of basal cell (bc) proliferation. To better understand these effects, a quantitative ^{3}H-thymidine study was undertaken. Normal (N) and HT animals were injected with ^{3}H-thymidine (5μCi/gbw) at 25d. Animals were sacrificed 1, 5 or 15d after injection. This timetable enables measurements of the rate of bc division (day 1) as well as the migration and survival of the developing neurons (days 5 and 15). Homologous regions of septal OE were cut into 2μ frontal sections and autoradiographs were studied by counting the total number of labeled cells and their density per mm in both the basal cell zone (bcz) and the receptor zone (rz). Initial results indicate that at 1 day post injection, the number of labeled cells in the rz was low in both N and HT groups while it was high in the bcz (20 cells/mm in the N group). HT rats showed a 30% reduction in this value. In HT rats, at days 5 and 15 post injection, the number of labeled cells was markedly decreased in the bcz and proportionally increased in the rz. Thus early thyroid deficiency reduces the rate of bc proliferation, but does not seem to affect significantly the survival and migration of the developing neurons. These results confirm our earlier observation that the thickness of OE and local density of olfactory neuronal nuclei was unaffected in 25d HT animals. The reduction in bc proliferation might be affecting the expansion of OE which was significantly reduced in 25d HT animals.
Supported by Univ. of Illinois Research Board; MP is an NIH Trainee Fellow.

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Spatial Patterns of Neurogenesis in the Olfactory Epithelium of the Adult Mouse. THOMAS A. SCHOENFELD (Clark University, Worcester, MA 01610 USA), DAVID S. REASNER AND ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 USA)

Immunohistochemical localization of 5-bromodeoxyuridine (BrDU) in S-phase basal cells was used to study the distribution of proliferative activity in olfactory epithelium of adult mouse. Labeled basal cells are not uniformly distributed across the receptor sheet but appear in scattered individual cells and patches of cells (2-10 per patch) interspersed among unlabeled segments of sensory epithelium. This pattern conforms well to the classical description of Graziadei and Monti Graziadei (1979), based on thymidine autoradiography, that receptor cell neurogenesis in the adult mouse olfactory epithelium occurs in interspersed zones of proliferative activity and quiescence. However, there is also evidence in our material for a topographic variation in proliferative activity across the olfactory epithelium. Dense collections of intensely stained basal cells are reliably found in the most recessed regions containing sensory epithelium, i.e., along the stems of turbinates and laterally situated cavity walls in the medial and lateral recesses. Intensely stained cells are also found in the most ventrally situated epithelia, including that of the septal organ. On the other hand, the dorsal meatus and the tips of turbinates situated near the dorsal meatus, particularly at and just caudal to the septal window, contain on average only sparse, lightly labeled basal cells. Thus, proliferative activity is greatest in lateral and ventral regions and least in dorsal and medial regions. Such regionally distinct active and quiescent zones in the mouse bear a close resemblance to rhinotopically distinct regions in the hamster and rat that are known to project exclusively to the ventral and dorsal main olfactory bulb, respectively.

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Unilateral Naris Closure Causes Increased Neurogenesis and Decreased Neuronal Survival in the Olfactory Epithelia of Adult Mice. FRANK COROTTO and JOEL MARUNIAK (Division of Biological Sciences, University of Missouri - Columbia, Columbia MO).

Unilateral naris closure in adult mice forces the animals to breath unilaterally and has been shown to cause decreased numbers of rostral receptor cells and increased numbers of caudal receptor cells on the open side of the nose (Maruniak, *et al., Brain Res.*, 490: 212-218). Here we investigated whether these changes are due in part to altered rates of neurogenesis. Last year we reported that naris closure in adult mice allowed increased numbers of olfactory epithelial basal cells to be labelled with bromodeoxyuridine (BrdU) on the open side of the nose. This finding indicated increased neurogenesis. Since then we have collected more data and subjected them to non-parametric statistical tests. In unoperated animals, we found no left-right or rostro-caudal differences in the numbers of labelled basal cells. In experimental animals, there was a significant increase in the number of BrdU labelled basal cells in the rostral region of the closed side olfactory epithelium four weeks after closure. Since naris closure is acheived through cautery and suture, cells in this region could have responded to growth factors released from the healing naris. Naris closure does not significantly change the number of labelled basal cells in the rostral region of the open side of the nose, although the coefficients of variation were approximatly double that of control values. This suggests that in the rostral region unilateral breathing is accompanied by increased amplitude waves of neurogenesis and quiescence but with little or no net increase in neurogenesis. In the middle and caudal regions of the olfactory epithelium on the open, the numbers of labelled basal cells were significantly above control values at 4, 12 and 16 weeks of closure. We conclude that receptor cell loss seen in the rostral olfactory epithelium on the open side of the nose is due not to decreased neurogenesis but to decreased neuronal survival. These findings argue against a strict, genetically determined receptor cell lifespan, explain the overgrowth of the caudal olfactory epithelium on the open side of the nose (previously described), and show that the mouse olfactory epithelium can increase basal cell mitosis to compensate for decreased neuronal survival caused by chronic, low-level trauma. However, these compensatory increases are visibly effective in the less traumatized regions.

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Effects of unilateral neonatal naris closure on the olfactory epithelia of mice. JOEL MARUNIAK and JEFF HENEGAR (Biological Sciences, University of Missouri, Columbia, MO 65211).

This study investigated the effects of unilateral naris closures of varying durations on the olfactory epithelia of developing mice. Naris closures were performed at 2 or 3 days of life and lasted for 6, 10 or 12 weeks. The effects of closure on numbers of olfactory receptor neurons in the rostral, middle and caudal regions were assessed. The mean number of olfactory receptor cells in the closed-side epithelia remained constant for all regions at all durations of closure. On the open side, the mean number of neurons in the rostral and middle regions decreased with increasing length of closure, but remained constant over time in the caudal region. In control animals, the mean number of neurons decreased between 10 and 12 weeks of age. In mice killed at 6 weeks, the effects of closure on the rostral, middle and caudal regions of the epithelium were similar. The control and open-side epithelia possessed the same mean number of neurons, while the number of neurons on the closed side was 21% lower. At 10 weeks of closure the effects on each region were more variable. In the rostral regions, the number of neurons on the open side had decreased to the same level as on the closed side, while controls levels remained 30% higher. In the middle regions, control levels remained 24% higher than closed-side levels, but the open sides showed losses that placed them 13% lower than control numbers. The caudal regions remained unchanged at 10 and 12 weeks. At 12 weeks of closure, in the rostral and middle regions the control and open-side epithelia showed losses of neurons, while the closed sides again remained unchanged. Thus it appears that unilateral closure caused the closed sides to develop with fewer receptor neurons than the open side or controls. By 12 weeks of age, normal(?) cell losses in control epithelia had wiped out the difference between control and closed-side epithelia. By 12 weeks of age, losses in the rostral regions of the open side had become significantly greater than seen in either the closed or control epithelia. Thus it wasn't until this closure period that the effects were similar to what we have reported for adult naris closure mice.

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The Effect of Odor Exposure on the Olfactory Epithelium of the Adult Mouse: Degeneration and Proliferation Following Short-Term Exposure to Filtered or Odorized Air. DAVID S. REASNER & ROBERT J. O'CONNELL (Worcester Found. for Exp. Biol., Shrewsbury, MA 01545 USA)[1]

Early work by Doving and Pinching established that chronic exposure to a single odorant during postnatal development causes a spatially selective morphological change in the mitral cells of the rat olfactory bulb. This pattern of alteration in the mitral cell layer may reflect reduced sensory input to topographically specified areas of the olfactory bulb. This interpretation is supported by Laing's observation of widespread but nonspecific alteration in mitral cells following prolonged exposure to filtered air. Thus, reduced olfactory stimulation leads to unpatterned mitral cell alteration whereas exposure to a single odorant can retain the normal appearance of mitral cells in a spatially selective manner. Presumably, these bulbar patterns result because subpopulations of olfactory receptor neurons vary according to their type, location, and projection pattern onto the bulb. Furthermore, the direct effects of odor exposure should occur earlier and perhaps more clearly in the periphery than in the bulb. Therefore, we are studying the effect of the odor environment on the olfactory epithelium of adult mice following short-term (6-7 day) exposure to filtered or odorized air. Initial analyses include light microscopy to determine rates of cell death and immunohistochemistry with a thymidine analog, bromodeoxyuridine (BrdU), to mark proliferating cells. Eight-micron, paraffin-embedded sections of the nasal cavity were stained with Hematoxylin & Eosin or immunohistochemically processed to recognize the BrdU incorporated into nuclei just before sacrifice. Mice exposed to a reduced olfactory environment (2 l/min of humidified filtered air; 1.4 l chamber) have higher numbers of pyknotic profiles in the most rostral olfactory epithelium (i.e., early dorsal meatus). The frequency of pyknotic profiles decreases caudally and is *not* accompanied by a wave of compensatory basal proliferation as measured by BrdU incorporation. In contrast, mice exposed to odorized air (2 l/min of humidified filtered air containing 0.2-0.6% of a saturated "lemon" odor) or colony-housed mice have few pyknotic profiles in the rostral olfactory epithelium or, in fact, throughout the rostral-caudal extent of the epithelium. Future studies will determine the time course and extent of this novel degeneration and, in particular, will examine the rate of basal proliferation following the animals return to an odorized environment.

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Stages in the Differentiation of Olfactory Sensory Neurons. JAMES E. SCHWOB (Department of Anatomy and Cell Biology and the Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse, NY 13210)

In the olfactory epithelium, new sensory neurons are born and differentiate throughout the life of the animal. Identifying markers that define stages in the differentiation of olfactory neurons is a necessary step toward better understanding this phenomenon. The growth-associated protein GAP-43 is found in a subset of olfactory neurons located deep in the normal epithelium (which are therefore younger than those more superficial) and in most olfactory neurons at chronic survivals after bulb ablation (when virtually all of the neurons are morphologically immature), suggesting that GAP-43 is a potential marker for immature olfactory neurons (Veerhagen et al, 1989; Schwob, 1991). These observations have been extended in four ways. First, the embryonic form of NCAM is also found in fewer neurons in normal as compared to ablated epithelium, suggesting that it too is a marker for immature neurons. Second, double immuno-labelling techniques have been used to directly demonstrate that GAP-43 expression is reciprocal with OMP expression, i.e. OMP is found in more mature neurons (defined by their position and their near absence after ablation), and only rarely do cells contain both OMP and GAP-43 in either normals or ablateds. Third, we have labeled GAP-43 (+) neurons by the retrograde transport of fluorescent latex beads from the olfactory bulb, indicating that the axons of some of these immature neurons have reached the vicinity of their synaptic target. Fourth, the timing of GAP-43 expression has been defined by combining immunohistochemistry with ^{3}H-thymidine labeling. In both ablateds and normals, GAP-43 expression ceases at some point between 5 d and 2 wk after a neuron is born. Thus, GAP-43 is indeed limited to immature olfactory neurons. Moreover, the cessation of GAP-43 expression appears to be regulated in concert with the onset of OMP expression.

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Effects of Neonatal Thyroid Deficiency in Rats on the Number and Size of Olfactory Bulb Glomeruli.
TIMOTHY SENDERA AND ESMAIL MEISAMI (Physiol. Dept., Univ. Illinois, Urbana, IL 61801)

We have found that in the absence of thyroid hormones, developing postnatal rats do not attain their final number of olfactory receptor neurons (Paternostro & Meisami, Int. J. Dev. Neurosci. 7:243-255,'89). To investigate how this reduction in olfactory receptor neuron may affect the size and number of the glomeruli of olfactory bulb (OB), we employed our recent morphometric method which reliably determines the size distribution and final number of OB glomeruli from area data of individual glomeruli (Chem. Senses 15:407-418,'90). This is also important in view of the recent surge of interest in the postnatal formation of OB glomeruli. Rat pups were made hypothyroid by adding the goitrogen PTU (propylthiouracil) from birth in the drinking water (0.1% w/v). At days 10, 25 and 50 postnatal, the OBs from the hypothyroid and control pups were isolated and frozen serial sections were cut frontally at 40u thickness and stained for cytochrome oxidase (CO) which intensely stains the glomeruli and enables their accurate delineation. Some of the OBs were also stained with Nissl (cresyl violet) and cut at 10u thickness. It was found that the OBs of the hypothyroid rats were significantly smaller in size compared to control animals. Qualitative observations of both Nissl and CO stained sections indicated that some of the OB layers, particularly the internal granular, external plexiform and glomerular layers were reduced in size. The detailed quantitative analyses of the glomerular data on the CO and Nissl series are currently in progress and the results will be presented.

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Early Olfactory Deprivation and Morphometry of Olfactory Glomeruli. A Cytochrome Oxidase Study in Rats
ERWIN THIMM AND ESMAIL MEISAMI (Physiol. Dept., Univ. Illinois, Urbana, IL 61801)

We had shown (Brain Res. 221:81-107,'81) that olfactory deprivation, caused by unilateral neonatal nare closure does not affect the size or total number of olfactory bulb (OB) glomeruli. That study was based on direct counts of glomeruli and their mean diameters in Nissl sections. We recently developed a more reliable morphometric method for determination of glomeruli number and size from area data of individual glomeruli (Chem. Senses 15:407-418,'90). Such data also permit more accurate measures of mean and maximal glomerular diameters. In the present study, we compare glomerular morphometric data obtained from complete serial sections of control and deprived OBs, stained with cytochrome oxidase, which permits accurate delineation of the glomeruli proper. Nare closure was performed by cauterization in 3-day-old rat pups. The left and right (experimental and control) bulbs and normal bulbs from control animals were isolated on days 10 and 30 postnatal. Complete series of frozen sections cut frontally at 40u thickness were prepared and stained for cytochrome oxidase. In general measurements before and after histology revealed that the olfactory deprived Obs were smaller in length, height and width and had reduced thickness of the external plexiform and internal granular layers. Glomeruli of the deprived bulbs were found to show no gross abnormalities in appearance or arrangements, and were adequately stained. Detailed morphometric measurements of the glomeruli as to size, total number and distribution and appropriate statistical analysis are currently in progress and the results will be presented.

Supported by Univ. of Illinois Research Funds

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Normal Development of the Olfactory Bulb is Dependent upon Constant Innervation by Olfactory Axons. JOE HERRERA and GAIL D. BURD (University of Arizona).

In *Xenopus laevis*, Byrd & Burd (Soc. Neurosci. Abstr. 1990) showed that olfactory axons reach the olfactory bulb (OB) by stage 30 and begin to form synapses between stages 36 and 38. By stage 44, all layers of the OB have formed. Several investigators reported that normal development of the OB is dependant upon innervation from the olfactory axons (Piatt, 1951; Clairambault, 1976; Stout & Graziadei, 1983). We have performed similar experiments in a effort to determine the cellular changes that take place in the developing OB following early deafferentation. *Xenopus* tadpoles (stage 45/46) were anesthetized and the nasal capsule, including the olfactory epithelium, was unilaterally or bilaterally removed. When the tadpoles reached stage 58, they were anesthetized, perfused with mixed aldehydes, and embedded in Durcupan. Serial, 2 μm sections were stained with toluidine blue and compared to sham and untreated controls. In OBs with bilateral denervation, the volume of the OB appeared to be greatly reduced, the normal laminated structure was lost, and there seemed to be many fewer neurons. In fact, it became very difficult to identify the boundaries of the denervated OB from that of the adjacent telencephalon. Fusion of the right and left OBs normally occurs at stage 50, but with bilateral denervation, the OBs were not fused. In a unilaterally denervated animal, the unlesioned side appeared normal and the OB was fused. There were some nerve fibers that crossed the midline, but they did not project far into the contralateral bulb and were unable to prevent the effects of ipsilateral denervation. In an animal with regenerated olfactory epithelium that sent axons to the OB, the innervated tissue was laminated. In future experiments, we plan to determine the fate of mitral/tufted cells in the denervated OB.

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Electron Microscopic Observations of Olfactory Axons and Bulbar Neurons during Development. A.G. MONTI GRAZIADEI, R. PEREZ and P.P.C. GRAZIADEI (Department of Biological Science, Unit 1, Florida State University, Tallahassee, FL 32306).

The presence during embryonic and early postnatal development of olfactory afferent fibers penetrating into the presumptive external plexiform and mitral cell layers of the olfactory bulb has been previously reported (Monti Graziadei et al., 1980). It has also been reported that these developing afferent fibers establish connections with TH-containing neurons and processes (Baker, 1989). In the attempt to characterize the mode of interaction between the olfactory fibers and the bulbar neurons, ultrastructural immunohistochemical techniques have been implemented. Rat embryos, 19 to 21 days old, and 1 to 2 day old newborns, were fixed by immersion and perfusion respectively using a mixture of aldehydes in phosphate buffer. The olfactory bulbs were embedded in gelatin and sectioned 50mμ thick on a vibratome. Sections were processed according to standard immunocytochemical procedures, osmicated, dehydrated and embedded in Araldite 506. Ultrathin sections were cut from selected areas based on light microscopic observations. The light microscopic observations revealed axons that appeared to terminate in proximity of the mitral cell layer, while others, after approaching the mitral cells, reconnected with distant glomeruli. Swellings were present on all penetrating fibers. Electron microscopic observations showed that these swellings contained densely packed vesicles.

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Neither CGRP nor the Olfactory Nerve is Necessary for Production of Tyrosine Hydroxylase in the Olfactory Bulb.

THOMAS E. FINGER, BARBEL BÖTTGER, (Univ. Colorado School of Medicine) and WAYNE L. SILVER (Wake Forest Univ.)

Catecholaminergic periglomerular neurons stop producing tyrosine hydroxylase (TH) shortly following lesion of the olfactory nerve. This has led to the hypothesis that contact with the olfactory nerve is required for the periglomerular neurons to produce TH. To test this hypothesis in vivo, we transplanted the olfactory bulbs from E15 rat fetuses into the anterior chamber of the eyes of adult rats. After 6-12 months, the transplants were fixed and processed for immunocytochemistry. In all cases, numerous multipolar TH-immunoreactive neurons (7-15μm) were found within the grafts. Co-localization for CGRP demonstrated that the location of TH-immunoreactive neurons in the grafts did not obviously correspond to the location of CGRP-immunoreactive fibers innervating the graft from the ground plexus of the iris. Recent work in vitro, however, has shown that TH expression in cultured bulbar neurons may be induced by superfusion with the neuropeptide CGRP. In order to test this hypothesis in vivo, we injected capsaicin into neonatal rat pups to eliminate the peripheral peptidergic innervation. After the animals were grown, they were processed for immunocytochemical analysis. As has been shown previously, neonatal capsaicin treatment eliminated virtually all CGRP-immunoreactivity from the olfactory epithelium, nerve and glomerular layer of the olfactory bulb. Despite the absence of this CGRP-immunoreactivity, numerous TH-immunoreactive periglomerular neurons were found in the capsaicin-treated rats. We conclude that TH-immunoreactivity can be demonstrated in vivo in the absence of CGRP as well as in the absence of olfactory nerve input. These findings suggest that multiple mechanisms exist which may impact on the expression of TH by bulbar neurons. The in oculo system permits further experimental manipulation of the factors controlling expression of TH in the olfactory bulb.

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NGF Receptors in the Olfactory System.

QIZHI GONG, MARY S. BAILEY, MICHAEL T. SHIPLEY (Univ. of Cincinnati)

Several studies have reported the presence of NGF receptors (NGFr) in the olfactory system, but the cellular elements bearing NGFrs are unclear. As NGF is a classical neurotrophic factor, the role of this molecule and its receptor during development, turnover and replacement of olfactory neurons in adults is of obvious interest. To address the possible role(s) of NGF in development and regeneration, we have assessed the cellular localization of NGFrs in adult and developing rats using two monoclonal antibodies directed against NGFrs (217C and 192).

In the adult, NGFr-immunoreactivity (NGFr-IR) is abundant in olfactory bulb glomeruli. In addition, there are many labeled fibers in deeper layers of the bulb. Streaks of NGFr-IR were also present in the olfactory nerve. In the olfactory epithelium, NGFr was present on fibers deep to the basement membrane. These fibers are probably trigeminal sensory and/or sympathetic axons. In developmental tissue there was high NGFr-IR in the epithelium and olfactory nerve, but almost no staining in the presumptive glomerular layer. NGFr-IR was observed in neurons of the basal forebrain and their axons coursing into the deep layers of the olfactory bulb.

These results suggest that NGFrs in the adult olfactory bulb glomeruli and deep layers is located on the cholinergic centrifugal afferents from the basal forebrain. This will be tested by lesioning the basal forebrain cholinergic neurons with ibotenic acid; this manipulation should eliminate NGFr from the adult olfactory bulb.

NGFr in the olfactory nerve may be located on Schwann cells. In the sciatic nerve, NGFr is present on immature, but not mature Schwann cells. Following nerve crush, NGFr is re-expressed on Schwann cells. The developmental regulation of NGFr in the olfactory nerve thus appears similar to that in the sciatic nerve. This similarity suggests that the likely cellular location of NGFr in the olfactory nerve is on Schwann cells. The "streaks" of NGFr seen in the adult olfactory nerve may thus represent fascicles transiently "denervated" by normal olfactory neuron turnover and replacement. We predict that NGFr will be robustly expressed in the olfactory nerve following lesion of the olfactory epithelium.

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Somatic Spines - Dendritic Spines: Ontogeny and Possible Function.

E. WEILER and R. APFELBACH (University of Tübingen, Dept. of Zoology, D-7400 Tübingen).

In the altricial ferret *Mustela putorius f. furo* (Carnivora) ontogenetic dependent changes do occur in the percentage of granule cells (interneurons in the olfactory bulb) bearing somatic spines as well as in dendritic spine density. As revealed by Golgi techniques, the percentage of cells with somatic spines increases from birth (72%), is highest in 10 days old animals (92%) and then decreases up to day 90 (75%), where adult levels are reached. Inaddition, there is a marked increase in the mean number of spines per dendritic segment (50 μm apical ending) from birth until the second month of postnatal life. This number remains constant during the third month but significantly (22%; $p<0.001$, Kolmogoroff-Smirnoff test) decreases thereafter, reaching adult levels by about 150 days. While there is little known about the meaning of somatic spines, dendritic spines are often regarded as related to learning (Cross and Perkel, 1985; Behav. Neural. Biol., 44, 151-185). Interestingly, in the ferret the maximum in spine density coincides with the age when olfactory food imprinting is possible (Apfelbach, 1986; Behav. proc., 12, 361-381).

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ABSTRACT WITHDRAWN

The Death of Olfactory Sensory Neurons Following Unilateral Bulbectomy is Attended by an Increase in TRPM-2, a Marker of Apoptosis, "programmed cell death".
H. G. COON, (National Institutes of Health, National Cancer Institute, Laboratory of Genetics, Bethesda, MD), E.Y. LAI*, and C. M. FULTON* (Brandeis University, Dept of Biology, Waltham, MA).

Testosterone Repressed Prostatic Messenger 2 (TRPM-2, a.k.a. SGP-2) is a message cloned from a library made from the rat prostate after regression had begun following orchidectomy (Leger, JG, Montpetit, ML, and Tenniswood, MP, **BBRC** 147, 196, 1987). It is known that this degeneration (and the appearance of TRPM-2) can be prevented by administration of testosterone. This marker protein, of unknown function, has also been found to increase and to mark the regression of tissues in other situations where the phenomenon of programmed cell death occurs *e.g.*, in the blocked kidney, or in lymphocyte cultures when IL6 was withdrawn (Buttyan, R. *et al.*, **Mol. Cell. Biol.** 9, 3473, 1989). We wondered if the massive death of sensory neurons in the olfactory epithelium after axotomy was of this "programmed cell death" type, and if so, would TRPM-2 serve as a good marker of these events? Two litters of three to four day old rat pups were anesthetized by hypothermia and the left olfactory bulb removed by suction through a small flap in the calvarium opened with a scalpel. The skin was closed by a drop of cyano-acrylic glue and the pup was allowed to recover under a lamp. Within an hour of the operation the animals were back nursing with their mother. One animal was killed on each day after the operation and a consistent portion of the olfactory epithelium was removed from the left (experimental side) and from the right (control side). The fragments of dissected olfactory epithelium were frozen in liquid nitrogen and stored for RNA extraction. After determining the amount of RNA recovered, 5µg of total RNA were dotted on blotting medium and then hybridized with ^{32}P-labelled probe prepared from the cloned TRPM-2 cDNA (kindly supplied by Tenniswood). The blots were washed and exposed to x-ray film. The density of the developed dots were evaluated by computer scanned densitometry. In the experimental (left) side the abundance of TRPM-2 mRNA incresed from five to seven fold in three to five days after bulbectomy, as compared to the control (right) side. Because the samples were evaluated double blind, and because the two experiments agree, we take this to be consistent with the notion that that the Wallerian degeneration following bulbectomy is due to a programmed cell death. Our next step will be to see if we can locate the dying cells in normal as well as intact animals using *in situ* hybridization. We would like to know the signal by which the underlying neuroblasts and basal cells are stimulated to replace the dying cells. We want to find out if the way in which the neurons die normally is the same as in regeneration following bulbectomy. Perhaps some product of the cell death programmed synthesis is the signal. Since there seem to be a number of functions conserved in this way of cell death among tissues, we may be able to test this hypothesis by irrigating unstimulated olfactory epithelium *in vivo* with extracts of cells undergoing apoptosis and with the purified proteins as they become available.

Heat Shock Protein HSP70 in Control and Bulbectomized Rat Olfactory Epithelium.
Virginia McM. Carr and Albert I. Farbman (Dept. of Neurobiology, Northwestern University, Evanston, IL 60208)

As part of an ongoing investigation of degenerative events in the rat olfactory epithelium (OE), we examined the immunohistochemical reactivity of a monoclonal antibody (Mab) directed against heat shock proteins (HSPs) in the OE of unilaterally bulbectomized (OB-X) rats. HSPs are highly conserved cellular proteins expressed in cells in response to a variety of physiological changes such as viral infection and elevated temperatures. They enhance the survival of stressed cells in an as yet unknown way. HSP70 is a 70kD HSP that is rapidly and transiently expressed in response to a variety of stresses. OB-Xd rats were perfused transcardially with Bouin's fixative at various post-operative times. Examination of control OE with an anti-HSP70 Mab revealed the presence of reactive receptor cells widely scattered along the OE. Reactivity was apparent in cell bodies, dendrites, and dendritic knobs but not the axons of positive cells. These cells were localized in the apical half of the OE and thus were considered mature receptor cells. Preliminary results in OB-Xd OE show that at one day post-op. reactivity occurs in most supporting cells. Immunoreactive mature receptor cells, such as those seen in control OE are very rare in the OB-Xd OE and, when seen, show much less intense staining than in the control OE. By 10 d. post-op. supporting cell reactivity is no longer apparent. The results support data of others showing the HSP70 response to stress in other systems is rapid and transient (Brown, Neurosci. Res. 27:247, 1990).

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Cell Dynamics in the Olfactory Epithelium
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It is well known that, during adulthood, olfactory receptor neurones can be replaced by division and differentiation of basal cells within the epithelium. Less well understood is the nature of the cell lineage by which new neurones arise. Is basal cell division a single event whereby a stem cell divides once, assymetrically, to provide one stem cell and one neuronal precursor? Is the process heterogeneous with both assymetric and symmetric divisions as suggested from *in vitro* studies (Calof and Chikaraishi, Neuron, 1989, 3:115-127)? Do any of the progeny of basal cell division survive beyond 30 days (presumeably at least the true stem cells must do so)? Answers to such questions were sought in the adult mouse olfactory epithelium using [^{3}H]-thymidine autoradiography. Mice were injected once with [^{3}H]-thymidine and their olfactory epithelia were examined 7, 14, 30, 60 and 90 days later. The number of silver grains over each nucleus was counted and the relative distance from the basement membrane was measured for each labelled nucleus. At 7 and 14 days the average number of labelled cells in each section was about 20 per mm. By 30 days, and for the following 60 days, the average number of labelled cells was only about 6 per mm. Therefore, most labelled cells died 2 to 4 weeks after injection. Labelled cells were compared by nuclear grain density, the time since injection (the "survival period") and the distance of the nucleus from the basement membrane (the "migration distance"). Under this analysis, there emerged a small population of "non-migrating" cells which remained close to the basement membrane. These cells, at first heavily labelled, divided a second time about 60 days after the [^{3}H]-thymidine injection, indicated by a significant decrease in nuclear grain density. We conclude that this non-migrating, slowly dividing, basal cell is probably the neural stem cell which divides assymetrically. Further comparison of the relative numbers of non-migrating and migrating cells indicates that after the asymmetric division there are at least two or three rapid, symmetric divisions of the precursor cells producing many immature receptor cells. Most of these die within 4 weeks of the [^{3}H]-thymidine injection. The results illustrate that olfactory neurogenesis is a heterogeneous process involving two classes of mitotically active basal cells with different rates of division and with different progeny.

Localization of D2 Dopamine Receptor Messenger RNA in Primary Olfactory Neurons.
MICHAEL T. SHIPLEY, WILLIAM T. NICKELL, ANDREW B. NORMAN (University of Cincinnati) and CHARLES GERFEN (Laboratory of Cell Biology, NIMH).

The glomeruli of the olfactory bulb are densely innervated by dopaminergic (DA) interneurons; however, the post-synaptic targets of these neurons are unknown. Most DA receptors in the bulb are of the D2 subtype; we recently showed that these receptors are selectively localized in both the glomerular and the *olfactory nerve* layers. This suggested the hypothesis that juxtaglomerular DA neurons target D2 dopamine receptors on olfactory nerve terminals. Consistent with this possibility, olfactory nerve lesions result in loss of D2 binding in both the nerve and glomerular layers. If D2 receptors are present on olfactory nerve terminals, then these receptors must be synthesized in the cell bodies of primary olfactory neurons (PONs). Here, we demonstrate that primary olfactory neurons contain RNA message for D2 receptors.

An ^{35}S-labelled oligonucleotide cDNA probe specific to D2 receptor was used to localize D2 receptor sequences on cryostat sections through the rat olfactory epithelium using *in situ* hybridization histochemistry. One olfactory bulb was removed from each rat by aspiration 10-14 days prior to sacrifice to cause degeneration of PONs on that side.

In all cases D2 mRNA labelling was present in all regions of the olfactory epithelium on the intact side; labelling was absent from the epithelium on the side of the lesioned bulb. Thus, D2 mRNA is abundant in the olfactory epithelium but disappears after bulbectomy. Since ablation of the olfactory bulb causes degeneration of the PONs, the present results strongly suggest that the D2 mRNA is localized in the PONs and not in other cellular components of the epithelium. The present findings, taken together with our earlier observation of olfactory nerve dependent D2 binding in the bulb, supports the hypothesis that juxtaglomerular DA neurons are presynaptic to olfactory nerve terminals.

If true, this hypothesis implies that there is presynaptic regulation of olfactory nerve terminals. If DA reduces the amount of transmitter released by olfactory nerve terminals, the DA neurons might function to increase the dynamic range of the olfactory system at the first level of synaptic integration. Synaptic targeting of olfactory nerve terminals by DA neurons might also explain the loss of DA and TOH in the bulb after peripheral deafferentation: Deafferentation would deprive juxtaglomerular DA neurons of their postsynaptic target. Target regulation of transmitter phenotype occurs in other model systems.

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Developmental Changes in Cytochrome Oxidase Staining in the Main and Accessory Olfactory Bulbs of Embryonic and Neonatal Garter Snakes. DAVID A. HOLTZMAN, EVAN GORDON, and MIMI HALPERN (State University of New York Health Science Center at Brooklyn, Brooklyn, N.Y.)

Garter snakes are good models for studying the development of the accessory olfactory system because this system has been shown to be functional at birth, and chemical substances that stimulate it are known. We have used the cytochrome oxidase (CO) method to demonstrate developmental changes in metabolic activity of the main olfactory bulbs (MOB) and the accessory olfactory bulbs (AOB) in garter snakes. By using an *in vitro* technique, we also presented a known vomeronasal stimulant, earthworm wash (EWW), to embryos. Our results show that increases in oxidative metabolism occur in the AOB of embryos, but no changes were seen in the AOB or MOB in response to stimulatory and control injections in the amniotic sacs of embryos. Throughout embryogenesis, the MOB showed very little CO staining. These results suggest that chemical stimulation from substances in the amniotic fluid may stimulate the MOB and AOB differentially. Alternatively, stimulation from central efferents into the AOB may be greater than those into the MOB during embryogenesis. After birth, both the AOB and MOB show similar CO staining. Although 7-12 day old snakes will preferentially tongue flick and bite EWW, no differences were seen in CO staining in the AOB and MOB of neonatal snakes exposed to EWW or saline.

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Tyrosine Hydroxylase Activity, Immunoreactivity and mRNA Decline in the Aging Rat Olfactory Bulb. HARRIET BAKER and DONNA STONE (Cornell Univ. Med. Coll. at The Burke Medical Research Institute).

The dopamine phenotype, normally expressed in periglomerular neurons of the main olfactory bulb, is subject to peripheral afferent regulation in both developing and young adult rodents. Specifically, activity, immunoreactivity and mRNA of tyrosine hydroxylase (TH), the first enzyme in the dopamine biosynthetic pathway, are dramatically reduced in animals deprived of receptor cell activation of the olfactory bulb. The phenomenon occurs subsequent to either chemical or surgical peripheral deafferentation and in animals lacking odorant access secondary to naris closure. Under appropriate experimental conditions, the reduction in TH expression is reversible indicating that the olfactory bulb retains plastic capabilities similar to the regenerative capacity of the olfactory epithelium. Two lines of evidence suggested that the dopamine phenotype also might be altered in aged rodents. First, several studies demonstrated that afferent innervation was reduced in old rats. Secondly, numerous reports exist indicating compromised olfactory function in both normal aged humans and especially in those with neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Thus, it was of interest to investigate the changes in neurotransmitter expression in the aged rodent olfactory system. In the studies reported here, TH activity in the olfactory bulb was measured in 6-, 18-, and 27-month old Fisher 344 rats. Activity (expressed as mean nmoles/bulb/15 min. $\pm$ SEM, n=6-8) was reduced significantly in 27-month old (4.88 $\pm$ 0.43) as compared to both 6- and 18-month old rats (6.28 $\pm$ 0.19 and 5.99 $\pm$ 0.22, respectively). Similarly, TH immunoreactivity and mRNA, the latter as indicated by *in situ* hybridization, exhibited a similar decrease. These data demonstrate that TH expression is compromised in old rats possibly as a consequence of a loss of afferent innervation.

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Development of the Olfactory Bulb in *Xenopus laevis* Tadpoles and Adults: a Morphological and Quantitative Study. CHRISTINE A. BYRD and GAIL D. BURD (University of Arizona).

The development of the olfactory bulb (OB) in *Xenopus laevis* was examined in tadpoles (stages 26 to 58) and in adult frogs. The morphology of the developing OB was analyzed with the light and electron microscope. The olfactory receptor cell (ORC) axons were first present below the basal lamina of the neural tube at stage 29/30, and the neural tube showed the first signs of differentiation at stage 32. Some of the differentiating neural tube cells began to extend processes by stage 34. Synapses were first seen at stages 36 and 38, but the glomerular layer was not present until stage 40. All the layers of the OB were present by stage 44, and by stage 48, the basic structure of the mature OB was evident. The only changes that occurred after this stage appeared to be an increase in size and in number of components. We also examined the quantitative relationship between the ORC axons and the mitral/tufted cells of the main and accessory OBs of tadpoles (stages 50 to 58) and adult frogs. The number of axons was obtained from EM montages, and the number of mitral/tufted cells was determined from serial, thick sections through the OB. There was a correlation between ORC axons and mitral/tufted cells throughout larval development (n=18; correlation coefficient=0.952). The average ratio of ORC axons to mitral/tufted cells in tadpoles was 5:1. This ratio increased to 32:1 in one adult frog. Therefore, the number of ORC axons and mitral/tufted cells did not increase linearly after metamorphosis; there was a 17-fold increase in ORC axons and only a 2.5-fold increase in mitral/tufted cells. Another adult frog is currently being quantified. The ratio of ORC axons to mitral/tufted cells in frogs is significantly lower than the ratio reported for mammals.

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Evidence for the "Early Formation" of Glomeruli in the Developing Mammalian Olfactory Bulb. E. MEISAMI (Dept. Physiology, U. of Illinois, Urbana, IL, 61801).

Studies by Meisami in the rat and Purves and associates in the mouse have indicated marked postnatal increases in glomeruli number, extending into early adulthood. This view, based in part on counting of glomeruli in Nissl sections, has been referred to as the "continuous construction" hypothesis. A major problem is that in Nissl sections, glomeruli are identified not as glomeruli proper but by their surrounding cell assemblies. Therefore, in young animals where the periglomerular cells are still migrating, reliable enumeration of glomeruli is difficult. To overcome this, we stained complete serial sections of bulbs of young and mature rats with cytochrome oxidase, which delineates the glomeruli proper, and applied our morphometric method (Meisami, Chem. Senses, 15:407-418,'90) to compare the glomeruli number. The results indicate that the bulk of the glomeruli are already present in the young neonate, postnatal increases occurring mainly in size and not number. These findings are consistent with the fact that mitral cells of the bulb, which contribute massively and importantly to the glomeruli, have reached their final number at birth, many showing fairly mature morphology and a glomerular tuft. We therefore propose an alternative hypothesis of "early formation", stating that in altricial mammals (rat, rabbit, etc.) glomeruli establish their final number and basic scaffolding, as relay stations, in the early neonatal period. Postnatally, arrival of periglomerular cells and their innervation of glomerular neuropil, will finalize the functional development of glomeruli as complete integrative units. It is this functional development that occurs largely after birth and may extend well into early adulthood.

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Denervation During Development Permanently Alters the Gustatory Epithelium.

BRUCE OAKLEY (Dept. of Biology, Univ. of Michigan)

Denervation shortly after birth prevents most rat vallate taste buds from forming, even if the vallate papilla is subsequently reinnervated by the IXth nerve. We wished to determine whether an early history of diminished nerve-epithelial interactions during the sensitive period resulted in the development of fewer taste buds because of alterations in the vallate papilla or in the IXth nerve. That is, did early denervation impair the gustatory founder cells or the taste axons? The vallate papilla was denervated from postnatal day 3 to 13 by avulsing one IXth nerve and crushing the other. Nerve crossing experiments, subsequently carried out when the rats were 75-180 days old, revealed that innervation by the chorda tympani nerve formed no more vallate taste buds (51 ± 10, n=8) than control regenerated IXth nerves (48 ± 12, n=10). In contrast, 214 ± 22, (n=7) taste buds formed after the chorda tympani cross-reinnervated a vallate papilla that had been spared early denervation. Early denervation must have caused gustatory founder cells in the vallate papillae to die or irreversibly adopt alternative fates. We conclude that there is a developmental window in the induction of taste buds when founder cells are committed to the gustatory system.

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A Model of Neural Activity-Dependent Alterations in the Developing Gustatory System: Neuroanatomical, Neurophysiological and Behavioral Changes Associated with Early Dietary Sodium Deprivation.

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Dietary sodium deprivation during early development has profound and enduring effects on the maturing gustatory system in rats. Neurophysiological responses from the chorda tympani nerve (CT) are reduced as much as 60% in deprived rats when the stimulus is a sodium salt. Taste responses to non-salt stimuli are unaffected. It appears that this suppression of presynaptic sodium activity affects both morphological and physiological development of the central gustatory system. Deprivation-induced alterations include expanded and dispersed CT terminal fields in specific regions of the NTS, increases in the length of "projection" neuron dendrites, increases in glial number at the level of the nucleus of the solitary tract (NTS) where CT axons terminate and a decreased responsiveness of NTS neurons to NaCl. Interestingly, although peripheral responses to NaCl "recover" to control levels when deprived rats are fed a NaCl replete diet, central morphological characteristics do not "recover". In addition, "recovery" consistently results in hyper-responsiveness of NTS neurons to NaCl. Such dramatic morphological and neurophysiological changes appear to influence salt taste preferences in that "recovered" rats have a lower preference for NaCl across all concentrations compared to controls. Since the interaction between activity and morphology has been shown to play a major role in developmental processes in other sensory systems, it is possible that such a relationship exists in the gustatory system. Consequently, we propose a model whereby the selective suppression of CT activity results in abnormal patterns of synaptogenesis in the NTS and leads to abnormal development of neurophysiological and behavioral taste responses.

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Spines are Lost from Neurons in Nucleus of the Solitary Tract during a Developmental Period of Functional Convergence and Complex Afferent Input.

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From previous work we know that there is a marked increase in convergence of chorda tympani taste fiber input onto second order taste neurons in the nucleus of the solitary tract (NST) between fetal and perinatal periods in sheep. During the period of convergence response frequencies to salt stimuli increase. In particular, convergence in NST apparently functions to maximize gain for processing neural responses to NaCl, compared to other salts. To determine possible relations between neuron geometry and development of convergence onto NST cells, we are studying Golgi preparations of neurons in a functionally defined region of the NST in several age groups of fetal, perinatal and postnatal sheep. From fetal to perinatal periods there is an increase in total dendritic length and volume for elongate and multipolar NST neurons. Number of branch points also increases in multipolar, but not elongate, cells. However, number of spines decreases in both cell types during these stages, and spine density on the highest order branch decreases. Therefore, spines are lost from NST cells during development of convergence. This suggests that some synapses might be selectively strengthened while others are eliminated, in the process of establishing central circuits for salt taste. To discern the nature of the chorda tympani projection to the NST during development, we have applied Lucifer Yellow to the intermedius nerve root near its entrance to the brainstem (Lasiter & Kachele, 1990, Dev. Br. Res.) and examined projections in the solitary tract (ST). In fetal and perinatal sheep, afferent input in the ST to second order neurons is organized in highly fasciculated bundles, not in a homogeneous network. Further, presumed terminal endings are complex and glomerular in appearance. We propose that the fasciculated fibers in the ST and complex terminals provide an anatomical basis for the functional convergence that emerges during development.

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Olfactory Discrimination of Nicotine-Enantiomers by Smokers and Non-Smokers

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HUMMEL C., and KOBAL G.

This study reports an investigation of smokers and non-smokers conducted in order to determine differences in the olfactory perception of the stereoisomeres of nicotine; 38 subjects participated (20 smokers, 18 non-smokers). The investigated parameters were: detection thresholds, hedonic ratings and intensity estimates, discrimination between enantiomers, degree of dissimilarity, and the odorous quality of the smell of nicotine enantiomers. Subjects were able to discriminate between the two stereoisomeres of nicotine. Differences in the subjects' perception of nicotine were found only with regard to S(-)nicotine, the main isomere in cigarette smoke. Whereas both groups reported the R(+)isomere to cause an unpleasant sensation, the S(-)isomere was perceived as pleasant by smokers but not by non-smokers. The differences in the hedonic ratings of S(-)nicotine between smokers and non-smokers might be due to the smokers' experience of the pharmacological action of S(-)nicotine.

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Physico-chemical Basis for the Production of Nasal Pungency in Humans by Non-reactive Chemicals.

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In a previous study (Physiol. Behav. 48(5) 719-725, 1990), we measured nasal detection thresholds for a homologous series of alcohols - from methanol to 1-octanol - in normals and anosmic persons. All anosmics reliably detected the alcohols via nasal pungency, albeit at much higher concentrations than the normals. We have extended our investigation to include a homologous series of esters from methyl acetate to octyl acetate, as well as decyl and dodecyl acetate. All anosmics reliably detected the series up to heptyl acetate. For the less volatile stimuli - octyl, decyl and dodecyl acetate - at least one anosmic failed to detect them reliably. Results from the new series closely resemble the previous ones. In both series: a) Odor and pungency thresholds decline with carbon chain length. b) When expressed as percent of saturated vapor - an index of thermodynamic activity - the span of thresholds across the series is drastically reduced for both odor and pungency. c) Pungency thresholds - expressed as thermodynamic activity - are strikingly constant across the series. When pungency thresholds for acetates and alcohols are plotted against saturated vapor (at room temperature) of each chemical, a single function described the data. Its slope implied that threshold pungency is evoked at a fixed saturated vapor percentage, regardles of the size or chemical functional group of the stimulating molecule. This suggests that nasal pungency elicited by nonreactive substances arises from a nonspecific, physical - rather than chemical - interaction between the stimuli and susceptible mucosal target sites within a lipophilic biophase.

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Effects of Adaptation on Perception of Similar and Dissimilar Odors*

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Adaptation among several odors was studied using a multiple-alternative forced-choice procedure where six concentrations of each odor and three blanks were presented in a random sequence before and following adaptation to each of the odors (one per session). Self-adaptation for each odor was demonstrated by a significant increase in identification threshold and a significant decrease in perceived suprathreshold intensities. In the first experiment, Galaxolide™ (a synthetic isochroman musk) and 5α-androst-16-en-3-one (androstenone) showed no cross-adaptation in spite of sharing, for some people, a musky note. In the second experiment, Galaxolide and Thibetolide™ (a synthetic macrocyclic musk) showed significant cross-adaptation, but the extent of cross-adaptation was asymmetric. When individuals were adapted to Galaxolide, identification threshold shifts and magnitude estimates for Thibetolide were not significantly different from those obtained for Galaxolide, suggesting that cross-adaptation was complete. When individuals were adapted to Thibetolide, identification threshold shifts and magnitude estimates for Galaxolide were significantly less than those obtained for Thibetolide, suggesting that cross-adaptation was incomplete. These data suggest that Galaxolide possesses a perceptual attribute that is not present in Thibetolide. This quality must be quite subtle since subjects did not distinguish between the two musks during random presentations. The odors of amyl acetate (banana) and *d*-limonene (orange) showed no cross-adaptation in either experiment.

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Mutual Cross-Adaptation of the Volatile Steroid Androstenone and a Non-steroid Functional Analog.

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Stimulation of similar olfactory receptors is believed to underlie the phenomena of iso- and cross-adaptation. In the present work, iso- and cross-adaptation were studied for 5α-androst-16-en-3-one (androstenone) and a synthetic functional analog, 4(4',4'-dimethyl-cyclohexyl)-2(R)-methylcyclohexanone. In Experiment 1, eight subjects received four randomized sequences of six concentrations of three odors (androstenone, analog, and amyl acetate; plus blanks) before and following adaptation to each of these odors in three test sessions (one adapting odor per session). Each odor produced strong iso-adaptation. Magnitude estimates and identification thresholds revealed clear reciprocal cross-adaptation between androstenone and the analog, but no cross-adaptation was observed for amyl acetate. The analog produced more complete cross-adaptation, presumably due to intensity differences across equivalent concentration steps. In Experiment 2, we chose to study people who were very sensitive to androstenone. Six subjects were tested in two sessions using the paradigm and odors of Experiment 1. An additional odor, Galaxolide™, a synthetic musk, was also included. Androstenone and the analog were used as adapting stimuli and each produced clear iso-adaptation. Cross-adaptation was demonstrated for virtually all test concentrations of analog and androstenone, but no cross-adaptation was noted for either amyl acetate or Galaxolide. These results demonstrate cross-adaptation for qualitatively similar odors, but not for dissimilar ones. They further suggest that androstenone and its analog share similar receptors.

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The Nasal Cycle: Relationship to Ultradian Rhythms and Unilateral Olfactory Thresholds.

RICHARD E. FRYE (Sensonics, Inc., Haddonfield, NJ) and RICHARD L. DOTY (Smell and Taste Center and Department of Otorhinolaryngology and Human Communication, University of Pennsylvania).

The nasal cycle is an ultradian side-to-side rhythm of nasal engorgement directly associated with cyclic autonomic activity. We have conducted three studies which suggest that the neurological oscillator which drives the nasal cycle may be associated with Kleitman's Basic Rest-Activity Cycle, as indexed by rhythms of verbal/spatial performance and sleep stage. In the first study, we demonstrate that nasal cycle phase can be predicted from parameters of sleep stage and body position in sleeping subjects. In the second study, we demonstrate that eating a meal influences the laterality of nasal resistance and verbal/spatial performance, presumably through systemic autonomic mechanisms. Finally, we present evidence linking the phase of the nasal cycle to coordinated changes in unilateral performance on an olfactory threshold test such that lower unilateral thresholds are present when airflow is proportionally greater through the right nasal chamber and higher thresholds are present when airflow is proportionally greater through the left nasal chamber. These results indicate that autonomic rhythms influence threshold values and contribute to their variability.

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Asymmetric Olfactory Function: Relationship to Handedness, Gender, and Nasal Resistance. RICHARD L. DOTY, RICHARD E. FRYE and PAUL SHAMAN (Smell and Taste Center, Department of Otorhinolaryngology and Human Communication, University of Pennsylvania, Philadelphia).

Using air-dilution olfactometry, 2-butanone odor detection thresholds were obtained in 16 right handed men, 21 right handed women, 17 left handed men, and 21 left handed women for each side of the nose in two test sessions. Nasal resistance was established before and after each olfactory measurement using anterior rhinomanometry. Testing was performed in 36 subjects with the contralateral nostril blocked, and 39 with it open. Both right and left unilateral thresholds were influenced by gender, the degree of handedness, and whether the contralateral nostril was blocked. A comparison between the thresholds of the left and right sides of the nose indicated that the left side of the nose was significantly more sensitive. The degree of olfactory asymmetry was influenced by the gender and the degree of handedness of the subject, but not by whether the opposite nostril was blocked during testing. Although airflow was significantly related to right and left thresholds and the right:left threshold asymmetry, removal of this covariate from the analysis did not influence the relationships of the main effects. This study suggests that a right:left asymmetry in olfactory thresholds exist and that this asymmetry is influenced by the gender and handedness of the subject.

This research was supported by Grant DC00161 from the National Institute of Deafness and Other Communication Disorders.

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Distribution of Immunoreactive Luteinizing Hormone-Releasing Hormone in the Nasal Epithelium and Ventral Forebrain of Embryonic and Neonatal Prairie Voles.
CLARE E. STRITTMATTER, SCOTT A. BURCHETT, JOHN J. LEPRI (University of North Carolina at Greensboro) and CELESTE R. WIRSIG-WIECHMANN (Wake Forest University, Bowman Gray School of Medicine).

In house mice, the intracerebral neurons that secrete luteinizing hormone-releasing hormone (LHRH) and control reproduction in the adult appear to be derived from the olfactory placode of the fetus. These neurons are "born" in the nasal epithelium and then migrate along the path of the terminal nerve to the ventral forebrain. We report here the developmental sequence of LHRH-immunoreactive (LHRH-ir) cell-bodies and fibers in embryonic and neonatal prairie voles, *Microtus ochrogaster*. We are particularly interested in examining the relationships of the LHRH-ir neurons to the development of the vomeronasal system, since adult female voles rely on that system for reproductive activation. The temporal pattern of origination and migration of the these neurons in voles is very similar to house mice, i.e., the first LHRH-ir neurons were seen in the epithelium of the vomeronasal organ at about embryonic day 12. In later embryonic stages, these neurons migrated along the nasal septum, and appeared within the ventral forebrain within two or three days. By examining the development of the LHRH-ir neurons, we hope to gain additional insights on their functional relationship to the vomeronasal system and the control of reproduction.

This research was supported by the UNCG Research Council and the North Carolina Board of Science and Technology (JJL), and by NIH grant NS27856 (CRW).

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Early Vomeronasal Lesions Cause Severe Deficits in Male Hamster Mating Behavior; Relieved, in Part, by Intracerebral LHRH Peptides.
GWENDOLYN FERNANDEZ, GAY HOWARD and MICHAEL MEREDITH. (Dept. Biol. Sci., Florida State Univ., Tallahassee, FL.).

Vomeronasal organ (VNO) sensory input, in rodents of both sexes, is followed by the release of LHRH, which is facilitatory to mating behavior. Previous results (Meredith et al, AChemS 1989) suggest that exogenous intracerebral LHRH might "substitute" for VNO input in sexually inexperienced male hamsters with mating behavior deficits following VNO removal (VNX) at 3 mo. In the present experiments we used prepubertal VNO removal and explored other routes of peptide delivery. Male hamsters whose VNOs were removed at 17 days had a more severe and reliable deficit in mating behavior when tested as adults than inexperienced animals VNXed at 3 mo. However, a previous hypothesis, that prepubertal VNX exacerbates behavioral deficits by preventing effective experience with maternal chemosensory cues, was not supported. Animals that had been isolated from adult females at 17 days of age did not show similar deficits to 17 day-VNX animals even if their VNOs were removed before experience, at 3 mo. Behavioral restoration by intracerebral LHRH was confirmed in early-VNX animals tested at 3 mo. LHRH, saline or an LHRH analog was injected (2 ul) through a 33 ga cannula and stereotaxically-implanted 26 ga guide tube into the lateral ventricles of VNX or intact (CON) animals using 3 pressure pulses 30s apart. Mating behavior was tested at 30 and 120 min after injection, in clear plastic cages with a naturally cycling, behaviorally receptive female. Investigation time, mounts, thrusts and intromissions were recorded for 5 min or until the male achieved 5 intromissions. VNX animals showed a significant increase ($p<0.05$) in the average number of intromissions/min and of mounts with thrusts/min, at 30 min after injection, indicating that intracerebral LHRH at a dose of 50 ng restores some behavior lost with VNX. Larger doses injected either subcutaneously (500mg) or intranasally (10 ug) had no significant effect. Nal-Glu, a potent antagonist of LHRH release, presumably acting at the pituitary LHRH receptors, appeared to produce a significant ($p<0.05$) increase in performance of both VNX and CON animals, at 30 min after injection (25 ng) - suggesting, as in female rat experiments, that analogs effective in facilitating mating behavior may act via receptors other than the pituitary type. The increases in the VNX and CON groups were not significantly different.

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PURIFICATION AND CHARACTERIZATION OF A LOW MOLECULAR WEIGHT CHEMOATTRACTANT TO GARTER SNAKES. PING CHEN, DALTON WANG AND MIMI HALPERN (State University of New York, Health Science Center at Brooklyn).

Garter snakes respond with characteristic tongue flicking and attack to objects covered with surface washes or secretions from earthworms. This discriminated response is mediated by the vomeronasal system. Aqueous earthworm wash or electric shock-induced secretion contains a number of proteins which elicit garter snake attack. We have recently isolated a low molecular weight snake-attractive protein from earthworm wash in addition to the sulfhydryl-containing protein reported earlier (Wang, D., Chen, P. and Halpern, M. Arch. Biophys. Biochem. 267, 459-466, 1988). This protein has been purified to homogeneity and has a relative molecular mass of 3,000 daltons as determined by SDS polyacrylamide gel electrophoresis. It consists of a single polypeptide chain with a blocked amino acid terminal. The purified protein is rather acidic with a pI of 3.3. The amino acid composition has been determined. This protein possesses the unique property that it remains as monomers in solution at room temperature or at 4^0 C., and will form oligomers if it has gone through several cycles of freezing and thawing during storage or if it has been treated with methylamine. The oligomers are not reversed to monomers under the action of urea, SDS, or SDS and urea. The nature of the linkage is unknown but appears to be through covalent bonds other than the disulfide bonds.

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Neural Projections of the Vomeronasal Organ in Voles.

SUSAN E. WISE and JOHN J. LEPRI (Department of Biology, University of North Carolina at Greensboro)

The vomeronasal organ (VNO) is strongly implicated in the chemoreception of social odors related to reproduction. Neuroanatomical research on this chemosensory system is being conducted in an effort to determine how chemical signals received at the VNO can lead to changes in hormone secretion and sexual behavior. We are focusing our research on prairie voles, *Microtus ochrogaster*, having shown the dependence of females of this species on the VNO for male-induced reproductive activation. Prairie voles are similar to other rodents in that the VNO is located in the rostral end of the nasal cavity. Using the lipid-soluble carbocyanine dye DiI, we observed that the sensory neurons from the VNO coalesce to form the vomeronasal nerves, which project between the main olfactory bulbs and synapse onto secondary neurons in the accessory olfactory bulb (AOB). When the DiI was placed on just one side of the VNO, it clearly diffused to the ipsilateral AOB, but we also saw smaller amounts of the dye in the contralateral AOB in some animals. We are attempting to discern whether these results suggest contralateral projections of vomeronasal neurons. An alternative explanation is that we simply observed retrograde diffusion of DiI from the point of parallel projection of the right and left vomeronasal nerves between the main olfactory bulbs.

This research was supported by the UNCG Research Council and the North Carolina Board of Science and Technology (JJL).

Trigeminal Chemoreception in the Nasal Cavity of Voles.

W. BRANT NIX, JOHN J. LEPRI (University of North Carolina at Greensboro, Department of Biology) and WAYNE L. SILVER (Wake Forest University, Department of Biology).

Among mammals, trigeminal chemoreception in the nasal cavity has been empirically demonstrated only in a couple of domestic species of rodents. We have extended these studies to include prairie voles, *Microtus ochrogaster*. We placed electrodes on the ethmoid branch of the trigeminal nerve and recorded its electrophysiological responses to the presentation of odorants delivered to the nasal cavity of anesthetized voles. The trigeminal responses of the voles were similar to those of Norway rats, i.e., cylcohexanone, propionic acid, and amyl acetate were effective stimuli. However, it appears that trigeminal thresholds of the voles to these stimuli were elevated compared to rats. We also observed that some stimuli caused a depression of ventilation in some animals, as seen in rats. We are currently examining the neural responses of voles to prolonged, "subthreshold" presentations of these stimuli.

This research was supported by the UNCG Research Council and the North Carolina Board of Science and Technology (JJL).

Neuropeptide and Glutamate Receptors Expressed by Trigeminal Sensory Neurons

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Trigeminal sensory neurons contain several neuropeptides and putative neurotransmitters including bombesin, calcitonin gene related peptide α & β, cholecystokinin, galanin, glutamate, neurokinin A, somatostatin, substance P and vasoactive intestinal polypeptide. Receptor autoradiography using radiolabeled ligands and in situ hybridization using radiolabeled 45 base pair oligonucleotide synthetic DNA probes were used to determine the types of receptors and mRNA coding for receptors that were expressed by trigeminal sensory neurons in several mammals including rat, rabbit, monkey and human. In all species examined cholecystokinin receptors were expressed by trigeminal sensory neurons, although both the pharmacology of the receptor expressed and the percentage of neurons expressing cholecystokinin receptors showed marked variation between the species examined. Of the other neuropeptides examined only galanin was found to be expressed by trigeminal sensory neurons and only in the rabbit. Glutamate receptors were abundantly expressed by trigeminal sensory neurons, in particular the family of AMPA-selective glutamate receptors.

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Do Fish Sniff? A Novel Mechanism of Olfactory Sampling in Fishes.

GABRIELLE NEVITT (Department of Neurobiology and Behavior, Cornell University).

Although olfaction is known to be a highly developed sensory modality in a variety of fishes, little information is currently available about behavioral mechanisms by which fishes sample the olfactory environment. The following study was therefore undertaken to asses the role of spontaneous jaw protrusion ("coughing") as a potential mechanism used by pleuronectid flounders to sample odorants.

Investigations were carried out using a combination of physiological, morphological and behavioral techniques. Physiological results show that: 1) typical respirations are coupled to measurable olfactory sac pressure fluctuations and that 2) during a coughing event, water is rapidly sucked into the olfactory sac. Morphological results indicate: 3) a direct linkage system between the protrusion apparatus and the olfactory or associated accessory sacs, and that 4) coughing is associated with a rapid expansion or stretching of these sacs. Lastly, behavioral studies demonstrate that: 5) coughing rates increase significantly over background activity when flounders are presented with attractive food odorants. From these results, I propose that coughing in pleuronectid flounders represents a behavior truly analogous to sniffing in certain air breathing organisms.

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Behavioral Discrimination of Binary Mixtures and Their Components: Effects of Mixture Interactions on Quality Coding. JACQUELINE FINE-LEVY AND CHARLES DERBY (Department of Biology, Georgia State University, Atlanta, GA)

We are interested in the way that the qualities of odorant stimuli are encoded and interpreted by the nervous system. There is mounting theoretical and physiological evidence that mixture interactions can act to alter the "predicted" physiological response to a mixture of components whose individual stimulatory capacities are known. This may ultimately lead to dramatic differences between the perceived qualities of a mixture and its components that would be expressed at the behavioral level. Toward examining this question, we have tested the ability of spiny lobsters to behaviorally discriminate between three odorant compounds (AMP, glutamate (glu), and taurine (tau)) and their binary mixtures through the use of a differential aversive associative conditioning paradigm. Six groups of lobsters were used, each being conditioned to avoid one of the single compounds or binary mixtures. Based on the results of analysis of variance and multidimensional scaling analysis incorporating observed responses to all stimuli and "predicted" responses to the binary mixtures, the following relationships between each binary mixture and its constituent components were determined: The mixture of AMP+glu was found to be different from either component, as well as the predicted value for this mixture. The mixture of AMP+tau was found to be intermediate between both components, and was similar to the predicted value. The mixture of glu+tau was found to be more similar to glu than to tau, and there was some indication that the glu was acting to suppress the response to tau. These behavioral results, especially for AMP+glu and glu+tau, are consistent with results of our electrophysiological analysis of the effects of mixture interactions on coding of stimulus quality and intensity by olfactory receptor cells. This provides further evidence for the effects of peripherally initiated mixture interactions on the coding and perception of the quality of odorant mixtures.

This work was supported by NIDCD Grants DC00312 and DC0002.

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Spatial information contained in the three-dimensional fine structure of an aquatic odor plume.
PAUL A. MOORE, NAT SCHOLZ*, JELLE ATEMA*, and GREG A. GERHARDT. *Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543, Departments of Psychiatry and Pharmacology, University of Colorado Health Science Center, Denver CO 80262

Turbulent odor plumes play an important role in many chemically-mediated behaviors, yet the fine scale spatial structure of plumes has not been measured in detail. With the use of a newly introduced microelectrochemical recording technique, we have measured in some detail the fine structure of an aquatic odor plume in the laboratory. We sampled a turbulent odor plume at 10 Hz with a spatial sampling area of .02 mm^2, approximately that of a chemoreceptor sensillum of the lobster, Homarus americanus. A three minute record was sampled at 63 different sites in three dimensions (x, y, z). As expected from time averaging models the mean values of pulse parameters such as height and onset slope were greatest near the source. However, what cannot be described by time averaging models is the instantaneous distribution of pulses: periodically high peaks and with steep concentration slopes (well above the local average and far above predictions from averaging models) can be found far away from the source. In addition, the probability of above average pulse heights decreases with distance from the source in x, y, and z directions. The most intense odor fluctuations occurred along the x-axis (the cross-sectional center of the plume). Odor profiles were analyzed with three different models of sensory filters; logarithmic, probability, and temporal filters. This analysis indicates that features contained within the plume structure could be used as directional cues for orienting animals. It remains to be demonstrated that animals use such sensory filters to extract biologically relevant spatial information from odor plumes.

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Effect of Flow Velocity on Chemical Signal Dispersal and Hermit Crab Orientation.
NAT L. SCHOLZ and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Marine Bioogical Laboratory, Woods Hole, MA 02543).

This study used the hermit crab *Pagurus longicarpus* as a model to examine the quantitative relationship between spatiotemporal patterns of odor distribution and chemically-mediated behavioral response. Experiments were conducted in a laboratory flow-through flume (75 x 120 cm) modelled after a saltmarsh tidal creek, with flows (1, 2, and 3 cm s^{-1}) chosen to emulate the changing velocities encountered during a tidal cycle. Homogenized mussel (*Mytilus edulis*) extract was used as a stimulus and constantly released at 50 ml min^{-1} from a pipette. For a successful response, a crab had to come within 2 cm of the pipette from a starting point 90 cm distant. Using this criterion, we obtained 85-95% response frequencies for 70 crabs at each flow velocity. Control runs substituted raw seawater for mussel extract; no successful responses were recorded, regardless of flow velocity. Further, crabs were not able to locate a chemical source in the absence of flow. Orientation paths were digitized with a computer frame-grabber and analyzed in terms of initial directional choice, path linearity, path distance, and search time. All four parameters indicated that crabs were more efficient at localizing the source of the turbulent odor plume at higher flow velocities. Temporal and spatial odor profiles were taken with electrochemical electrodes sensitive to dopamine (Moore *et al.*, 1989, *Chem. Senses* 14(6):62-74). Recordings were made in the center axis of the plume at 15, 45, and 75 cm from the pipette tip. Results indicate that two odor peak parameters, maximum rise values (peak slopes) and absolute concentrations (peak heights) decrease with 1) distance downstream from the source, and 2) decreasing flow velocities. Crabs may rely on mechanical input to direct upstream movement ("rheotaxis") while monitoring spatiotemporal changes in the local odor signal to direct lateral movements within the odor plume ("chemotaxis"). At higher flow velocities, more signal is available to both modalities. This may explain why resulting orientation is more precise at higher flow rates.

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Avian repellents: towards an understanding of taxonomic, class-specific repellency. PANKAJ S. SHAH (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104). J. RUSSELL MASON (Denver Wildlife Research Center, USDA/APHIS/S&T). LARRY CLARK (Denver Wildlife Research Center, USDA/APHIS/S&T).

Methyl anthranilate (MA) is an ester derivative of anthranilic acid that is repellent to birds but palatable to mammals. Ortho-aminoacetophenone (OAP) has an odor similar to that of MA, is structurally similar and is also repellent to birds. Because so little is known about avian chemosenses in general, we set out to empirically construct a hypothesis about the nature of chemical repellency for birds. We selected isomers of acetophenone, benzoic acid moieties, and hetero cyclic ring structures of benzene to determine the correlation with repellency in drinking and feeding trials. Empirically, we found all the isomers of aminoacetophenone to be highly repellent to birds. Methoxyacetophenones and the ortho isomer of hydroxyacetophenone were moderately repellent. The more acidic anthranilic acid (aminobenzoic acid) was also moderately repellent. On the strength of these findings we hypothesized that resonance of lone electron pairs, basicity, planarity of the pi cloud and electron richness of the phenyl ring contributed to repellency. This interpretation was supported when we tested heterocyclic atoms. These findings form the basis of planned experiments to determine important structural, electron and configurational features associated with inter-class differences in repellency.

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Odors Mediate Social Affiliation And Sexual Receptivity But Not Maternal Behavior In Female Prairie Voles.
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Female prairie voles are spontaneous ovulators and become sexually receptive after 1-2 days of exposure to a male, are spontaneously maternal and, of particular interest to our investigations, show strong affiliative behaviors toward familiar males. The role of olfaction in these behaviors was examined in sham lesioned controls (n=10), unilateral bulbectomized (n=11) and bilateral bulbectomized (n=13) naive adult female prairie voles. Behavior was assessed using observation and video recordings of social interactions.

Sexual Receptivity
Ninety percent of the shams (9/10), 82 percent (9/11) of the unilateral and 31 percent (4/13) of the bilateral bulbectomized females mated after 48 hrs of exposure to a male ($p<0.005$).

Maternal Behavior
All voles showed normal maternal behavior toward 1-3 day old foster pups and differences among groups were not significant for retrieval, grooming, or crouching over pups.

Affiliative Behavior
All sham controls showed a strong preference for the familiar male in a choice test. In contrast, bilaterally bulbectomized voles spent more time alone and, while they investigated both males, showed no preference for the familiar male. Interestingly, the unilaterally bulbectomized females also did not exhibit a preference for the familiar male but, like controls, spent little time alone.

In summary, olfactory bulbectomy in female voles dramatically alters social affiliation (a behavior not assessed in prior studies of anosmia and social behavior of mice and hamsters), reduces, but does not eliminate mating behavior and, in contrast to prior reports with mice, has no discernible effect on maternal behavior.

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Effects of Chemosensory and Physical Stimuli on Oxygen Consumption during Reproductive Activation in Female Voles.
RHONDA R. GARDNER and JOHN J. LEPRI (Department of Biology, University of North Carolina at Greensboro).

Female prairie voles (*Microtus ochrogaster*) do not express spontaneous cycles of follicular maturation and ovulation; rather, contact with males and/or their odors "activates" the female's reproductive system. This mechanism of reproductive activation may help to conserve metabolic energy until females gain the resources and experience needed for successful reproduction. To address this issue, we measured oxygen consumption in adult female voles before and after activation by chemosensory and/or physical contact with males. Oxygen consumption was measured in animals at approximately 29.7°C. The four treatment groups were: physical contact with a male followed by exposure to his odors (soiled bedding); contact with a male followed by exposure to unsoiled bedding; no contact with a male followed by exposure to male-soiled bedding; and a control group with no exposure to males. Preliminary data suggest that exposure to males increases oxygen consumption, but it remains unclear whether chemosensory stimulation can, by itself, lead to an increase in metabolic rate.

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Chemical Characterization of MHC-Determined Body Odors.
HIRONORI TSUCHIYA, KUNIO YAMAZAKI, GARY K. BEAUCHAMP, and ALAN G. SINGER (Monell Chemical Senses Center, Phila., PA)

Previously we demonstrated that the extremely polymorphic Major Histocompatibility Complex of genes (MHC), critical in immune function, also provides each mouse with a characteristic odor called an odortype. Perception of MHC-determined odortypes influences reproductive behavior in the contexts of mate choice and maintenance of early pregnancy, tending to favour the propagation of one MHC type over another.

With the goal of characterizing the chemical signals in mouse urine responsible for the olfactory discrimination of MHC type, we have used a variety of liquid phase separation techniques and evaluated fractions with trained mice in the Y-maze bioassay. The results indicate that proteins are unnecessary for discrimination, that the active compounds are not extracted by low polarity solvents such as ether, and that they are soluble in water or ethanol. By sequential high performance liquid chromatography (HPLC) under four different conditions, evidence has also been found that very reliable discriminations can occur with several different and distinct fractions consisting of only a few compounds. It is tentatively concluded that there are multiple and redundant simple olfactory cues signalling MHC type in mouse urine.

Supported by NIH grant #GMCA-32096 and Richard Lounsbery Foundation

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Development of Olfactory Exploration in Hamsters Involves "Stretching" but Not Breaking the Nest Tether.
THOMAS A. SCHOENFELD AND AMY FAFARD (Clark University, Worcester, MA 01610 USA)

Individual golden hamster pups ranging from 3 to 18 days of age huddle readily with a warm, furry surrogate conspecific covered with home nest shavings. However, pups younger than 12 days old huddle within 1-2 minutes after placement in the test box, whereas pups at 12 days and older spend 5 or more minutes exploring the rest of the test box, filled with fresh pine shavings, before huddling. Exploration by the older pups habituates with repeated exposure to the pine shavings and dishabituates upon exposure to lemon-scented pine shavings. These data extend previous observations with litters of 12-18 day old pups that locomotor exploration of novel odors ends reliably in group huddling (Schoenfeld and Corwin, 1985). Thus, nascent exploration of novel, non-nest odors, first appearing in hamsters before the eyes and ears are open, adds to and competes with but does not replace responsiveness to and even preference for nest-associated olfactory and thermotactile stimuli.

Supported by the University College Summer Research Apprenticeship Program at Clark.

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Sources of individual odors in golden hamsters
ROBERT E. JOHNSTON, Cornell University

How do individuals recognize other individuals by scent? Are some sources of scent specialized so as to be individually distinctive or are all scents individually distinctive? This question has apparently never been systematically investigated for any mammalian species. Either hypothesis could be true - any secretion or excretion could differ in components or ratios of components across individuals and thus have a distinctive odor quality or it could be that only certain scents vary significantly in their components across individuals due to selection for such distinctive individual differences. Using habituation techniques we investigated a number of possible sources of individual odors in golden hamsters, including flank glands, urine, feces, ear glands, vaginal secretions, foot-pad secretions, and saliva. Each scent was tested in at least two habituation-like experiments, using 10 hamsters as subjects in each experiment. The two experiments differed in using either 15 minute or 24 hour intervals between trials, thus providing information on memory for individual odors as well as ability to distinguish them. The results indicated that some scents are probably not used for individual discrimination (saliva, secretions from the feet) but that most scents may be (urine, ear glands, feces, vaginal secretions and flank glands). There were differences in the magnitude and robustness of the effects among the later group of scents, however, indicating that some scents may have especially salient individual differences. All of these scents were discriminated in both experiments , that is those with 15 minute and 24 hour intervals between trials, suggesting that memory for the individual characteristics of the different scents was equally good. The meaning and interpretation of these and other habituation-like experiments used in the individual discrimination/recognition literature will be discussed.

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Effects of Nickel Sulfate Hexahydrate on Tests of Olfactory Function in Rats.
JAMES EVANS (University of Cincinnati) and
LLOYD HASTINGS (University of Cincinnati)

Clinical reports suggest a relationship between loss of olfactory function and occupational exposure to nickel compounds. In addition, inorganic, sulfated nickel compounds (Ni^{2+}) have been shown to produce histological lesions in the nasal mucosa of rats and, more specifically, atrophy of the olfactory epithelium. Although these lesions reportedly lead to the formation of carcinomas, they could produce deficits in olfactory function prior to the onset of tumor formation, as is seen after exposure to compounds producing similar lesions. To investigate this, a project was undertaken to determine the effects of inhalation of nickel sulfate hexahydrate on the rat olfactory system. Function was assessed utilizing behavioral measures of odor detection threshold and odor discrimination. Rats previously trained to perform a go-no-go discrimination paradigm were exposed to either 0, 100 or 500 $\mu g\ Ni/m^3$, 5hrs/day for 12 consecutive days. Throughout the exposure period, subjects were tested on their ability to discriminate between two suprathreshold odorants, ethyl acetate and amyl acetate, and between varying concentrations of a single odorant, ethyl acetate. In addition to exposure related effects, tests subjects were also monitored for 24 days after exposure ceased to determine the capacity of the system to recover from the insult.

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Experience Alters The Neurobehavioral Responses Of Rat Pups To Different Odor Concentrations
OSNAT CARMI (University of California, Irvine)
MICHAEL LEON (University of California, Irvine)

Neonatal rats acquire an olfactory preference following daily pairing of an odor with simultaneous tactile stimulation (Coopersmith and Leon, 1984). This odor preference is associated with physiological, anatomical and neurochemical changes in the olfactory bulb (Coopersmith and Leon,1984; Wilson and Leon 1988, and Woo and Leon, 1987). Previous studies have shown that on PND 19 there are focal regions of high 2-DG uptake in the glomerular layer along the lateral aspect of the olfactory bulb of rat pups which were trained with peppermint for 10 min/day on PND 1-18. Since previous studies of this phenomenon used only one concentration of peppermint (1:10 of a saturated vapor), we determined the neural and behavioral responses of pups trained and tested with different concentrations of an odor. We trained and tested pups with a concentration of 1:10 (high) or 1:1000 (low). During PND 1-18, all animals were exposed for 10 min/day to either peppermint (1:10 or 1:1000) or air while receiving perineal stimulation. On PND 19, half of the pups from each litter were used for behavioral preference testing in a Y-maze, given a choice between the high or low concentration of peppermint. To determine the neural responses under the different training conditions, 2-DG testing was done as previously described (Coopersmith and Leon, 1984), with the remaining pups. Animals trained with the low concentration of peppermint exhibited a preference for the low over the high concentration ($p<0.05$). This suggests that these pups *can* recognize and prefer a particular odor concentration. However, rat pups that were trained at high concentration of peppermint showed an equal preference for the two concentrations. Rat pups trained at the high peppermint concentration preferred peppermint over air, regardless of the odor concentration (Carmi and Leon, 1990). Training with the low concentration of peppermint does not increase the preference for the high odor concentration. Examination of the 2-DG autoradiographs revealed that animals receiving similar training conditions exhibited similar neural activity regardless of the odor concentration during testing. The 2-DG response, therefore, appears to be more sensitive to previous experience than to the immediate odor stimulus characteristics.

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Use of Li and Mutants to Classify Chemical Stimuli. J. L. VAN HOUTEN, M. FRANTZ, M. V. WRIGHT, (University of Vermont, Dept. Zoology, Burlington, VT 05405)

The chemosensory transduction pathway of *Paramecium* begins with stimulus binding to receptor and continues with a change in Vm that affects ciliary beating and results in attraction or repulsion by indirect kinesis mechanisms. The change in Vm is independent of resting potential, external K, and external Na, and there is indirect evidence that, for some stimuli, an increase in Ca pump activity creates the hyperpolarizing current. LiCl displaces internal K , inhibits Ca efflux from whole cells and inhibits the Ca-ATPase activity of cell surface membranes. LiCl also profoundly affects chemoresponse to folate, acetate, lactate and cAMP but not to NH_4Cl. A mutant defective in Ca homeostasis, likewise, is unaffected in its attraction to NH_4Cl but does not respond to other attractants. These and other studies imply that attractants such as acetate, folate, lactate and cAMP cause hyperpolarizations through modulation of the Ca-ATPase and that stimuli NH_4^+ and probably OH^- hyperpolarize by a different mechanism, possibly an alteration in internal pH.

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Pharmacodynamics and Kinetics of Propanol Actions on Fly Receptor Cell Responses to Sucrose. MATTHEW E. ROGERS and LINDA M. KENNEDY (Department of Biology & Neuroscience program, Clark University, Worcester MA 01610).

The 'taste modifiers', hodulcin, gymnemic acids, and ziziphins, selectively suppress sweetness perception in humans and behavioral and receptor cell responses to sucrose in flies. Hodulcin and gymnemic acids show a pattern of kinetics that is characteristic of action on a later step in a sequential-step transduction process and involves a necessary, but not necessarily sufficient physicochemical mechanism (Kolodny & Kennedy, Chem. Senses 15, 1990, 602; de los Santos et al., Chem. Senses 15, 1990, 565). The modifiers may penetrate into the receptor cell membrane and affect a membrane molecule involved in a transduction cascade (Kennedy, Chem. Senses 16 (1), 1991). It is well known that alcohols affect neuronal membranes by physicochemical and penetration mechanisms. Therefore, to better understand the role of physical chemistry in the modifier suppression, we have been conducting comparative studies of the effects of normal alcohols on fly (Phormia regina) receptor cell responses to taste stimuli. The alcohols produce biphasic effects similar to those of the modifiers (firing is first suppressed and then increased and irregular), and physicochemical mechanisms play a role (Kennedy. Olfaction and Taste IX, 1987, 409). Moreover, the physical chemistry of propanol actions are different for responses to sucrose or NaCl (Bourassa & Kennedy, Chem. Senses 13, 1988, 703). However, while the concentration-effect curves for the modifiers are bell-shaped, with peak effects of 29 - 58% suppression (Kennedy & Halpern, AChemS II, 1980, 24; Kolodny & Kennedy, 1990), the effect after propanol treatment increases from no suppression at 0.012 - 0.024M, to 47% suppression at 1.2M, 75% suppression at 2.4M, and 100% at 4.8 and 9.6M ($p<0.0001$, Two-Way ANOVA). Currently, we are conducting Michaelis-Menten kinetic studies with propanol. Preliminary results show mixed kinetics (decreased V_{max}, increased K_m) similar to the mixed kinetics found at low concentrations of hodulcin and the gymnemic acids.

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Electrophysiological Evidence for Synaptic Interaction in Single Peg Sensilla of Scorpion Pectines. D.D. GAFFIN, P.H. BROWNELL (Oregon State University, Corvallis, OR 97331) and J. GÖDDE (University of Regensburg, D-8400 Regensburg, Germany).

The pectines of scorpions, two large mid-ventral appendages unique to this arachnid group, may be a particularly useful system for studies of chemosensory synaptic integration. Morphological studies of the pectines reveal what appear to be multiple synaptic contacts between axons of sensory neurons within each of the numerous, short ($<5\mu$) sensilla called "pegs". In a previous report we used extracellular recordings from individual pegs to show these sensilla are primarily chemosensory. We now report physiological evidence of synaptic interactions between sensillar neurons. Long term extracellular recordings from individual peg sensilla of scorpions (3 species, 2 families) were examined with regard to interactions between spiking units. Individual units were segregated on the basis of spike shape and firing frequency by a computer algorithm. The proximate temporal environment (200 msec before and after firing) for a given category of spike was then analyzed with respect to activity of other identifiable units. In records containing clearly discernable spike types the activity of larger biphasic spikes was inhibited during the 15 msec period following the spontaneous firing of a small monophasic spike. The patterns of activity for larger spikes showed little evidence of interaction although there was some indication that larger units may briefly facilitate the firing of smaller units. In previous studies we have shown that small units are differentially sensitive to some chemical stimuli. Thus, it appears that chemosensory information from the pectines of scorpions is processed within single sensilla prior to its relay to the CNS.

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Changes in Salivary Protein Composition of Isoproterenol Treated Mice. JOHN L. BEIDLER (Florida State University, Tallahassee, Florida 32306)

Studies By Whitney and others have demonstrated in mice a genetic relationship between the genes coding for the salivary proline-rich proteins (PRP) and the ability to taste sucrose octaacetate. Glendinning and Soliwoda have recently shown that mice treated with the β-adrenergic agonist isoproterenol, which elevates the levels of PRP in mice, significantly reduces tannic acid sensitivity. Utilizing ELISA techniques, we followed the levels of the salivary proteins amylase (amy), somatostatin (som), PRP, lipase (lip), NGF, EGF, and kallikrein (kal), along with the total protein concentrations of the parotid, submandibular and von Ebner's gland saliva of mice during thirty days of IPR stimulation. All proteins except the PRP's dropped to residual levels within three days of IPR stimulation. PRP levels increased five-fold in ten days, followed by a slight drop (10%) during the final twenty days in the parotid gland secretions. A four-fold and two-fold increase in PRP levels were observed in the submandibular and von Ebner gland secretions, respectively. Change in total protein concentrations were 6-fold, 4-fold, and 0.75-fold in the parotid, submandibular, and von Ebner secretions, respectively. Thus, repeated exposure to isoproterenol creates a dramatic change in the protein composition of salivary secretions, the most pronounced being the large increase in the levels of PRP's secreted.

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Analysis of Human Von Ebner's Saliva.
A.I. SPIELMAN, S. D'ABUNDO, H.C. LIN, F. CHUIERI, G. TURNER, (New York Univ. Coll. of Dentistry) and **H. SCHMALE** (Univ. Hamburg, German Fed. Rep.)

The lingual serous glands of von Ebner are located in close proximity to the foliate and circumvallate papillae. Saliva secreted by these glands provide the immediate environment to taste buds and have been hypothesized to modulate taste perception (Gurkan and Bradley, *Chem. Senses* 13:655, 1988). The purpose of this study was to develop a technique to collect resting and stimulated saliva from human von Ebner's glands in an attempt to identify the factor(s) responsible for such modulatory activity. Saliva was collected under resting and under various gustatory stimuli (sweet, sour, salt, and bitter) by insertion of perio-strips into the foliate papillae. Stimulated saliva was also collected using Drummond glass microcapillaries. The collected volumes of saliva were determined using a Periotron^R. The flow-rate of resting von Ebner saliva was 2.3 ± 0.6 μl/min, and 4.5 ± 1.2 μl/min under 1% citric acid stimulation. The protein content was 2.5 ± 0.5 mg/ml. The SDS gel electrophoretic profile showed that although von Ebner saliva shares a number of bands with whole saliva it also contains a set of unique proteins that include two VEG proteins (Schmale *et al.*, Nature, 343:366, 1990) as demonstrated by western blot. No qualitative differences in protein expression were observed when different gustatory stimuli were applied. **This collection technique should prove useful in analysis of salivary factor(s) associated with taste.**

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Stereospecific Activation of Stimulus-Gated Cation Conductances in Isolated Catfish Taste Epithelial Membranes. TAKASHI KUMAZAWA (Monell Chemical Senses Center), JOHN H. TEETER (Monell Chemical Senses Center and Univ. of PA) and JOSEPH G. BRAND (Monell Chemical Senses Center, Univ. of PA, and Veterans Affairs Medical Center, Philadelphia, PA 19104).

The taste system of the channel catfish, Ictalurus punctatus, is highly sensitive to the L-isomers of alanine and arginine, which interact with independent high affinity receptor sites. In addition, low affinity receptor sites for L-proline have been inferred from neural recordings (Wegert and Caprio, AChemS 1988; Kohbara et al., 1990). Current evidence indicates that relatively nonselective L-Ala receptors are coupled via a G-protein to the formation of both cAMP and IP3. We have recently shown that L-Arg and L-Pro selectively and directly activate independent cation channels in catfish taste epithelial membranes reconstituted in lipid bilayers suggesting the existence of receptor-channel complexes for each stimulus (Teeter et al., 1990; Kumazawa et al., AChemS 1990). The L-arginine-gated conductance activated near 0.5 μM and saturated between 100 and 200 μM, with half-maximal increase in conductance at 15 μM. The L-proline-gated conductance activated at about 100 μM, saturated between 2 and 4 mM, with half-maximal conductance at 450 μM. D-Arg, which had no effect on bilayer conductance at concentrations up to 200 μM, suppressed the increase in conductance produced by 100 μM L-Arg at relatively low concentrations (IC_{50} = 6 μM D-Arg). Similarly, D-Pro did not elicit an increase in bilayer conductance, but blocked the conductance produced by 1 mM L-Pro (IC_{50} = 150 μM). Neither L- nor D-Arg had an effect on the L-proline-gated conductance. Conversely, L-Pro had no effect an the L-arginine-activated conductance. The channels associated with the L-Arg and L-Pro receptors displayed similar ion selectivities and unitary conductances (45 pS for L-Arg and 49 pS for L-Pro). Although L-arginine-activated channels were usually observed in clusters of 5-20, individual L-proline-gated channels were frequently observed. These results indicate that independent receptors for L-Arg and L-Pro in the catfish taste system are part of or coupled to nonselective cation channels which open for significant periods of time only in the presence of their respective ligands. The D-isomers of both amino acids appear to compete for the L-isomer binding sites, but fail to trigger opening of the associated channels.

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Patch clamp recordings from cells in intact taste buds in thin lingual slices. ALBERTINO BIGIANI (Colorado State University and the Rocky Mt. Taste and Smell Center) and STEPHEN ROPER (*ibid.*).

Patch-clamp recording on isolated cells has proven to be a powerful technique for investigating the microphysiology of taste receptor cells. For example, several laboratories have now collected details about voltage-gated ion conductances and taste transduction mechanisms using this methodology. We have perfected a variation on these techniques by applying **patch pipettes** onto **intact** taste cells in thin (100-200 μm) **slices of lingual epithelium** from *Necturus maculosus*. The important advantages include: (1) interrelationships among cells within the taste bud are preserved, and (2) tissue is never exposed to enzymes or other treatments that might affect chemosensory transduction.

Daston, *et al.* (Chem. Senses **13**: 682, 1988) introduced the lingual slice technique. We have extended their approach by removing the lingual epithelium along with a thin layer of lamina propria from the tongue with blunt dissecion, fixing this tissue to a block of firm mounting medium (carrot slice) with cyanoacrylic glue, and cutting sections with a vibrating razor blade (ladies Remington electric shaver). In fortuitous sections, entire taste buds and individual cells can be clearly seen under the microscope at 160-400X magnification. We applied patch pipettes to taste cells that are exposed at the cut surface or to cells embedded deeper within the slice. We included Lucifer Yellow fluorescent dye in the patch pipette to identify cells after the recording.

To date, we have been able to record large inward and outward currents in response to step depolarizing pulses, confirming data from isolated taste cells. In contrast to data from isolated cells, however, in the slice preparation we have observed deflections in the current traces that appear to be activation of adjacent cells via **electrical synapses**. We are correlating these unique signals with **dye-coupling** between cells, utilizing Lucifer yellow as a tracer. If this correlation holds, it will allow us to study electrical coupling between cells in the intact taste bud and how it is affected, if at all, by taste stimulation, neuromodulators, or by other factors (e.g. ΔpH, ΔV_m, $\Delta[Ca^{++}]_i$ etc.).

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Metabolism of Inositol-1,4,5-Trisphosphate in Catfish Gustatory Epithelium. TAUFIQUL HUQUE (Monell Chemical Senses Center, Philadelphia, PA), JOSEPH G. BRAND (Monell Center, VA Medical Center and Univ. of PA) and JOSEPH L. RABINOWITZ (VA Medical Center, Univ. of PA, Philadelphia, PA).

The metabolism of inositol-1,4,5-trisphosphate was studied in the taste organ (barbel) of the catfish, Ictalurus punctatus. Homogenates of epithelial barbel scrapings were incubated with 3H-IP3, whose dephosphorylation or phosphorylation was assayed under appropriate conditions by measuring the production of either 3H-IP2 (IP3-phosphatase) or 3H-IP4 (IP3-kinase). Both enzymes were predominantly cytosolic, magnesium-dependent and had pH maxima at 6.4. For IP3-phosphatase, Km = 6 μM and Vmax = 10.5 nmol/min/mg. For IP3-kinase, Km = 0.23 μM and Vmax = 0.05 nmol/min/mg. Neither enzyme was significantly affected by the presence of taste stimuli (amino acids), GTP[S], cAMP or phorbol esters. In the presence of physiological levels of free calcium, IP3-phosphatase was moderately activated while IP3-kinase was moderately inhibited. IP3-phosphatase was moderately activated by MnCl2, unaffected by various exogenously-added lipids and LiCl, and strongly inhibited by 2,3,-diphosphoglycerate, Na-pyrophosphate, CdCl2, HgCl2, CuCl2, FeCl3 and ZnSO4. IP3-phosphatase activity was rapid (first-order rate constant = 6.9/sec) and time-course studies revealed typical precursor-product kinetics, with IP3 being dephosphorylated to IP2, which in turn was degraded to IP1. This enzyme may participate in taste transduction since calculations, based on both rate constant data and the mass of IP3 in homogenates, indicate that it is capable of dephosphorylating basal levels of IP3 with a half-life of 100 milliseconds or less.

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Rat Taste Epithelial cDNA Library: Molecular Genetic Approach to Taste Transduction. P.M. HWANG AND S.H. SNYDER (Depts. of Neuroscience, Pharmacology and Molecular Sciences, Johns Hopkins Univ. Sch. of Med.).

The mammalian taste receptor cells utilize diverse signal transductory mechanisms for different taste modalities. These range from direct interaction with apical membrane channels by ionic tastants to the generation of intracellular second messengers by other types of tastants. Scarcity of taste receptor cells limits biochemical studies of taste signal transduction. One way to circumvent this difficulty is to use molecular genetic techniques. We have recently constructed a cDNA library from rat tongue epithelial tissue highly enriched in taste receptor cells. We will present the characterization of the cDNA library as well as the different methods we are employing to isolate molecules involved in taste receptor cell signal transduction.

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The Effect of Modulators of the Adenylate Cyclase System on Taste Responses in Gerbil. SUSAN S. SCHIFFMAN[1], LARRY A. GATLIN[2], MARK S. SUGGS[1], SHIRLEY A. HEIMAN[2], WILLIAM C. STAGNER[2], AND ROBERT P. ERICKSON[1] ([1]Duke University and [2]Glaxo Inc.)

The adenylate cyclase system has been implicated in both bitter and sweet taste transduction. The purpose of this study was to determine whether application of modulators of the adenylate cyclase system to the tongue alter taste responses. Integrated chorda tympani (CT) recordings were made in gerbils to bitter, sweet, salty, sour, and glutamate tastants before and after a four minute application of three types of modulators of the adenylate cyclase system. The compounds tested were: urea (2.0 M), quinine HCl (0.03 M), $MgCl_2$ (0.03 M), sucrose (0.03 M), NaCl (0.03 M), KCl (0.3 M), and HCl (0.005 M and 0.01M), and monosodium glutamate-MSG (0.05 M). The three types of modulators tested were: 1) forskolin, a powerful stimulant of adenylate cyclase; 2) dibutyryl cyclic 3'-5' AMP (DbcAMP) and 8 bromo-adenosine 3'-5 monophosphate (8BrcAMP), two membrane permeable forms of cAMP; and 3) H-7, a protein kinase inhibitor. Forskolin (0.12 mM) did not alter CT responses to any stimuli. 8BrcAMP (1.16 mM) reduced the responses to bitter-tasting quinine HCl and $MgCl_2$ but not to urea. It also blocked the responses to KCl and HCl which have bitter components. It had no significant effect on NaCl, sucrose, or MSG. Similar results were found for 5 mM DbcAMP. H-7 (300 uM) slightly reduced responses to several stimuli. These data suggest that cAMP modulates the intensity of some bitter taste responses.

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The Mechanism of Sucrose Octaacetate (Bitter Taste) Signal Transduction.

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The peripheral mechanism of mammalian bitter taste has been the focus of recent investigations (Spielman et al., Brain Res., (1989) 503:326-329). The purpose of this study was to understand the nature of the molecules and second messengers involved in the signal transduction of bitter taste of sucrose octaacetate (SOA).

Mice belonging to the B6.SW and C57Bl/6J strains are congenic and differ ONLY in their tasting ability of SOA (Whitney and Harder, Behav. Genet. (1989) 16: 559-574). Taste tissue from vallate and foliate papillae from both strains, taster and non-taster of SOA, were used to obtain membrane preparations (Spielman et al., Chem. Senses, (1989) 14:841-846). Accumulation of IP_3 was monitored in the presence of SOA (10^{-4}M) alone, or in association with hydrolysis resistant GTP analogues (10^{-5}M) or AlF^{-4} (10^{-2}M).

Membrane preparations derived from taste tissue of the SOA taster strains demonstrated a sharp increase in IP_3 accumulation over nontaster mice and non-gustatory tongue tissue preparations upon stimulation with sucrose octaacetate, but only in the presence of SOA, GTPγS and 278 nM Ca^{2+}. IP_3 was also increased when sucrose heptaacetate, strychnine and denatonium benzoate were applied. IP_3 appears to be the second messenger of the bitter taste of a number of bitter compounds. SOA tasting is a Ca^{2+} dependent process that requires the presence of a GTP-binding protein which is pertussis toxin insensitive.

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In situ recording from hamster taste cells: responses to salt, sweet and sour.

P. AVENET (Colorado State University and the Rocky Mt. Taste and Smell Center), S. KINNAMON (*ibid*) and S. ROPER (*ibid*).

We used the non-invasive technique described by Avenet and Lindemann (1991, Biophys. Abstr.) for recording action potentials from hamster taste cells *in situ*. Whole tongues were excised from hamsters that had been killed with CO_2. A glass pipette of 100 um tip diameter was pressed onto fungiform papilla of the isolated tongue and the interior voltage-clamped to 0 mV. This allowed us to observe action currents, if any, generated by the underlying taste cells. We perfused the interior of the pipette with the following stimuli (in mM and in a control solution containing 30 NMDG-Cl and 10 Hepes, pH 7): 200 NaCl; 20 NaCl; 200 KCl; 20 Na saccharin; 3 citric acid (pH 3, no buffer). In 25% of 82 buds, action currents of 10 ms duration and up to 100 pA amplitude were elicited in response to one or more of the taste stimuli. 13 taste buds were tested with citric acid. Of these, 92 % responded with a train of biphasic currents of 10-20 pA amplitude at a typical frequency of 4/sec. Although the frequency of the response was maintained, the amplitude of the transients typically declined on prolonged stimulation. The biphasic shape of the action currents elicited by citric acid suggests a blockage of an apical conductance. 21 taste buds were tested with NaCl, of which 71% responded with a burst of transients at about 2/sec. The response completely adapted within 10 sec or in some cases declined to a tonic rate of 0.2/sec. The amplitude of NaCl action currents was up to 100 pA. Their monophasic shape suggests that the action currents are conducted through Na-selective channels in the apical membrane. KCl produced action currents in only 16 % of the taste buds tested with this salt (n=19). Their amplitudes were small (5-10 pA) and their frequency was about 0.5/sec. 15 taste buds were tested with 20 mM Na saccharin. Because some responses could be mimicked by 20 mM NaCl, we could only attribute 26% of the responses to saccharin *per se*. The transient currents elicited by saccharin were of large amplitude (up to 100 pA), typically biphasic, and the frequency of the response adapted within 30 sec. Thus, these data confirm that taste cells generate action potentials *in situ* in response to chemical stimulation. This technique can be used to study transduction mechanisms for the 4 basic taste qualitites.

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Regeneration of Rat Glossopharyngeal Nerve Through Sieve Electrodes. ROBERT M. BRADLEY, SUAT GURKAN, BRUCE E. BRADLEY AND KHALIL NAJAFI (Dept. Biologic and Materials Sciences, School of Dentistry, and Center for Integrated Circuits, College of Engineering, University of Michigan).

In an attempt to make long term chronic recordings from peripheral taste fibers we are regenerating the glossopharyngeal nerve through an electrode array consisting of small diameter holes etched in a silicon wafer. The initial design of this electrode contains 13 holes ranging in size from 5 to 20 microns. This wafer, measuring 1 x 1 mm, is cemented between two lengths of silastic tubing with an internal diameter of 0.3 mm. The glossopharyngeal nerve, exposed in the neck, is cut and the proximal and distal ends sutured into the tube opposed to the electrode array. Following a survival time of at least 40 days to permit regeneration, the implants are removed and proximal and distal ends of the nerve fixed, embedded in plastic, and sectioned a 1 micron. Both myelinated and unmyelinated fibers are found in both ends of the nerve. The number of regenerated fibers distal to the electrode is small, probably due to the small number of holes. Currently, several other electrode designs with a large number of varying sizes of holes and incorporating an integral connecting lead are in the fabrication stage. These results demonstrate that axons of the glossopharyngeal nerve will regenerate through an array of small holes.

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Electrophysiological responses of the chorda tympani nerve to bitter and salt solutions in inbred strains of mice. K. S. GANNON and R. J. CONTRERAS (Florida State University, Department of Psychology, Tallahassee, FL 32306-1051)*

When given the opportunity to self-select between water and a taste solution, inbred strains of mice have been found to display unique fluid preferences. For example, the inbred strain, SWR/J, responds to the bitter-tasting compound, sucrose octaacetate (SOA), with total rejection, while other strains (e.g., C57BL/6J) behave as if it is tasteless and consume it like it was water. Whitney and his colleagues (*Chemical Senses* 15: 243-252, 1990) have shown that the autosomal gene, *Soa*, has a determining effect on the aversion to SOA. The inbred strain, C57BL/6J, which carries the Soa^b allele (SOA taste blind), consumes equal quantities of SOA and water in a 48-h two-bottle test. The inbred strain, SWR/J, which carries the Soa^a allele (SOA taste aversion), consumes water to the exclusion of SOA. Congenic mice, B6.SW-Soa^a, derived from selective backcrossing between C57BL/6J and SWR/J parent strains, have the Soa^a allele transferred from the SWR/J donor strain onto the genomic background of the C57BL/6J inbred partner. As a consequence of having this allele in their genome, congenic mice respond like the SWR/J donor strain with an aversion to SOA. In contrast to these inbred strains that distinguish SOA, 129/J mice exhibit remarkably strong preferences to NaCl (Beauchamp, AChemS Abstr., 1990) compared to other inbred strains. Little electrophysiological data exist which could aid in the interpretation of the above-mentioned strain differences in fluid consumption. The purpose of the present study, therefore, is to examine possible underlying neural gustatory mechanisms of fluid preferences in inbred strains of mice. The electrophysiological responses of the chorda tympani in four inbred strains of mice (SWR/J, C57BL/6J, B6.SW-Soa^a, 129/J) to various concentrations of SOA, quinine sulfate, NaCl, and KCl are currently being examined.

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Salt Taste Responses and Paracellular Junction Potentials: The "Anion Effect" Explained. HARRY WMS. HARPER (Duck Engineering Design, 500 E. 63rd St., New York, N.Y. 10021)

Taste responses to a given cation, when paired with different anions, vary in magnitude: this is the "anion effect". A quantitative account of this phenomenon emerges naturally from a model of salt taste transduction (the Diffusion Potential Model) based on selective ion channels in taste cell receptor membranes. In fact, this explanation of the anion effect is a necessary consequence of any transduction model based on current flow through receptor membranes. Such generator currents must form a loop: current flowing through the receptor membrane will spread through the cell interior and out through the lateral cell membrane, returning to the receptor membrane by way of the interstitial fluid of the taste bud, and the taste pore. Since the contents of the taste pore are variable, a variable potential must arise between these contents and the interstitial fluid of the taste bud, in the region of the paracellular junctions which form the taste pore floor. Treating this potential as a liquid junction potential (E_j), which can be calculated, it is found that the variation in responses to a given cation, paired with different anions, is well accounted for by changes in E_j that the anions produce (due largely to differences in electrical mobilities). In the hamster *chorda tympani*, 83% of the variance in responses to 0.1 M NaCl, paired with 11 different anions, is accounted for by variation in E_j, which covered a range of 12.2 mV ($r = .91$, $p < .001$). This result supplies a powerful biophysical tool for use in developing models of transduction: the response sensitivity of the system is thereby given an absolute voltage calibration! Building upon this, one can experimentally confirm the existence of Nernst potentials at receptor membranes when varying concentrations of NaCl or KCl are applied as stimuli, as predicted by the DPM.

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Salivary Ions and the Response of the Hamster Chorda Tympani Nerve to Taste Stimuli. BRADLEY G. REHNBERG, THOMAS P. HETTINGER, AND MARION E. FRANK (University of Connecticut Health Center).

The most peripheral events in mammalian gustation normally take place in a variable salivary environment. Yet most experiments in taste physiology use distilled water to rinse the tongue between stimulus presentations. The relevance of these data to normal gustatory functioning has been questioned by Matsuo and Yamamoto (Neurosci. Lett. 108: 121, 1990), who showed significant differences in taste responses between water-adapted and saliva-adapted conditions in rats. We tested the effects of using artificial salivas as rinsing solutions on whole-nerve responses of the chorda tympani nerve in hamsters (Mesocricetus auratus). "Low-flow" artificial saliva consisted of 20 mM Na^+, 90 mM K^+, 1.5 mM Ca^{++}, 0.5 mM Mg^{++}, 89 mM Cl^-, 20 mM HCO_3^-, 0.5 mM $SO_4^{=}$, and 4 mM $H_2PO_4^-$. "High-flow" artificial saliva consisted of 70 mM Na^+, 25 mM K^+, 3 mM Ca^{++}, 1.5 mM Mg^{++}, 64 mM Cl^-, 35 mM HCO_3^-, 1.5 mM $SO_4^{=}$, and 1 mM $H_2PO_4^-$. We measured responses to 0.1 M NaCl, 0.3 M NH_4Cl, 0.3 M sucrose, and 0.003 M quinine.HCl under three successive tongue-rinsing conditions: (1) artificial saliva rinses, (2) distilled water rinses, and (3) artificial saliva rinses again. Responses to NaCl and NH_4Cl were somewhat lower during artificial saliva rinses and responses to quinine were virtually eliminated. Responses to sucrose were not clearly affected by rinsing with water or either artificial saliva. In current experiments, pilocarpine-stimulated saliva from donor hamsters is being used to evaluate the effects of natural saliva on taste function. Preliminary findings suggest that responses to NaCl, NH_4Cl, and quinine.HCl are stronger using water rinses whereas sucrose responses are stronger when natural saliva is on the tongue. The effects of saliva on sucrose responses in hamsters seem to be smaller than those observed by Matsuo and Yamamoto in rats, and the effects on responses to ionic stimuli are probably explainable on the basis of adaptation to salivary cations.

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The Effect of Calcium Chloride on the Gerbil's Sucrose-best Gustatory Chorda Tympani Neurons. LATCHMAN SOMENARAIN and WILLIAM JAKINOVICH JR. (Department of Biological Sciences, Lehman College and the Graduate School, City University of New York, Bronx, NY 10468, USA).

When 0.5M $CaCl_2$ is rinsed from the gerbil's tongue it's sucrose-best chorda tympani neurons respond vigorously. The $CaCl_2$ solution by itself is not very stimulatory. This effect was not observed in other "best neurons". The effect of $CaCl_2$ on the water rinse on the gerbil's whole nerve has been reported by Jakinovich and Oakley (1975). These observations agree with the suggestions that calcium channels are involved in gustatory transduction. Supported by NIH NINCDS grant #DC00434.

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Comparison of Chorda Tympani and Trigeminal Nerve Responses to Astringent Compounds in Rodents. SUSAN S. SCHIFFMAN, SIDNEY A. SIMON, MARK S. SUGGS, ANN L. SOSTMAN.(Duke University)

The mechanism by which astringent compounds impart a sensation in the oral cavity is not well understood. Historically, astringency has been considered by some to be a fundamental taste quality analogous to sweet, sour, salty, and bitter (Boring, 1942, Sensation and Perception in the History of Experimental Psychology). Others have suggested that astringency is a tactile sensation that results from precipitation of salivary proteins or activation of oral or lingual mechanoreceptors (Lyman and Green, Chem. Senses, 1990, 15, 151-164). The purpose of this study was to determine whether astringency is mediated by taste nerves or the trigeminal nerve. Integrated recordings from the chorda tympani nerve in gerbils and the trigeminal nerve in rats were obtained in response to astringent compounds. The stimuli applied to the gerbil tongue were: tartaric acid (0.43mM to 112mM), tannic acid (0.37mM to 96mM), gallic acid (1.87mM to 120mM), and aluminum ammonium sulfate dodecahydrate and aluminum potassium sulfate dodecahydrate (1.25mM to 160mM). Hydrochloric acid was also tested at 0.78mM to 200mM. The pH values for all of the compounds tested ranged from pH 0.93 to pH 3.74. Tannic, tartaric, and gallic acids were also tested at pH 6.0 which is above the pKa of these astringent weak acids. Each of the astringent compounds at both low and high pH values stimulated the chorda tympani nerve of gerbil, and the integrated responses increased in magnitude with increasing concentration. These same compounds were tested on the rat trigeminal nerve. No responses to trigeminal compounds were found over the same range of concentrations used in the chorda tympani experiments. These data suggest that in rodents, the chorda tympani nerve (a taste nerve) rather than the trigeminal nerve transduces the signals for astringent compounds.

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Responses of Lingual Trigeminal Nerve and Lingual Epithelia to Hydrophobic Stimuli.
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Previous studies of oral trigeminal chemoreception indicate that hydrophilic compounds, such as salts, probably diffuse across intercellular tight junctions and directly stimulate intraepithelial trigeminal endings. To determine if hydrophobic compounds also directly stimulate nerves or if they initially interact with surrounding epithelial cells, the effects of mucosal application of six n-alcohols, phenyl ethanol, toluene and amyl acetate on the lingual whole nerve response of rat and on the short circuit current (Isc) across isolated canine lingual dorsum were studied. There were three major findings. First, threshold concentrations for the trigeminal nerve responses, ranging from 12.4 M for methanol to 4 mM for octanol, correlated inversely with the stimulus partition coefficients ($r = -0.97$). This suggests that these compounds achieve their effects by partitioning into epithelial and neural cell membranes. Second, the different compounds produced different trigeminal responses: methanol and ethanol only increased activity; longer chain n-alcohols initially increased but then suppressed activity below baseline; phenyl ethanol and toluene only suppressed it. Third, in canine lingual epithelium, the concentrations required to reduce Isc by 50% also correlated with their partition coefficients ($r = -0.94$). For the smaller alcohols, these concentrations were lower than the trigeminal threshold concentrations, while for the larger alcohols, they were similar. Since direct application of alcohols to nerves blocks action potentials when the membrane activity of anesthetic reaches a critical concentration, these results suggest that alcohols of low lipid solubility (eg. methanol) never achieve a sufficiently high activity within the axolemma to suppress action potentials, but instead interact with the epithelial cells to produce a change in the composition of the perineural space - for example, by inhibiting Na^+-K^+-ATPase and increasing the extracellular K^+ concentration. By contrast, more lipid soluble alcohols, after producing an initial epithelially mediated increase of nerve activity, are able to achieve a sufficiently high axolemma activity to exert an anesthetic effect and suppress action potentials.

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Structure/activity relations of the L-proline taste receptor site in the channel catfish. S. WEGERT, B. ANDREWS AND J. CAPRIO (Louisiana State University).

An independent L-proline receptor site was previously identified from electrophysiological cross-adaptation experiments in the facial (Wegert and Caprio, 1988) and glossopharyngeal-vagal (Kanwal and Caprio, 1983) taste systems of the channel catfish. Independent confirmation of the L-proline receptor site was recently obtained by recording from purified fragments of membranes from channel catfish taste epithelia that were incorporated into azolectin bilayers at the tips of patch-pipettes. (Kumazawa et al., 1990). Recordings from single facial taste fibers indicated further that L-proline taste information is transmitted centrally by a subset of the L-arginine-best fibers (Kohbara et al., 1990). The present report is an initial electrophysiological, structure/activity characterization of the L-proline receptor site in the facial taste sytem of the channel catfish. Multiunit, electrophysiological recordings were obtained from facial nerve fibers innervating the maxillary barbel. Thirteen compounds structurally-related to L-proline were tested (L-thioproline, L-azetidine-2-carboxylate, pyrrole-2-carboxylate, L-proline methyl ester, N-methyl-L-alanine, prolinamide, betaine, L-proline benzyl ester, D-proline, sarcosine, L-pipecolate, L-hydroxyproline and cycloleucine). Cross-adaptation experiments in which L-proline and the test compounds were adjusted in concentration to become equipotent stimuli indicated that many of the proline-related compounds were not major agonists of the L-proline receptive pathway. Thus, prediction of receptor agonists based on similarity of molecular structure is unwise since glycine betaine (N-trimethyl glycine) was cross-adapted to control level by L-proline, while some structurally more-similar compounds to L-proline (e.g. thioproline and hydroxy-L-proline) remained significantly above control level. Molecular factors that govern a molecule's ability to interact with the gustatory L-proline receptor site will be presented.

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Sensory Coding by Peripheral Chemoreceptor Cells of Invertebrates and Vertebrates. CHARLES DERBY (Department of Biology, Georgia State University, Atlanta, GA)

The major topic to be discussed will be coding of chemical quality and intensity by invertebrate and vertebrate chemoreceptor cells. The focus will be on mixtures, both simple (binary) and complex, since most natural signals for animals are mixtures. Issues include the tuning of chemoreceptor cells to mixtures and its implications for coding, and the influence of mixture interactions and inhibition on coding. The specificity of the excitatory responses of chemoreceptor cells to single compounds can be quite high for some vertebrates, including fish and mammals, but such specificity generally tends to be higher in the invertebrates. However, the excitatory response specificity of invertebrate chemoreceptor cells is much lower to natural and artificial mixtures than to single compounds, which probably precludes these receptor cells from functioning as labeled lines in the quality coding of mixtures. The ability of animals to behaviorally discriminate among mixtures often parallels the neural discrimination of mixtures according to the across-neuron patterns, suggesting that across-neuron patterns might code chemical quality. Mixture interactions, in which the response to a mixture deviates from the response expected based on responses to the mixture's components, have been observed in chemoreceptor cells of both invertebrates and vertebrates. These mixture interactions can influence the neural coding of and subsequent behavioral discrimination of chemical quality or intensity, such that there may be an improvement in contrast between the quality of mixtures and their components. In addition to excitation, chemically-evoked inhibition is also observed in both invertebrate and vertebrate chemoreceptor cells, and it may significantly contribute to the responses of receptor cells to mixtures.

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WORKSHOP

COMPARISONS BETWEEN INSECT AND VERTEBRATE OLFACTION

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Molecular mechanisms of olfactory signal transduction. BREER, H., BOEKHOFF,I., KRIEGER, J., RAMING, K., STROTMANN, J. and E. TAREILUS (Institute of Zoophysiology, University Stuttgart-Hohenheim), 7000 Stuttgart 70, FRG

The chemo-electrical transduction process in olfactory receptor cells comprises a chain of events including the transfer of hydrophobic odor molecules to perceptive membranes where they interact with specific receptors inducing the generation of receptor potentials; second messengers are supposed to provide the critical link between the initial receptor binding and subsequent ion channel activity.
Towards an elucidation of the 'perireceptor events' pheromone binding proteins (PBP), characteristic proteins in the sensillum lymph of insects which are supposed to catalyse the transfer of hydrophobic pheromones through the sensillum fluid to the denrites, have been cloned, sequenced and expressed. Analysis of the secondary structure as well as the hydrophobicity profile suggest that the polypeptide displays a hydrophilic surface as well as hydrophobic pockets.
In preparations from insect antennae and rat olfactory epithelia the formation of second messengers, induced by pheromones or odorants, was monitored in a subsecond time range using a rapid kinetic methodology. It was established that in insect antennae pheromones activate phospholipase C via specific G-proteins leading to elevated IP_3-levels within 50 msec after stimulus application. In vertebrate olfactory cilia individual odorants were found to induce an rapid formation of either cAMP or IP_3. The rapid kinetic of second messenger signalling as well as the essential role of G-proteins favour the concept that the chemoelectrical transduction in olfactory receptor cells is mediated by receptor-activated chemosensory reaction cascades.

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Specialized Forms of G Proteins, Adenylyl Cyclase and Ion Channels Mediate Odor Detection.

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Previous biochemical studies have demonstrated that certain odorants stimulate a GTP-dependent increase of cAMP levels in olfactory neuronal cilia. Our laboratory has identified a novel $G_{s\alpha}$ subunit termed "$G_{olf\alpha}$" expressed exclusively in olfactory sensory neurons (Science 244, 790-795). Recently, we have cloned a bovine brain adenylyl cyclase (type I, Science 244, 1558-1564). Low stringency hybridization of a rat olfactory cDNA library with the type I clone revealed a distinct form of cyclase, adenylyl cyclase type III. The two cyclases share considerable amino acid similarity and each has a primary protein sequence which predicts 12 membrane spanning domains. The role of the membrane spans in protein function has not beeen determined. Northern analysis indicates that type III mRNA is confined to the sensory neurons of the olfactory epithelium. This olfactory specific adenylyl cyclase appears to be a component in a signal transductory cascade specialized for odorant detection. Specifically, the type III enzyme appears to have a low basal activity relative to the level that can be maximally attained. This property could provide for increased sensitivity in odorant detect. Changes in intracellular cAMP levels lead to depolarization of the sensory neurons. An olfactory neuron-specific ion channel has been identified that shares significant protein homology to a visual photoreceptor channel of similar function. The availability of cDNA clones encoding each of the components of this signal transduction cascade may allow the identification of olfactory receptors

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Dual Activation of a Sex Pheromone Dependent Ion Channel from Insect Olfactory Dendrites by Protein Kinase C and cGMP.

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Male noctuid moths detect species-specific sex-pheromones via specialized olfactory receptor neurons (ORNs) located in their antennae. In order to investigate the primary sensory transduction mechanism of pheromone detection we recently described the ionic currents of ORNs maintained in long term cell culture (Zufall et. al. J. Neurosci., 1991). One important criterion which must be met for an ion channel to be involved in the primary transduction process is that it is present in the dendritic membrane of ORNs. Here we describe single channel recordings obtained from the outer dendritic plasma membrane of adult silkmoth (A. polyphemus) ORNs in situ. Under control conditions without pheromone application, neither voltage activated nor any type of Ca^{++} or ATP gated single channel currents were found. After preincubation with the pheromone (AC_1, 0.1 ng/ml), openings of a non-specific cation channel (66 pS) could be recorded not previously described in the cultured neurons. Open time and burst length of these currents depended on the pheromone concentration. In inside-out patches, ATP was required to maintain openings of the active AC_1 channel. The open probability of the AC_1 channel could be increased by application of activators of protein kinase C (DAG or phorbolesters) and, surprisingly, also by cGMP, whereas Ca^{++}, IP_3 and cAMP where without effect. Our results strongly indicate that phosphorylation of a specific ion channel via a protein kinase C could be the crucial step in sex pheromone transduction. They also suggest an extensive cross-talk between different second messenger pathways.

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Molecular Cloning and Sequencing of a Candidate Pheromone Receptor Protein from the Silk Moth *Antheraea polyphemus*.

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MICHAEL R. LERNER (Yale University School of Medicine)

A membrane protein of the sensory dendrites of the silk moth *Antheraea polyphemus* was previously identified as a candidate pheromone receptor protein (Vogt, Prestwich and Riddiford, *J. Biol. Chem.* 263:3952, 1988). A photoaffinity analog of the pheromone was shown to bind to a 69kda protein, pheromone was shown to displace the analog, and the protein was shown to be antennal specific. We have purified this 69kda from the sensory dendrite membranes isolated from 800 antennae. PCR primers were designed based on N-terminal sequence of the protein, and the PCR product was used to screen a Northern blot and a random primed cDNA library. Clones isolated from this library were sequenced and the full length amino acid sequence of the 69kda protein was thus obtained. The 69kda protein does not show significant sequence identity to any database proteins, including membrane associated proteins (receptors, cyclases, lipases, channels, etc). A current view suggests that an odorant receptor should be homologous to rhodopsin type receptors (receptors containing 7 transmembrane domains), yet the 69kda protein shows no homology to this group. Either the photoaffinity experiment was inadequate in identifying a receptor, or the pheromone receptor is not a rhodopsin-like protein. While we are beginning localization and functional studies, progress on this project will be presented, and the properties of this 69kda protein will be discussed.

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Odor induced single channel activity in membrane patches from salamander olfactory receptor neurons. STUART FIRESTEIN, FRANK ZUFALL* and GORDON M. SHEPHERD. (Section of Neurobiology, Yale University Medical School, New Haven, CT and *Physiological Institute, Technical University, Munich)

We report here the first recordings of odor induced single channel activity from membrane patches of salamander olfactory receptor neurons. In on-cell patches we recorded inward cationic single channel currents which were activated in a dose dependent manner by pulses of an odor mixture. The activation of the channels was also timed to the odor pulse; within 0.5 to 2 seconds after the onset of the stimulus, channel activity was detected and the activity persisted for longer times with longer stimulus pulses. In most recordings a large depolarization and an action potential could be detected within 200-300 msecs of the stimulus pulse and this was followed by increased channel activation. When extracellular Na+ was replaced with choline (except in the membrane patch) the depolarization and action potential were blocked, but the channel activity remained. In the same patch increased channel activity could also be elicited by treatments which increased the intracellular concentration of cAMP, such as the addition of IBMX, a phosphodiesterase inhibitor, or membrane permeable cyclic nucleotide analogs. These results suggest that the channels activated by odor stimulation are the same as those gated by cyclic nucleotides. These membrane patches were from the soma and dendrites of isolated receptor neurons. Previously, macroscopic currents activated by cyclic nucleotides have been recorded in patches of membrane taken from the cilia, but single channel currents could not be resolved, presumably due to the high density of channels in the cilia. We hypothesized that the same channels might exist in dendrite and soma but at a much lower concentration. From the results presented here it appears that the soma/dendrite channels are the same as those found on the cilia, and further that this type of channel is responsible for generating the electrical response to odors in olfactory receptors.

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Localization of transduction to olfactory receptor cilia. GRAEME LOWE and GEOFFREY H. GOLD (Monell Chemical Senses Center)

Last year we described experiments using local odorant stimulation to show that odorant sensitivity is limited to and uniformly distributed throughout the ciliary region of tiger salamander olfactory receptor cells. These observations showed that the odorant receptive sites are localized to the cilia. However, because of the evidence that olfactory transduction is mediated by second messengers, i. e., cAMP and IP_3, the ion channels which are activated by odorants need not be limited to the cilia. To provide direct evidence regarding the localization of the transduction current carried by these channels, we measured it under voltage clamp (V_H=-70 mV) with whole-cell recording, while simultaneously recording membrane current from defined regions of the cell with a suction electrode.

As expected, no odorant-induced current was recorded across the somatic membrane under voltage clamp, demonstrating that the transduction current originates in the cilia and/or the dendrite. When the suction electrode enclosed the ciliary and apical dendritic membranes, an inward current, I_S, was detected which was less than or equal to the whole-cell current, I_{WC}, by a constant scale factor (range 0.5-1.0). Experiments in which $I_S = I_{WC}$ constitute direct evidence that the transduction current originates entirely within the cilia. Observations in which $I_S < I_{WC}$ were also consistent with the transduction current originating from the cilia, since the observed ratio, I_S/I_{WC}, could be predicted from the measured seal and pipette resistances and the geometry of the suction electrode; the fact that $I_S < I_{WC}$ reflected leakage of membrane current across the low resistance seal (2-6 MΩ) between the dendritic membrane and the suction electrode. Because the calculated ratio, I_S/I_{WC}, depends on the spatial distribution of current along the cilia, these measurements also provided evidence that the transduction current is uniformly distributed along the cilia. The observed values of I_S/I_{WC} were inconsistent with a significant fraction of the transduction current originating from the dendritic membrane.

These results, combined with our previous results with local odorant stimulation, demonstrate that the cilia contain the complete transduction apparatus, from the odorant binding sites to the ion channels which carry the transduction current.

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Olfactory-Specific Cytochromes P450 (P450olf1, P450olf2) and Their Possible Functions in Olfaction.

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Microsomal cytochromes P450 are products of a large superfamily of genes. These monooxygenases display broad and sometimes overlapping substrate specificities. P450s utilize electrons from NADPH and molecular oxygen to hydroxylate many lipophilic compounds including drugs, carcinogens and steroids as well as odorants. One of us (PN) has recently identified more than eight different P450s expressed in the rat olfactory epithelium by cDNA cloning, RNA and protein blot analyses. Two of them, P450olf1 and P450olf2, are novel enzymes which are olfactory-specific. P450olf1 and P450olf2 gene activity is first detected between days 2 to 7 postpartum, reaches a maximum level of activation around day 21 and persists in the adult. This temporal pattern of activation coincides markedly with an increased sensitivity to odorants in the rat (P. Nef, et al. (1990) J. Biol. Chem. 265:2903-2907). On the basis of these results and the substrate specificity of P450olf1 and P450olf2, we propose that these P450s are involved in the molecular inactivation or activation of odorants. In addition, other P450s expressed in nasal epithelial tissue could serve a functional role to protect olfactory neurons from airborne xenobiotics.

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Ultrastructural Localization of the Transduction Apparatus in the Rat's Olfactory Epithelium

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Electron-microscopic immunocytochemistry was used to localize important, only recently characterized, components of the olfactory chemosensory transduction apparatus, i.e., $G_{olf\alpha}$ (*Jones and Reed, Science, 244: 790 (1989)*), $G_{s\alpha}$ (*Mania-Farnell and Farbman, Devl. Brain Res., 51: 103 (1990)*) and olfactory adenylyl cyclase (*Bakalyar and Reed, Science, In Press (1989)*). G-proteins and adenylyl cyclase act, together with among others cyclic nucleotide-gated channels, to transduce the olfactory signal in a message which can be transported to, and understood by, the brain. Rapid-freeze, freeze-substitution, post-embedding (embedding in Lowicryl K11M) immunocytochemistry was used, with protein G-gold as secondary probe, to determine sites of binding of the polyclonal antibodies. All three antibodies co-localized in majority in the same cellular compartment, i.e., antigens were mainly found in the long distal parts of rat olfactory cilia. For $G_{s\alpha}$ we used two antibodies. One was made against a peptide region which, according to the manufacturer's leaflet (Dupont, NEN), shows great overlap with $G_{olf\alpha}$ (*Simonds et al., Proc. Natl. Acad. Sci. USA, 86: 7809 (1989)*). Another one (*Gilman, Ann. Rev. Biochem., 56: 615 (1987)*) was made against a peptide region which was more different from $G_{olf\alpha}$. If at all, the latter one labeled all components in the olfactory epithelial surface rather diffusely, whereas the first one mainly labeled the same distal elements of olfactory cilia as $G_{olf\alpha}$, suggesting the presence of overlap indeed. Antibodies to olfactory marker protein (*Menco, Cell Tissue Res., 256: 275 (1989)*) or tubulin are a suitable positive control, and labeled dendrites, dendritic knobs and, only very sparsely, olfactory cilia. Thus, in contrast to previous ideas, which suggested that it are mainly the proximal parts and knobs which are involved in olfactory transduction (*Getchell et al., Biophys. J., 29: 397 (1980)*), the present studies provide the first hard evidence that it are the long, special modified distal parts of olfactory cilia which mainly contain the sites of initial olfactory transduction. *Drs. Heather Bakalyar, David Jones, Susan Mumby and Randy Reed are thanked for sharing their antibodies. Supported by NSF (BNS-809839).*

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Stimulus intensity filters in chemoreception: the transfer function.

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The olfactory receptors of the lateral antennules of the American lobster *Homarus americanus* are surrounded by a relatively thick boundary layer created by the aesthetasc sensilla. Antennule flicking temporarily sheds a large part of this boundary layer. Intensity fluctuations of chemical signals are filtered by the boundary layer before they reach the receptor surfaces. In Vivo Electrochemistry (IVEC) techniques allow us to measure a more precise temporal correlation between stimulus arrival and cell response than previously possible. Lobster lateral antennules were excised and placed in a recording chamber. An IVEC probe approximately the size of an aesthetasc hair was placed in the receptor tufts and 10^{-4}M hydroxyproline was introduced into the chamber. Simultaneous measurements of the chemical signal and the neural response to the stimulus were made. The antennule was stimulated both with well-defined pulses (1-8 s duration) and with more natural random patterns. All pulse durations regardless of stimulus length caused phasic responses lasting for less than a second. This suggests that stimulus onset, more than duration, is an important feature of the chemical signal. The occurrence of spikes in relation to the instantaneous stimulus profile allows us to determine how chemoreceptor cells in situ translate the chemical intensity signal into a neural signal (transfer function). The data confirm results from the pluse study and predictive computer models based on information contained in odor plumes (Moore & Atema, 1988 *Biol. Bull.*): stimulus onset slope is a more important parameter for cell responses than stimulus duration and height. The boundary layer surrounding the receptor organs serves as a filter for incoming chemical signals.

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Effects of Capsaicin on the Negative Mucosal Potential (NMP) Evoked by Chemical Irritants in Rats

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In 1985 Kobal (Pain 22:151-163) described a negative potential, recorded from the human respiratory mucosa after painful stimulation with carbon dioxide (CO_2), which correlated with subjective pain sensations. The aim of the present study was to determine the origin of the negative mucosal potential in an animal model (rat). NMPs were recorded after stimulation with different concentrations of CO_2. After control stimulation with non-painful hydrogen sulphide no signals could be recorded. Amplitudes of the NMPs correlated with CO_2-concentrations of the stimulus. Latencies from the onset of the stimulus to the onset of the NMP were within 120-500 msec. In order to determine if either functionally unimpaired sympathetic nervous structures or intact C-fibers are requisite for NMPs, the influence of systemic (200 mg/kg capsaicin s.c., cumulative dose, 1% w/v alcoholic capsaicin solution) and local (3 mg in 0.3 ml 1% w/v alcoholic solution) treatment with capsaicin and the influence of systemic treatment with guanethidine (50 mg/kg s.c., in 0.3 ml 0.9% NaCl) was examined in different groups of rats. The NMP was reduced by systemic treatment with capsaicin and eliminated by local adminstration of capsaicin whereas guanethidine did not eliminate this signal. After administration of the local anesthetic lidocaine (31 mg in 0.3 ml solvent) the NMP was abolished. In order to exclude a vascular origin of the NMP blood flow changes were measured. Onsets of blood flow changes appeared significantly later than onsets of potentials. On the basis of these results a sensory neurogenic origin for the NMP is proposed.

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Inspiratory and Expiratory Airflow Patterns in a Large Scale Human Nasal Cavity Model

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Nasal airflow patterns were studied by using a large scale(20 X) human nasal cavity model which has been introduced at AChemS X, 1988.(Abstract #74) The model was constructed to be anatomically correct with a healthy human nose from the naris to the beginning of the nasopharynx. Airflows through the model were produced by a d.c. voltage-controlled fan, and, by applying the principles of fluid mechanics, were designed to be kinematically similar to those occuring in the real human nose. The fan was located at the nasopharynx for inspiratory airflows and at the naris for expiratory ones. Three airflow rates through the model were studied: a low flow rate typical of normal breathing and two higher flow rates typical of medium and vigorous sniffs(265 ml/sec, 1140 ml/sec and 2000 ml/sec in the real human nose.) A hot-film anemometer probe(TSI Inc.) was used to measure local air velocity in different regions of the nasal airway by insertion into the various drilled reference holes. Due to the small size and measurement area(0.01 mm) of the probe, it was possible to obtain local velocities with a spatial resolution of 1mm in the nose model(equivalent to 0.05 mm in real nose.) For both inspiratory and expiratory nasal airflows, detailed maps of velocity profiles were made for various cross sections of the nasal airways including the naris and the olfactory slit. For inspiratory flows, it was confirmed that a constant fraction of inhaled air(about 15%) flows through the olfactory slit for all flow rates studied, and that the velocity in lower olfactory region is appreciable and comparable to those in the middle and lower nasal airways. The intensity of turbulence was also measured.

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Single and Mixed Solution Behaviour of Sucrose, Glucose, Fructose and Citric Acid

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The solution properties of glucose, fructose, sucrose and citric acid were examined in single and mixed solutions by pmr pulse relaxation and precision densitometry in an attempt to illuminate some of the sensorial reports of such mixtures. The pmr results are more sensitive to differences in solute structure than are the precision densitometry results. However, they are subject to greater experimental error. Spin-spin relaxation times (T_2 values) of the sugars (5-10% w/v solutions) were typically in the range 2.3 - 3.4 seconds (pH 4-6) whereas 1% citric acid was 4.8 seconds.

Mixtures of sugars and citric acid usually gave T_2 values close to or > 4.0 seconds, showing that citric acid dominated the proton behaviour. 5-10% solutions (w/v) of sugars displayed apparent specific volumes in the range 0.61-0.62 cm^3g^{-1} which were close to the apparent specific volume of citric acid solutions (0.61 cm^3g^{-1}). Mixtures of sugars with citric acid were therefore practically indistinguishable using this parameter. These results may help to explain the psychophysical effects reported for the major solutes in soft drink formulations. They support the idea that one solute in a mixture may be taste-dominant, and underline how solute concentration affects total proton order and water structure.

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Effect of salivary flow rate on temporal perception of bitterness and astringency

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Eleven subjects (S) rated bitterness and astringency in 18 wines varying in ethanol (1% ,8%, 14% v/v), pH (3.0 and 3.6) and added phenolic compounds (none, 1500 mg/L catechin or tannin acid) by time-intensity methodology (TI). In a separate test, while the Ss rated bitterness, saliva was collected for 2 min from one parotid gland. When Ss were grouped on the basis of accumulated flow, four high-flow Ss reached their maximum saliva flow-rate (Max S Rate) sooner than four medium or three low-flow Ss. For all groups, Max S Rate was reached after expectoration of the stimulus at 10 sec and did not correlate with the time to maximum intensity of either bitterness or astringency. Average bitterness and astringency TI curves showed differences in perception among the flow groups. Low-flow Ss took a longer time to reach maximum intensity and had a longer duration for both bitterness and astringency, and rated astringency intensity at maximum higher than high-flow Ss. For all flow groups, the decay constants for bitterness were higher than those for astringency. The log intensity decay curves were linear, suggesting that first order kinetics governed the decay function. Because the decay constants for the two attributes were different, the primary effect is considered to be desorption, rather than diffusion across a liquid film. Reciprocal plots of salivary flow rate vs time were linear suggesting 2nd order kinetics, and that the salivary response was not mediated solely by perception of taste or irritation.

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Foliate Papillae Taste Perception in Humans.
F.A. CATALANOTTO, Y. LECADRE, F. DEVONSHIRE and L. BARTOSHUK (UMDNJ-New Jersey Dental School and Yale University)

Unilateral chorda tympani nerve block results in increased whole mouth, suprathreshold scaling, magnitude matching estimates for taste stimuli. We studied potential mechanisms for the increase in this current study. Following unilateral mandibular block injections, 19 subjects provided whole mouth magnitude estimates (without auditory tones) and 14 subjects provided similar estimates of Q-tip delivered taste stimuli applied to various locations on the tongue. The overall effects of anesthesia on the whole mouth magnitude estimates, analyzed by ANOVA, were significant, $F(1,18)=4.69$; $p<0.05$. The results generally agree with the earlier findings but highlight important procedural issues that will influence future testing. More interestingly, the results of the spatial testing showed that magnitude estimates from ipsilateral fungiform and anterior foliate areas, but not posterior foliate areas, reduced essentially to zero after anesthesia, $F(1,13)=303.181$; $p<.00001$), demonstrating that the anterior foliate areas are innervated exclusively by the chorda tympani rather than the glossopharyngeal nerve. This contradicts some recent literature on the innervation of the tongue. Supported by Foundation of UMDNJ and NJDS Alumni Association.

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Peripheral Source of Taste Phantom (i.e., Dysgeusia) Demonstrated by Topical Anesthesia.
LINDA M. BARTOSHUK and JOHN KVETON (Yale University School of Medicine)

"Dysgeusia" refers to a chronic taste without obvious stimulation. The taste often results from a real but unrecognized stimulus in the mouth but it can also originate within the nervous system. In the latter case we suggest the term "phantom." We report such a case. EO is a 36 year old female with a history of chronic otitis media and mastoiditis. She has had a mastoidectomy on the right (11/89) and left (2/90), as well as a right revision mastoidectomy and tympanoplasty (6/90) at which time a portion of her right chorda tympani nerve was removed along with debris from her middle ear. After the initial right mastoidectomy, EO experienced a salty taste presumably produced by damage to the chorda tympani. The taste is still present although it diminished in intensity after the second right surgery. EO was evaluated periodically with spatial taste tests which initially showed no taste on the right front but some taste on the left front (abolished after the left mastoidectomy). To study the source of the salty taste, EO's mouth was exposed to a topical anesthetic (.5% dyclonine and .5% diphenhydramine in .9% saline) on six occasions with similar results. Anesthetization of her mouth produced a dramatic increase in the intensity of the salty dysgeusia. When the anesthetic wore off, the salty dysgeusia returned to its original intensity. We conclude that the neural responses from the glossopharyngeal nerve usually inhibit neural responses from the chorda tympani nerve. By abolishing the glossopharyngeal input, we removed that inhibition. Anesthesia diminishes a dysgeusia produced by a taste stimulus in the mouth and increases a dysgeusia produced in the nervous system.

We thank NIH Grants DC 00283 and DC 00168 and EO for her generous cooperation and careful observations.

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Differential Sensitivity of Tongue Areas and Palate to Electrical Stimulation: A Suprathreshold Cross-Modal Matching Study
JAMES A. SALATA, JAYA M. RAJ, and RICHARD L. DOTY (Smell and Taste Center, Department of Otorhinolaryngology and Human Communication, University of Pennsylvania, Philadelphia, PA)

A cross-modal matching procedure was used, in twelve subjects, to evaluate regional differences in suprathreshold sensitivity of the oral cavity to electrogustometric stimulation. Stimulation of five loci on each side of the oral cavity was performed: tongue tip (one cm from the midline), anterior tongue side (2.5 cm from tip on lateral margin), posterior tongue side (region of the foliate papillae), posterior medial tongue (one cm from midline on circumvallate papillae), and soft palate (one cm from midline, one cm above superior pole of anterior palatine arch). The tip of the tongue was significantly more sensitive than the other areas to electric stimulation, as evidenced by the slope and absolute position of the psychophysical power functions. Strong correlations were observed in the sensitivity measures across tongue loci and between tongue and palate sides. No effects of subject gender or mouth side were found.

This research was supported by Grant DC00161 from the National Institute of Deafness and other Communicative Disorders.

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Taste Preference in Parkinson's Disease Patients.
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Neuromuscular dysfunction is a prominent sequelae of Parkinsonism but sensory systems can also be affected. Sensory deficits in Parkinson's disease (PD) patients have been reported in both the visual (Bodis-Wollner, '90) and olfactory systems (Doty et al, '88). No studies have reported gustatory dysfunction in PD patients but clinical observations (G. Paulson) suggest that this population has an increased craving for sweet foods. The present study was undertaken to determine if PD patients (n=27) varied in their preference ratings for sweet stimuli compared to an age and sex matched control group (n=15). Seven concentrations of sucrose (0.04 M - 1.0 M) and NaCl (0.02 M - 1.0 M) were randomly presented and subjects rated their preference on a 6 point category scale ("extremely liked" to "extremely disliked"). The results indicated that PD patients did not differ significantly from the control group in their preference for NaCl (ANOVA). The preference-concentration function for sucrose, however, varied for the PD and normal groups. The preference curve for the normal group was an inverted U-shaped curve which peaked at 0.3M sucrose. In contrast, the preference function for the PD patients was a monotonically increasing function of concentration. Analysis of variance for the sucrose ratings revealed no main effect for Group but there was a highly significant Group X Concentration effect ($P<.0005$). Post hoc tests indicated that PD patients preferred the lower concentrations of sucrose less than the control group but preferred the higher concentrations more. Future studies will examine whether this shift in preference for sucrose is accompanied by a shift in magnitude ratings for this stimulus.

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Altered Suprathreshold Quality and Intensity Judgements in Patients with Liver Disease.

SHERI SOMMERVILLE (Monell Chemical Senses Center).
JOAN HAVEY (Monell Chemical Senses Center).
MARK I. FRIEDMAN (Monell Chemical Senses Center).

Compared to healthy subjects, patients with liver disease show a decreased preference for foods having a pronounced bitter component, are more likely to rate foods as being bitter, and yet report decreased taste intensities for foods having a predominantly bitter component. To explore these apparent perceptual differences, we examined suprathreshold intensity and quality judgements for four taste qualities in 21 patients with liver disease and in gender- and age-matched controls. Each subject was asked to identify the quality and to categorically rate the intensity of five suprathreshold concentrations of sucrose (sweet), sodium chloride (salty), citric acid (sour) and quinine (bitter) using a whole-mouth, sip-and-spit procedure. Subjects also were asked to identify and rate the intensity of the highest concentration of each tastant when it was applied to discrete areas of the tongue and palate with a Q-tip. There was no significant difference between controls and patients in their intensity judgements of sucrose and sodium chloride or in their ability to identify these two tastants as, respectively, sweet and salty. However, liver disease patients reported diminished intensity ratings across all suprathreshold concentrations for citric acid and quinine and made more errors in identification of these tastants as, respectively, sour and bitter. Patients made more errors in the identification across all four taste qualities and had significantly lower intensity judgements of stimuli applied to discrete areas of the tongue and palate. The results suggest that the altered response of patients with liver disease to bitter foods may reflect a change in their perception of bitter and sour.

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Does Sensory Processing Contribute To The "Personality" Of Eating ?

NICOLETTE VAN DER KLAAUW, GREGORY SCHAFFER & ROBERT A. FRANK (University of Cincinnati).

It has been suggested that sensory factors contribute to an individual's liking for or willingness to try foods. This hypothesis was evaluated by assessing taste and smell perception in subjects with an unusually large number of food likes, dislikes or an unusual number of foods they were unwilling to try. Threshold data revealed no significant differences in the detection thresholds of PTC and quinine among groups. In addition, intensity and hedonic ratings for suprathreshold concentrations of sucrose, NaCl, citric acid and quinine were not found to be different. In a smell experiment, subjects made intensity and pleasantness ratings of 25 common odors (foods and non-foods). Subjects with a large number of food likes (likers) gave higher intensity and pleasantness ratings than subjects who were generally unwilling to try foods (won't try-ers). The same pattern of results was found in a replication of the initial study and in a final study using 20 artificial odorants. These experiments support the hypothesis that differences in the perception and/or evaluation of olfactory stimuli are related to people's liking of or willingness to try foods.

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The Effects of Flavor and Macronutrient Composition on Satiety

JILL JOHNSON (University of Minnesota)
ZATA VICKERS (University of Minnesota)

The effects of consuming foods with different macronutrient compositions and flavors on hedonic changes and the development of satiety were investigated. Subjects rated their hunger and liking of a set foods (rating set) before and after eating a serving (preload) of one of the foods in the rating set. The liking of the preload foods dropped more than the liking of the uneaten foods. Foods having the same flavor as the preload generally dropped more in liking than a food having a similar macronutrient. The drops in liking increased with the caloric content of the preload, but were unrelated to specific macronutrients. Less weight and calories of food were eaten after the high calorie preloads. Eating the high protein or the high carbohydrate preload decreased hunger more than eating the high fat food. Eating a high protein preload decreased the weight of food eaten more than eating a high fat or a high carbohydrate preload and decreased total caloric intake more than eating a high fat preload. However, macronutrient intake was not differentially affected by the macronutrient composition of a preload.

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Dietary Restraint and Responsiveness to Sensory-based Food Cues.

BEVERLY J. TEPPER and PATRICIA SWALES (Rutgers University).

The regulation of food intake in humans is a complex process and the mechanisms that control it are not well understood. Sensory-related food cues may play an important, but as yet, undefined role in this process. This study examined the relationship between dietary restraint, i.e., the conscious limiting of food intake to control body weight and two well documented behavioral indices of the sensory properties of foods; cephalic phase salivary response and sensory specific satiety. Sensory specific satiety measures the extent to which the consumption of a food affects its pleasantness. Eighteen, normal-weight adults (9 men; 9 women) participated. Six subjects displayed high restraint scores (13.2 $\pm$ 2.4) as measured by the 3-Factor Eating Questionnaire. To measure cephalic phase salivary (CPS) flow, subjects salivated into a funnel for 5 min under 2 conditions; unstimulated (no food present) and stimulated (pizza present). To monitor sensory specific satiety, subjects were presented with a tray of 9 common foods. Subjects tasted the items and rated their pleasantness using a 15 cm. line scale. They then consumed a meal of either cheese & crackers or cookies then tasted the 9 items again at 2, 20 and 40 min after the meal. The rating tray included the eaten items. Unstimulated CPS flow rates were similar in unrestrained and restrained subjects. With stimulation, CPS flow rates increased by only 20% in unrestrained subjects as compared to 31% in restrained subjects. As expected, unrestrained subjects rated the eaten foods as less pleasant than the foods which were only tasted. In contrast, restrained subjects displayed a striking 30% increase in their pleasantness ratings of the eaten foods as compared to the tasted foods. These findings suggest that dietary restraint is associated with heightened responsiveness to the sensory properties of foods. Further examination of sensory-related food cues may provide additional clues for understanding the dietary behaviors of various subgroups of the population.

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Investigations of Human Taste Response Using Microdrop Stimulation of Fungiform Papillae.

ANN M. TENNISSEN (State University of New York, Albany, N.Y.)

Microdrop stimulation was used to determine the smallest number of fungiform papillae needed to elicit a reliable salty, sweet, sour, and bitter taste response. Individuals differ in the number of papillae required for each taste quality response, but, overall, no one taste quality required more stimulation than another. This study also examined the effects of amiloride on the reduction of salt and sweet intensity responses in these papillae. Results indicate the response reduction is not present in all subjects nor is it uniform across the subjects who show the reduction. An additional study used bretylium tosylate to examine the possible role of sodium channels in sweet taste perception. Results of these studies are discussed in terms of possible coding mechanisms in taste perception.

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Differential Context Effects and Role of Similarity in Taste Perception. KRYSTYNA M. RANKIN (John B. Pierce Laboratory, Yale University, and Department of Psychology, Stockholm University, Sweden) and LAWRENCE E. MARKS (John B. Pierce Laboratory and Yale University).

In a series of experiments we used the method of magnitude estimation to investigate the effects of multiple stimulus contexts (defined as the subsets of possible stimulus concentrations in the set) and stimulus similarity on the perception of taste intensity (differential context effects). In hearing, Marks found that sounds judged equally loud in one contextual condition were judged substantially unequal in another contextual condition when the stimuli differed sufficiently in quality (quality defined as difference in sound frequency). In the present experiments subjects judged the intensity of one tastant, such as NaCl or sucrose, within the context of another tastant, such as saccharin, quinine, or NaCl-sucrose mixture. Results support the hypothesis that the magnitude of the differential context effect (i.e the changes in the relative judgements due to context) depends on the degree of qualitative similarity between the stimuli.

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Time-Intensity Studies of the Intensity and Hedonics of Prolonged Gustatory Stimuli

WILLIAM E. LEE III (University of South Florida), DENISE M. BARRICK (Youngstown State University), and EDWARD S. WELLING (University of South Florida).

Time-Intensity (T-I) techniques were used to study sweet (sucrose in water), bitter (caffeine in water), sour (citric acid in water) and salty (NaCl in water) solutions. Concentrations of the respective stimuli were selected to provide a range of "weak" to "strong" impressions. Subjects evaluated the solutions for characteristic intensity (for example, "sweetness intensity") and like/dislike. Room temperature 20 ml solutions were presented every 10 seconds for a total time of 180 seconds. The experiments were designed to observe any adaptive behavior and transitions from "like" to "dislike" as a function of concentration in addition to any correlations between the characteristic intensity and like/dislike responses. It was found that the weak intensity solutions typically did not exhibit any transition from like to dislike. However, such transitions were observed for higher concentrations, with both the transition time and degree of dislike correlationg to solution concentration. Other correlations were also observed between the various response curves. Sensory fatigue behavior was present in almost all situations.

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Oral Capsaicin Desensitization and its Effects on Thermal, Tactile, and Chemical Stimuli. TRACY KARRER (Yale University), LINDA BARTOSHUK (Yale School of Medicine)

It has been previously reported that a 10 min application of 10,000 ppm capsaicin (CAP) to the human tongue resulted in desensitization to subsequent applications of mustard oil and zingerone (chemical irritants), and in an increase in the difference threshold for 45°-49°C temperatures, whereas there were no changes in cool thresholds or tactile perception (Szolcsanyi & Jancso-Gabor, 1973). It has also been reported that applications of CAP over 10 min decremented the intensity of a subsequent piperine (irritant) application (Green, 1990). This abstract reports that a 15 min application of 100 ppm CAP produces decrements in suprathreshold heat, while suprathreshold cold and tactile perception are not decremented. Tests of the perceived intensity of 0°, 10°, 20°, 36°, 42°, 48°C and 0.5, 1.1, 1.9, 4, 4.9, 27 g pressure (monofilament) were performed before and after CAP application. As a control, all stimuli were also tested on a finger. Only oral 42° and 48° showed decrements in intensity post-CAP. This abstract also reports that a 15 min application of 10 ppm CAP results in the decrement of the perceived intensity of several chemical irritants. The CAP concentration was 10 ppm, rather than 100 ppm, in order to more closely match the moderate intensities of some of the other irritants. Tests of the perceived intensity of 350 ppm piperine, 5000 ppm ginger oleoresin, 40% ethanol, and seltzer water (CO_2) were performed before and after CAP application. All irritants showed a decrement in intensity post-CAP, though the CO_2 effects were less dramatic than the others. All irritants but CO_2 showed complete recovery one day after CAP. Both temperatures showed complete recovery one day after CAP. Thus, the effects of CAP desensitization on suprathreshold thermal and tactile stimuli and those on alternative irritants mirror the effects found in the above experiments which used higher and lower CAP concentrations.

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Sensory Threshold Evaluated from Dose-response Curves

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Three methods of threshold estimation are compared for the detection of 1,8-cineole added to Concord grape juice presented in a semi-ascending paired difference test (Lundahl *et al.*, 1986). Two methods consider dose-response behavior to be a discontinuous function with the threshold at the point of discontinuity. Method A tests the significance of obtaining a non-zero response and method B determines the transition point of a 2-phase linear model fitted to the data. An alternative method fits a logistic model to the data and assumes dose-response behavior is a continuous function. Method C defines a threshold at the point of maximum curvature, C_{max} on the dose-response curve. Method A yields thresholds which are inversely related to the error in the data and systematically decrease with increasing sample size. Methods B and C yield thresholds which are independent of sample size, are a property of the dose-response data, and are similar in value.

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PTC/PROP and the Tastes of Milk Products.

SUSAN MARINO, LINDA M. BARTOSHUK, JILL MONACO, (Yale University), JEAN ANN ANLIKER (University of Connecticut), DANIELLE REED (University of Pennsylvania), and SALLI DESNOYERS (Pierce Foundation).

Calcium chloride (.32 M) tastes more bitter to some tasters of PTC/PROP than to nontasters. This suggests the possibility that some concentrated milk products (e.g., dry milk powder and cheeses which contain amounts of calcium comparable to .32 M) might taste more bitter to tasters. Adult subjects were asked to rate a variety of cheeses (monteray jack, american, mild cheddar, sharp cheddar, brie, mozzarella, swiss, muenster, blue) for several sensory attributes (bitterness, sweetness, saltiness, sourness, and creaminess). ANOVAs and t-tests showed that tasters rated the sharp cheddar and the swiss cheese as more bitter than nontasters did. Sweetness, sourness, and creaminess showed no taster/nontaster association but tasters rated all but the sharp cheddar to be saltier than nontasters did. Dry milk powder also tasted more bitter to some tasters than to nontasters. Five to six year old children were given eight foods, including cheddar cheese and milk, and asked to order them by preference. Cheese was selected significantly earlier by nontasters than by tasters (ranks 1.4 and 3.7, resp.) and milk was selected significantly later by nontasters than by tasters (ranks 5.9 and 4.2, resp.). This suggests that the bitter taste perceived in concentrated milk products may significantly affect preference in children. Casein (the protein found in milk) was tested with adult subjects. Some tasters found the casein to be more bitter than nontasers did. Since protein molecules are too large to stimulate taste, the bitter taste may originate from fragments of the proteins (e.g., amino acids) resulting from the processing of the protein.

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The Transfer of Alcohol to Human Milk: Sensory Implications.

JULIE A. MENNELLA,[1] KIKUE KUBOTA,[1,2] GARY K. BEAUCHAMP[1] ([1]Monell Chemical Senses Center and [2]Ochanomizu University).*

The present study investigated the transfer of the odor of ethanol and its metabolites to human milk. We tested 12 lactating females on two days separated by one week. On each day of testing, the mother expressed a baseline sample of approximately 15 ml of foremilk after which she drank either orange juice or a small dose of ethanol (0.3g/kg body wt) in orange juice. Milk samples were then taken 0.5, 1, 2 and 3 hours after drinking the beverage. Evaluation of the milk samples by a sensory panel revealed there was no perceived change in the odor of the milk on the day the orange juice alone was consumed. In contrast, the consumption of ethanol in orange juice significantly and consistently increased the perceived intensity of milk odor as judged by adult panelists; this increase in odor intensity peaked in strength one hour after ingestion and decreased thereafter. Such a time course in the alteration of the odor of milk was consistent with the changing concentrations of ethanol in the milk, as determined via an NAD-ADH enzymatic assay. In an attempt to determine which odors the breast-fed infant may be experiencing in the milk, static headspace gas-chromatographic analyses was performed to quantify and identify the metabolites of ethanol present. In addition, adult panelists evaluated various solutions with known quantities of ethanol to determine if the enhanced sucking responses exhibited by the infants (see accompanying abstract, Mennella and Beauchamp) could be explained because the milk was perceived as tasting sweeter.

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The Transfer of Alcohol to Human Milk: The Effect on the Recipient Infant.

JULIE A. MENNELLA AND GARY K. BEAUCHAMP (Monell Chemical Senses Center)*

Previous work in our laboratory has shown that flavors (e.g., garlic) consumed by a lactating female alter the sensory qualities of her milk and, in turn, the behavior of her infant during nursing. The present study investigated the effects of alcohol consumption by the nursing mother on the feeding behavior of her infant. We tested 12 mother-infant dyads on two days separated by one week. On each day of testing, the mother expressed a baseline sample of approximately 15 ml of foremilk after which she drank either orange juice or a small dose of ethanol (0.3g/kg body wt) in orange juice. Milk samples were then taken 0.5, 1, 2 and 3 hours after drinking the beverage. In addition, each mother fed her infant on demand and we monitored milk intake, time attached to the nipple and the patterning of sucking for each feed. Because alcohol consumption altered the sensory qualities of the milk (see accompanying abstract, Mennella et al.), we predicted that the suckling behavior of the infant would be altered following alcohol ingestion by the mother. The data revealed a significant decrease in the number of feeds ($p < 0.05$) and amount of milk consumed ($p < 0.005$) and an increase in sucking ($p < 0.05$) during those 3-hour testing sessions in which mothers drank alcohol in orange juice when compared with sessions in which orange juice alone was consumed. Although we suggest that the changes in suckling were due, in part, to the altered milk flavor, other explanations, including pharmacological effects on mother and/or infant, are also possible.

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A Test of Flavor Sensitivity. VALERIE B. DUFFY (John B. Pierce Laboratory, New Haven, CT, and University of Connecticut, Storrs, CT), WILLIAM S. CAIN (John B. Pierce Laboratory and Yale University, New Haven, CT), JOSEPH C. STEVENS (John B. Pierce Laboratory and Yale University, New Haven, CT), and ANN M. FERRIS (University of Connecticut, Storrs, CT)

Standardized tests of olfactory functioning employ orthonasal smelling and thereby tap perception of environmental odors. We see the need for a test of retronasal smelling that relates to the perception of food. Such a test would reveal possible disparities between orthonasal and retronasal functioning. Hyposmics, for example, sometimes claim more retronasal than orthonasal loss. The elderly and including denture wearers often complain of food flavor. For a stimulus in a retronasal test, we used a gelatin sweetened like commercial products and laced with various concentrations of orange flavoring. Gelatin offers little taste of its own, allows for uniform dispersal of flavoring, holds volatile flavoring better than do water solutions, and handles easily. Subjects, tested with a two-alternative forced-choice (2AFC) variations on the ascending method of limits, perceived a sweetened gelatin base that varied in "orangeness" from subthreshold to threshold levels. Tests with confirmed anosmics uncovered no non-olfactory contribution from the flavoring, even at high levels. When tested to a criterion of four correct in a row at any given concentration, elderly subjects (n=20, av. age 72 yr) exhibited a threshold 16 times above that of young subjects (n=20, av. age 24 yr). The flavor test-retest reliability (r) over successive days for the entire group of young and old equalled 0.80 (young: 0.64 and elderly: 0.73). An orthonasal smell test (odorant: 75% t-butyl mercaptan/25% dimethyl sulfide) on these subjects, but to a criterion of five correct in a row, yielded a threshold five times higher in the elderly than in the young. The test-retest reliability for the orthonasal test equalled 0.53 (young: 0.58 and elderly: 0.37). The correlation between the orthonasal and retronasal thresholds equalled only 0.11 (young: zero and elderly: -0.22), which implies a difference between the two types of sensitivity. Perhaps the difference lies in how well subjects can employ mouth movements and retronasal breathing for active perception of flavors in the mouth. In this regard, pilot results suggest that the retronasal test may discriminate eudentulous from edentulous flavor perception.

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Sensitivity to 6-n-propylthiouracil predicts hedonic response to sucrose. HEATHER LOOY & HARVEY P. WEINGARTEN (McMaster University).

Evaluation of the hedonic response to sucrose solutions (.05 to .83 M) in 117 adults and 60 children revealed two distinct populations: "sweet likers" showed increasingly positive ratings with increasing concentration, while "sweet dislikers" reported increasingly negative ratings with increasing concentration. These hedonic responses were obvious to naive observers: Subjects were videotaped while tasting the solutions. Raters blind to the subjects' hedonic ratings were able to reliably classify the subjects as sweet likers or dislikers on the basis of their facial responses to the taste of .83 M sucrose. The sweet liker/disliker distinction was strongly related to the genetic ability to taste 6-n-propylthiouracil (PROP). In both adults and children, PROP nontasters were sweet likers, and sweet dislikers were PROP tasters:

	Adults		Children	
	Liker	Disliker	Liker	Disliker
Taster	20	67	21	22
Nontaster	28	2	17	0
	$X^2=45.62$, $p<.0001$		$X^2=13.73$, $p<.0005$	

Subjects were asked to describe the taste quality of sucrose solutions to explore whether a difference in quality mediates the relationship between PROP sensitivity and hedonic response to sucrose. Sweet dislikers tasted a "purer" sweet quality than likers, who reported the presence of other qualities apart from sweet in the solutions.

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Apparent Specific Volumes and Tastes of Sugars and Polyols

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The well-established increase in apparent molar volume (ca 10%) which accompanies hydrogenation of sugar molecules has been investigated by precision densitometry of various pyranoses and polyols in aqueous solution. The volume increase is due to loss of cyclic structure rather than conversion of a carbonyl to an hydroxymethyl function. Introduction of an hydroxymethyl group to a polyol containing α-glycol groups causes a smaller increase in apparent molar volume (ca 16.3 $cm^3.mol^{-1}$) than introduction to a polyol without α-glycol groups (ca 18.5 $cm^3.mol^{-1}$). Introduction of an hydroxymethyl group to a pentopyranose molecule causes an increase of ca 16.3 $cm^3\ mol^{-1}$ to form the corresponding hexopyranose which accords with the multi α glycol systems of the pentopyranoses and leads to a constant ratio (1.17) of the apparent molar volume of hexopyranose to corresponding pentopyranose. This in turn suggests that the hydroxymethyl substituent does not interfere with the subtle interplay of hydrogen bonds between α-glycol groups which might generate sweetness differences in these types of compound. Introduction of the hydroxymethyl substituent into D- lyxose causes a rather large increase in apparent molar volume (16.9 $cm^3\ mol^{-1}$). The resulting hexopyranose, D- mannose, is the only simple aldopyranose exhibiting bitterness. These results may refine the prediction of taste by physicochemical methods.

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The Effect of Sweeteners on Bitter Taste Thresholds. SUSAN S. SCHIFFMAN[1], LARRY A. GATLIN[2], ELIZABETH A. SATTELY[1], BREVICK G. GRAHAM[1], SHIRLEY A. HEIMAN[2], WILLIAM C. STAGNER[2], AND ROBERT P. ERICKSON[1] ([1]Duke University and [2]Glaxo Inc.)

Many compounds have both bitter and sweet tastes which have led to the hypothesis that sweet and bitter receptor sites are proximate on taste cells (Birch and Mylvaganam, 1976). The purpose of this study was to determine if the addition of sweeteners to solutions of bitter compounds modified the detection and recognition thresholds for these compounds. Detection and recognition thresholds were determined for six bitter compounds (quinine hydrochloride, caffeine, urea, magnesium chloride, sucrose octaacetate, and denatonium benzoate) in the absence and presence of several suprathreshold concentrations of five sweeteners. The sweeteners were: aspartame (3.36×10^{-4}M, 6.72×10^{-4}M, and 1.12×10^{-3}M), sucrose (0.195M and 0.39M), sodium saccharin (2.83×10^{-4}M and 5.66×10^{-4}M), sorbitol (0.921M and 1.84M), and mannitol (0.837M and 1.67M). The concentrations of the sweeteners were fifteen and thirty times greater than the detection threshold for that sweetener. Aspartame was also tested at fifty times detection threshold. In general, the detection and recognition thresholds for bitter compounds mixed with sweeteners were not significantly different from the detection and recognition thresholds of the bitter compounds alone. There was a slight trend for higher recognition thresholds with quinine hydrochloride and urea. This finding is noteworthy since sweeteners are often used to mask bitter taste. These data also suggest that bitter and sweet receptor sites do not necessarily overlap.

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Covariance of Panelist Stimulus Concentration/Response Functions Among Thirteen Sweeteners and Implications Regarding Receptor Multiplicity.

GE DUBOIS[1], SS SCHIFFMAN[2], ZS WARWICK[2], S PECORE[1], B BOOTH[1], DE WALTERS[1], T CARR[1], K GIBES[1], AND L BRANDS[1] ([1]NutraSweet Company and [2]Duke University)

If the activities of all sweet tastants are mediated by a common cellular activation system, then all of the sweetener Concentration/Response (C/R) functions should be highly correlated between subjects. On the other hand, if the activities of different sweeteners are mediated by different cellular activation mechanisms or different receptors, then correlations should be observed only between sweetener pairs mediated by common pathways. The purpose of this study was to compare C/R functions obtained with a diverse group of sweeteners. Eight men and five women were trained to anchor sweetness intensity ratings to sucrose standards (2-16% wt/vol). Sweetness ratings were obtained from all subjects for at least seven concentrations of each of the following sweeteners: Sodium Saccharin, Acesulfame-K, Sodium Cyclamate, Aspartame, Glycine, Neohesperidin Dihydrochalcone, Rebaudioside A, Stevioside, Thaumatin, Fructose, Glucose, Maltitol and Lactitol. The C/R functions for the first nine of these sweeteners are best fit by a hyperbolic function of the form $R = (R_m \times C)/(K_d + C)$. The last four of the above-mentioned sweeteners, all of which are carbohydrates of low sweetness potency, give linear C/R functions. It is concluded as a consequence of the linearity of these functions that the carbohydrate sweeteners are mediated, at least in part, by a pathway not available to the other sweeteners. For the sweeteners exhibiting hyperbolic C/R functions, K_d values were determined for each subject for each sweetener. Correlation coefficients (r) were then calculated for the K_d values of sweetener pairs. The results of this analysis are consistent with the conclusion that activities of the nine sweeteners are mediated by a multiplicity of pathways. The most plausible explanations for multiple pathways are different molecular recognition units (receptors) and/or different transduction processes.

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Individual Differences in Effects of Mouth Movements on Intensity of Sweetness.

DAVID A. STEVENS (Clark University)

Previous work has shown that individual differences in levels of self-produced cueing and private body consciousness (PBC) predict individual differences in psychophysical responses to tastants and oral irritants. These measures classify people on the basis of the extent to which they utilize cues from internal sources and actions relative to those from external, situational sources. Self-produced cuers and people with high PBC show psychophysical functions with steeper slopes than do situational cuers and those with low PBC. The present work tested the hypothesis that some of the cues used differentially by these people are related to mouth movements. Subjects, classified for PBC, made magnitude estimates of the intensity of sweetness of sucrose solutions ranging in concentration from .12M to .79M while tasting with normal and exaggerated mouth movements. Analysis of the magnitude estimates by ANOVA showed a significant interaction between level of PBC and type of mouth movements. Exaggerated mouth movements produced lower estimates of intensity than normal movements for low PBC subjects, while the opposite was found for high PBC subjects.

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Cephalic Phase Insulin Release: relationship to post-prandial insulin and glucose levels.

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KARL ENGELMAN (Hospital of the University of Pennsylvania)

In humans, the functional significance of cephalic phase insulin release (CPIR) is not known. Animal experiments suggest that CPIR may be involved in the regulation of blood glucose although few human studies have attempted to address this issue. This experiment examined the relationship between insulin released during the cephalic phase time period (0 to 10 minutes post-ingestion) and insulin released post-prandially (12 to 240 minutes post-ingestion).

Normal weight male subjects (n=11) with no family history of diabetes were administered an aspartame-sweetened dessert composed of gelatin and dairy fat, twice over a 3-day period. Four baseline blood samples were taken prior to food administration, followed by collections at 2 minute intervals for 14 minutes. Samples were then taken every 15 minutes for 225 minutes. Insulin released during the cephalic phase time period was significantly increased over baseline when both area under the curve ($p < 0.05$) and individual timepoints were compared ($p < 0.001$). The cephalic phase insulin area was found to be highly correlated with post-prandial insulin areas at 90 minutes post-ingestion (r=0.93, Day 1; r=0.84, Day 2). CPIR area was also significantly correlated with post-prandial glucose areas at 90 min (r=0.64; r= 0.54). These descriptive data suggest that a significant relationship exits between pre- and post-absorptive insulin release although the physiological function remains to be elucidated.

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Developmental Changes in the Response of Human Infants to Bitter Tastes.

H. KAJIURA[1,2], B.J. COWART[1] AND G.K. BEAUCHAMP[1] ([1]Monell Chemical Senses Center; [2]Kirin Brewery Co., Ltd.)*

Human sensitivity to sweet tastes appears to be well developed at birth, whereas there are indications of increasing sensitivity to the taste of salt in the first months of life. There have been no comparable, published studies of sensitivity to bitter or sour tastes. We examined ingestive responses in infants $\leq$ 6, 14-90 and 91-180 days of age to a stimulus typically described by adults as bitter. Infants were presented solutions of 0.07 M sucrose with and without urea (either 0.12 M, 0.18 M or 0.24 M in a between subjects design) and allowed to consume as much as they wished of each in a 30 sec period. Total volume ingested and a variety of sucking parameters (recorded via a pressure transducer inserted into the nipple) were assessed. Both infants less than 7 days of age and 14- to 90-day-old infants consumed equivalent amounts of the sucrose solution alone and of solutions to which 0.12 M or 0.18 M urea had been added, although both groups tended to reject the 0.24 M urea solution relative to the diluent. Older infants, however, consumed less of all 3 concentrations of urea than of the diluent alone. Similar patterns of developmental change were observed in some, but not all, parameters of sucking behavior. These data suggest that human sensitivity to bitter, like that to salt, increases in the first several months of life.

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Regional Differences in Sensitivity to Astringent Compounds in the Oral Cavity of Humans. SUSAN S. SCHIFFMAN, SIDNEY A. SIMON, BREVICK G. GRAHAM (Duke University)

Regional differences in sensitivity to chemical stimuli have been reported on the tongue and oral cavity. For example, the anterior tongue is more sensitive to salts, and the posterior to bitter. The purpose of this study was to determine if there are regional differences in sensitivity to astringent compounds. Seven and one half microliter drops of six different astringent compounds were applied at suprathreshold concentrations to a circumvallate papilla, to the anterior tongue and to the buccal mucosa. Seven dilutions of each of the following astringent compounds were tested: tannic acid (4×10^{-4} M to 2.56×10^{-2} M), tartaric acid (6.25×10^{-3} M to 4×10^{-1} M), gallic acid (1.1×10^{-3} M to 6.73×10^{-2} M), catechin (1.56×10^{-4} M to 1×10^{-2} M), aluminum ammonium sulfate (1.56×10^{-3} M to 1×10^{-1} M), and aluminum potassium sulfate (2×10^{-4} M to 1.28×10^{-2} M). NaCl (1.56×10^{-1} M to 5.0 M), quinine HCl (4.87×10^{-5} M to 1.56×10^{-3} M), citric acid (3.9×10^{-3} M to 1.25×10^{-1} M), and sucrose (5×10^{-2} M to 1.6 M) were used as controls. On each test day, the drops were applied to the same area of the tongue or oral cavity. Subjects were more likely to report an astringent sensation in the circumvallate region than on the anterior tongue or buccal mucosa. For each of the three regions, subjects were also more likely to report an astringent sensation when drops larger than 7 1/2 microliters were applied. The regional differences found in this experiment suggest that the glossopharyngeal nerve is more sensitive to astringent stimuli than the nerves innervating the anterior tongue.

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Detection Thresholds of Potassium Salts Are Related to the Molar Conductivity of the Anion. SUSAN S. SCHIFFMAN, ALVIN L. CRUMBLISS, ZOE S. WARWICK (Departments of Psychology and Chemistry, Duke University)

In a previous study (Schiffman et al., AChemS-XI), human taste detection thresholds for sodium salts were found to be linearly correlated with molar conductivity values at infinite dilution of their anions. The purpose of this study was to determine if the same relationship holds for potassium salts. Detection thresholds were found for nine potassium salts with the same anion as the sodium salts previously tested. The mean detection thresholds for these potassium salts are: K Acetate (0.00311 M), K Carbonate (0.00286 M), K Chloride (0.00242 M), K Citrate (0.000295 M), K Phosphate (0.00196 M), K Sulfate (0.0009 M), K Tartrate (0.00164 M), K Glutamate (0.00153 M) and K Ascorbate (0.00375 M). The rank order correlation between the detection threshold values for sodium and potassium salts was 0.88. Like the sodium salts, detection threshold concentrations for potassium salts were found to be linearly correlated ($r = -0.92$) with the molar conductivity of the anion of the salt. Molar conductivity values (expressed in $ohm^{-1}cm^2mol^{-1}$) were obtained from data tabulations at infinite dilution and 25°C. Molar conductivity is directly proportional to both ionic mobility and ionic charge, i.e. electrical charge carried by the anion per unit time. This suggests that detection thresholds for both Na and K salts are determined by the charge mobility of the anion.

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The Taste, Odor and Flavor Modifying Effects of NaCl and MSG

SARAH E. KEMP and GARY K. BEAUCHAMP (Monell Chemical Senses Center, Philadelphia, PA)

NaCl and MSG are added to foods in the belief that they enhance taste and flavor. Little research has been reported on the taste and flavor modifying properties of NaCl and, although more research has been done using MSG, the results are confusing. It is unclear whether enhancement actually occurs or whether any taste and flavor modification is caused by the presence of the tastes of NaCl and MSG. In order to address this issue, a ranking procedure was utilized. Subjects were given five solutions which contained the same concentration of a tastant or flavor but different concentrations of NaCl or MSG, corresponding to zero, just below, at and above the approximate average detection threshold of the subjects and a level which might normally be used in foods. Subjects were asked to rank the solutions in order of strength of the taste, odor of the flavor or the flavor. NaCl showed no obvious enhancement effects on taste. For MSG, sweetness and bitterness were ranked as higher at detection threshold concentration, possibly indicating enhancement. Due to the addition of components of their tastes, saltiness increased with concentration for NaCl and MSG and, for NaCl, sweetness was ranked as slightly higher at detection threshold concentration. At supra-threshold levels, NaCl suppressed sweetness, sourness and bitterness, and MSG suppressed sweetness and bitterness. NaCl and MSG showed no effect on the odor or flavor of a variety of flavorings except for mint, where suppression occurred as concentration increased. This may be due to modifying effects on the trigeminal component of mint flavor.

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Salt Preference in Anglo and Hispanic Preschoolers RANI NIJJAR, KRISTY STRAITS, JILL SNIFFEN, MAGGIE GILMORE, and CLAIRE MURPHY (San Diego State University and UCSD, San Diego, CA)

We characterized salt preference among 50 Anglo and 50 Hispanic preschool children (mean age = 4.4) and one parenttof each child. All participants tasted three different diluents (deionized water, low-sodium chicken soup, and no-sodium V-8 juice) mixed with five different concentrations of salt (0.0, .05, .10, .20, and .40M NaCl). Participants rated their judgements of the pleasantness or unpleasantness of the stimuli on a bipolar line scale (visual analog scale) with a zero midpoint and no endpoints. Training preschoolers for this task began with asking the children to indicate by the span between their hands how much they liked or disliked self-reported familiar foods. Children then were asked to build lines on the visual analog scale with wooden toy blocks, to indicate taste preferences. Finally, they drew lines on the scale to correspond with preferences. When the child demonstrated competence in drawing lines on the visual analog scale that corresponded with hand-span ratings and paired comparison ratings, training was complete. The stimuli were then presented twice, in random order, and the scaling data were obtained. ANOVA on the preference ratings showed Hispanics to have higher salt preference than Anglos, and preschoolers to have higher salt preferences than adults. Approximately two years later, follow-up testing was conducted on 88 children (mean age = 6.5) to investigate the possibility of developmental shifts in salt preference. Results will be discussed from this perspective. Understanding the development of salt preference in young children may suggest strategies for behavioral interventions which will reduce salt intake.

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A Cross-Cultural Study of Preferences for Common Tastes

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As part of a large scale, cross-cultural study of the taste preferences we evaluated the preferences of groups of Japanese and Australians using seven common tastants. Thirty five panellists at laboratories in Tokyo and Sydney rated their liking for sweet (sucrose), salty (NaCl), sour (citric acid), bitter (caffeine), and *umami* (MSG, IMP, GMP) solutions at 6 widely spaced concentration levels.

Both groups showed similar patterns of hedonic ratings across the concentration range for sweet and bitter solutions. For all other tastants, Japanese showed a greater preference at the highest concentration. For the salty and two of the *umami* (IMP and GMP) solutions, the Japanese showed greater preference across most of the concentration range.

Given that high concentrations of these tastants were relatively unpleasant, these results also show that Japanese will tolerate higher levels of most of the tastants.

Whether these differences are maintained within the context of food preferences (e.g., higher salt levels in cooked food) remains to be determined and is currently under investigation.

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Learned Food Aversions: A Family Study.

RICHARD D. MATTES (Monell Chemical Senses Center)

A genetic basis for susceptibility to food aversion learning (LFA) has been identified in rats and evidence in humans suggests a familial association. To explore this latter observation further, 100 families with two parents and at least two of their biologically related offspring completed a questionnaire eliciting health, demographic and dietary information. A significant familial association (X^2=16.8, P<0.001) was noted for history of aversions. This was strongest for fathers and offspring (X^2=8.8, p<0.005 father-son; X^2=5.1, p<0.025 father-daughter). Associations between mothers and offspring were not significant but the difference in proportions between offspring and each parent was not significant. A stronger concordance was observed between brothers than sisters for food targets. This study also allowed expanded characterization of LFA in the general population. It was found that 37.5% of the sample had formed a LFA at some point in their life and 26.3% currently held an LFA. Through adolescence, there is a similar gender distribution, but from the third decade of life onwards, the female:male ratio is approximately 2:1. The mean aversion duration is about 17 years. Meats are the most common target for LFA. Associations were observed with allergy (X^2=12.0, p<0.001) and medication use (X^2=6.9, p<0.01). LFA was related to body mass index (a measure of body fatness) among adults (X^2=7.4, p<0.01), but not children or adolescents. These observations suggest there may be a sub-group of individuals at elevated risk for food aversion formation. This trait may hold direct or prognostic nutritional implications.

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Time Course of Adaptation in Moth Pheromone Receptor Neurons. Paola F.BORRONI and Robert J. O'CONNELL (Worcester Found. for Exp. Biology, Shrewsbury, MA 01545 USA)[1]

The temporal pattern of response in chemoreceptor neurons reflects several processes: 1) the physical and temporal distribution of the effective stimulus, 2) the time course of the biophysical and biochemical steps underlying transduction and 3) the subsequent sequence of events associated with spike generation and propagation. The patterns of response elicited by temporally controlled pulses of pheromone were evaluated in extracellular recordings from moth pheromone receptor neurons. Two olfactory neurons can be identified in the HS sensilla trichodea of *Trichoplusia ni*: the "A" neuron, which responds to the major component of the pheromone blend (Z7,12:AC) and the "B" neuron, which responds to Z7,12:OH. These neurons were stimulated with three doses of these materials at two durations (10s for the two higher doses and 40s for the lower dose). The "B" neurons were classified into two types (HR=High Response; LR=Low Response), based on the magnitude and temporal pattern of their response to Z7,12:OH. Most "A" and HR "B" neurons responded to all of the stimuli tested with a short phasic component (50-70ms and 40-50ms respectively), followed by a slowly declining tonic component which lasted as long as the stimulus. In contrast, the LR "B" neurons appear to lack a phasic response component. Spikes were counted in four 1s windows: three, at the beginning, middle and end of the stimulus, and one just after the end of the stimulus. Response duration was defined from the beginning of the response (first spike after stimulus onset) to the last spike with an interspike interval < 3 s. A small but significant decline in response (10-20%) was observed in the "A" and HR "B" neurons, for the two higher doses, i.e. responses were adapting while the stimulus was held constant. Responses declined rather quickly (~60%) in the first few seconds after the stimulus was turned off. In the LR "B" neuron the number of spikes counted at different times was fixed, i.e. adaptation was *not* observed. Future studies will describe the time course of adaptation with brief test pulses of a pheromone component superimposed, at various times, on a long duration background exposure to a lower concentration of the same compound.

[1] Supported by NRSA Fellowship DC00011-02 to PFB, and NIDCD Grants DC00131 and DC00371 to RJO.

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Differential Responses from Antennal Sensilla in the Female Arctiid Moth *Utetheisa ornatrix* (Lepidoptera: Arctiidae). ALAN J. GRANT, ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545), and THOMAS EISNER (Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853).

The Ornate Moth, *Utetheisa ornatrix* possesses bilateral filiform antennae each containing a variety of sensory structures, including many sensitive to olfactory signals. We previously have shown that the short sensilla basiconica on the female antennae are innervated by one or two spontaneously-active receptor neurons. One of these neurons responds to low doses of the male-produced close-range pheromone, hydroxydaniadal (HD). HD is a biochemical derivative of the pyrrolizidine alkaloid, monocrotaline, which is found in the larval host plant, *Crotalaria spp*. The longer sensilla trichodea, which have classically been associated with pheromone detection, are also innervated by at least two receptor neurons. However, neither of these neurons respond to HD. This raises the question: To what array of chemical signals do the female sensilla trichodea respond? When disturbed, many aposematically colored arctiids, including *U. ornatrix*, exude from their thoracic glands a frothy material believed to be defensive in nature. The exact chemical composition of this material is unknown; however it is thought to be composed largely of blood. For s. trichodea, the neuron producing the larger amplitude action potential is consistently stimulated by the thoracic froth produced by female moths. The neuron producing the smaller amplitude action potential, on the other hand, responds to froth collected from male moths. These findings suggest that intraspecific chemical signals may also be released with the froth. Interestingly, blood collected from male and females moths also elicits this dichotomous response pattern from trichoid sensilla, although larger amounts of blood are required to elicit responses comparable to those evoked by the thoracic froth. Additionally, a neuron housed in these s. trichodea can also respond to low concentrations of one of the major components of the female produced blend, (Z,Z,Z)-3,6,9-heneicosatriene. These studies demonstrate: (1) S. trichodea, classically associated with detection of sex pheromone components, can also respond to compounds presumably not emitted from the pheromone gland. (2) This class of sensilla contains neurons specifically responsive to male or female emissions. (3) Neurons in these female sensilla respond to low concentrations of a component found in her own pheromone blend.

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Responses of Three Olfactory Pheromone Specialist Neurons of Male Trichoplusia ni (Hübner) to Six Pheromone Components at Concentrations Emitted by Calling Females. M. S. MAYER (USDA, ARS, Insect Attractants, Behavior and Basic Behavior Research Laboratory, Gainesville, FL. 32604)

Three ensembles of sensilla on the antenna of Trichoplusia ni (Hübner) house an olfactory receptor neuron that responds to only one component of virgin female volatile emissions at naturally emitted concentrations. The HS(a) neuron responds to Z-7,12:Ac at concentrations ranging between 1 X 10^{-15} to 1 X 10^{-10} M; the LS(b) neuron responds to Z-7,14:Ac concentrations ranging between 1 X 10^{-15} to 1 X 10^{-10} M. The threshold of a recently identified neuron (designated NS(a)) for Z-9,14:Ac is near 1 X 10^{-18} M. At concentrations exceeding that emitted by the female, each of these neurons responds to at least five other compounds. The olfactory pheromone receptor neurons of the cabbage looper are, consequently, concentration-tuned specialists. The EAG predicts the number of specialists on the antenna when the stimulus concentration of the various emission components is accounted for. The volatiles emitted from freshly excised virgin female sex pheromone glands elicit a response from each specialist that is representative only of its most effective stimulus, i. e., there is no evidence of peripheral mixture interactive effects by other compounds within the female effluvium. None of the other neurons within the sensilla housing the specialists respond reliably to the volatile emissions from freshly excised sex pheromone glands.

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Mechanisms of cumulative and background adaptation in lobster olfactory receptor cells. W.C. MICHEL, M. WACHOWIAK and B.W. ACHE. Whitney Lab. and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

Although numerous studies have considered the influence of adaptation on olfactory receptor cells, the physiological basis of adaptation remains poorly understood. Whole-cell recording was used to study adaptation in lobster olfactory receptor cells. Discontinuous and continuous odor presentation mimicked cumulative (*i.e.*, influence of previous response history of a cell) and background adaptation, respectively. Both the receptor current and the spike generator were considered as sites of adaptation. Excitatory receptor potentials showed two patterns of adaptation during continuous stimulation. Approximately 70% (27/39) of the cells tested repolarized slowly and only partially during 2-4 min of continuous stimulation with 0.01 - 1 mM odorants. Twelve cells, in contrast, repolarized more rapidly and completely within the first 30 sec. of continuous odor presentation. The two patterns of adaptation may be odor dependent in that 8 of 11 taurine-sensitive cells adapted completely, whereas only one each did so of the 6 arginine, 7 betaine, 9 glutamate and 6 proline sensitive cells. Dendritic application of ouibain during continuous odor presentation blocked the slow repolarization in two cells that were tested, implicating an electrogenic Na^+/K^+ pump in slow adaptation of the receptor potential. The involvement of an electrogenic pump has not yet been investigated on cells which repolarized completely during continuous odor presentation. Inhibitory receptor potentials repolarized slowly and only partially during continuous odor stimulation similar to the majority of excitatory responses. The encoded action potentials are characterized by a high frequency but rapidly adapting phase of < 500 msec. followed by tonic output which adapted in less than 30 sec. of continuous stimulation. Discontinuous stimulation simulating natural sampling (*i.e.*, duration < 2000 msec.) cumulatively adapted of the phasic, but not tonic, component of the action potential train. Peak instantaneous frequencies of *ca.* 70 Hz only occurred in the phasic component of the first of a series of 3-5 stimulations. Subsequent stimulations never evoked this level of activity unless the frequency of stimulation was below 0.1 Hz. This rapid adaptation of the output of lobster olfactory receptor cells cannot be accounted for by the slower adaptation of the receptor current. Presumably, the cumulative adaptation of cell output is a function of the inactivation of voltage-dependent sodium channels involved in action potential generation whereas a dendritic electrogenic pump is involved in background adaptation. Experiments testing these hypotheses will be reported.

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Calcium-Related Conductances in Frog Olfactory Receptor Neurons. STEVEN J. KLEENE, RAYMUND Y.K. PUN, and ROBERT C. GESTELAND (University of Cincinnati College of Medicine, Cincinnati, OH 45267).

An influx of Ca^{2+} may play a role in odorant transduction in some olfactory receptor neurons. We have measured the effects of cytoplasmic Ca^{2+} on the conductance of single cilia excised from frog olfactory neurons. When free cytoplasmic Ca^{2+} is buffered at 0.1 μM, ciliary conductance is low. As Ca^{2+} is increased, ciliary conductance increases. Maximal conductance averages 6-fold higher than that measured in the absence of Ca^{2+}. We estimate that the $K_{1/2}$ for Ca^{2+} is about 3 μM. Activation by Ca^{2+} is rapid and fully reversible. The reversal potential of the Ca^{2+}-activated current shifts 57 mV for each tenfold change in cytoplasmic Cl^- concentration, suggesting that Cl^- is the principal current carrier. The current persists in the absence of Na^+ and K^+. Analogues of the Cl^--channel inhibitor diphenylamine 2-carboxylate reduce the Ca^{2+} current by as much as 90%. We cannot yet define the role of the Ca^{2+}-activated ciliary Cl^- conductance, since the intraciliary concentrations of Ca^{2+} and Cl^- are unknown.

To look for Ca^{2+} currents in the intact neuron, we apply 25 mM tetraethylammonium ion (TEA) externally. This depolarizes the membrane and prolongs the duration of the action potential several-fold. Under voltage-clamp with K^+ currents blocked, a sustained non-inactivating inward current is recorded in the presence of 5 mM extracellular Ca^{2+}. This current activates near -20 to -10 mV and peaks near +10 to +20 mV. The sustained current shows no inactivation even with 500-msec pulses, but shows marked rundown, disappearing within 10 min after the onset of whole-cell recording. Rundown of the fast inward current is not seen.

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Voltage-Sensitive Dye Confocal Microscopy of Living Olfactory Epithelia. JAN N. BROUWER, PEGGY FARMER, & ROBERT C. GESTELAND (University of Cincinnati College of Medicine, Cincinnati, OH 45267) & JUDY DRAZBA (Laboratory of Neurobiology, NINDS, Bethesda, MD 20892).

Different cell types of the olfactory epithelium are surprisingly selective in their affinities for voltage-sensitive dyes. A given cell type in different animal species differs in its affinity for particular dyes. These differences can be exploited to selectively measure odor-evoked changes in receptor neurons and in supporting cells. Dye uptake and stimulus responses were compared in the tiger salamander (*Ambystoma tigrinum*), grass frog (*Rana pipiens*) and California newt (*Taricha torosa*). Olfactory epithelia were stained with voltage-sensitive styryl dyes RH414 and RH795, which are of the membrane-binding type. Fluorescence of thin optical sections was examined with a laser-scanning confocal microscope. The epithelial surface and epithelial slices were also examined with a conventional fluorescence microscope. Responses to odors were displayed by subtracting the video image of the fluorescence intensity of the unstimulated preparation from that acquired following stimulation. Sensory neurons and supporting cells can be selectively stained, depending upon the type and concentration of the dye used. In the frog, RH414 in low concentrations predominantly stained supporting cells, including the surfaces of intracellular secretory granules. At higher concentrations the apical portions of neuron dendrites and their motile cilia were the most intensely stained elements in the epithelium. In the frog, RH795 lights neurons more brightly than supporting cells. With RH414, the contrast is lower. In the salamander, both RH414 and RH795 stain neurons more intensely than supporting cells with higher contrast between cell types than in the frog. At all concentrations we used, the differences between the cell types was more pronounced with RH795. In the newt, both RH414 and RH795 have much higher affinities for neurons than for supporting cells. The most contrast was obtained with RH795 in the newt. Uptake by supporting cells was so small that neuron dendrites were brilliant against a dark field when viewing the epithelial surface with conventional fluorescence microscopy. In all preparations neither dye had any apparent effect on ciliary movement, which was used as a criterion for preparation viability. We expect that appropriate dyes will be found which allow clear discrimination of differential odor responses of separate neural elements in vertebrate olfactory epithelia.

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Perireceptor events: Direct determination of diffusion coefficients in the olfactory mucus layers of salamanders.
MARILYN FRIEDEMANN, PAUL A. MOORE, THOMAS E. FINGER*, WAYNE L. SILVER+, and GREG A. GERHARDT. Departments of Pharmacology and Psychiatry, *Department of Cellular and Structural Biology, University of Colorado Health Sciences Center, Denver, CO 80262, +Department of Biology, Wake Forest University, Winston-Salem, NC 27109.

Perireceptor events are important aspects of chemoreception. These include the movement of chemicals by turbulence and diffusion, the binding of odorants to transporter proteins and diffusion to receptor sites. In terrestrial olfaction, odor molecules must diffuse through a mucus layer before binding with receptor proteins and subsequently back through the mucus layer to be removed from the olfactory epithelium. Thus, the diffusion properties of molecules in mucus can be important in the perception of odor. Yet, due to the problems associated with the direct measurement of chemicals at the spatial and temporal scales relevant to chemoreceptor cells, the transport and fine scale structure of chemicals in air, water, and mucus has remained relatively unknown. With the introduction of new recording technology to chemoreception, we now have the capability to measure chemical dynamics at high spatial (30μm) and temporal (up to 200 Hz) resolution. Using *in vivo* electrochemical recordings combined with local drug application techniques, we have determined the effective diffusion coefficients of positively charged, negatively charged, and neutral molecules in the mucus layer covering the olfactory epithelium of the salamander. In addition, natural odorants were measured. These measurements were performed by securing single carbon fiber electrodes (diameter 30 μm) to single-barrel micropipettes (tip diameters 10-15μm) at known distances (150-350μm). Concentrations of chemical signals are directly proportional to the current produced by the oxidation or reduction of those molecules at the electrode surface. These studies are needed to understand the role that diffusion and transporter proteins play in determining the structure of chemical signals as they arrive at the receptor cells.
Support by USPHS (AG00441) and NSF 2534920 to GAG.

Effects of Unilateral Occlusion of Nasal Cavity on Transport of Cadmium During Inhalation Exposure.
LLOYD HASTINGS (University of Cincinnati)
JAMES E. EVANS (University of Cincinnati)

While the potential of the olfactory system to serve as a route of entry for cadmium into the CNS has been demonstrated using intranasal instillation of radiolabeled compounds (Hastings et al., Chem. Senses 19, 1988), the importance of this route during inhalation exposure has not been explored. The significance of entry and transport of cadmium into the CNS via the olfactory system would be strengthened if it was demonstrated that transport of cadmium occurred when cadmium entered the nasal cavity naturally, i.e. via inhalation, as opposed to a liquid gavage. Moreover, limiting the exposure primarily to only one side of the system with the other side serving as a control preparation would also be advantageous. Occluding one nostril during exposure via inhalation accomplished this goal. (Some crossover may occur via the "septal window"; the extent will be determined empirically.) Two groups of rats were exposed to CdO via inhalation. One group (10 rats) was exposed to filtered air only. A second group of 10 rats was exposed to 500 $\mu g/m^3$ CdO for 5 hrs/day, 5 days a week, for 10 weeks. Prior to exposure, one nostril in each rat was occluded by placing a small length of PE 20 tubing flared at one end, into the anterior naris. At the end of exposure, the rats were sacrificed and the right and left olfactory epithelium, bulb, and brain were saved for determination of cadmium content by atomic absorption spectrometry. Carnosine analysis of the bulbs as well as histological examination was also performed.

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SYMPOSIUM
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Olfactory Competence Over the Life Span

JOSEPH C. STEVENS, INGRID K. JOHNSTON, CONNIE M. NICKOU, AMY M. RUTHRUFF, and WILLIAM S. CAIN (John B. Pierce Laboratory, New Haven, CT 06519

This study examined (a) olfactory sensitivity (thresholds to butanol, measured by a forced-choice version of the method of limits), (b) odor identification (OID) of common household objects, and (c) picture identification (PID) by the Boston Naming Test; (b) and (c) were tested with and without name prompting. There were 125 Ss, 25 each in five age groups: (1) childhood (25 Ss, 8-14 yrs), (2) young adulthood (18-28 yrs), (3) early middle age (32-47 yrs), (4) late middle age (48-62 yrs), and (5) old age (66-90 yrs). The purpose was to try to disentangle more or less purely sensory from cognitive changes with age. Threshold sensitivity proved to be the same in childhood as it was in young adulthood, but it declines systematically thereafter with age group, by about 10-fold altogether on the average. OID and PID turned out to be relatively poor in the childhood years--probably underdeveloped because of limited experience. Performance on OID and PID reached their maximums in young adulthood and changed little therafter, except in old age, when both fell off considerably, OID more so than PID. Prompting facilitated OID and PID (an old finding), and OID more so than PID. Thus, OID seems to be limited in childhood by cognition (experience with odor naming) and in old age by both insensitivity and cognitive decline, which is attributable in part but not whole to difficulty in retrieving names. Supported by NIH Grant AG04287.

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Aging, olfaction and food preferences.

MARCIA LEVIN PELCHAT (Monell Chemical Senses Center). CARYN STOESS (University of Oregon).

It is important to understand how age and the olfactory deficits which often develop with age affect food preferences because the elderly are such a rapidly growing segment of the population. Elderly (65 or older) and young adult (18-35) subjects were given a food preference test using foods that varied in pleasantness of odor and in familiarity and were given an olfactory function test. Old age and poor olfaction were associated with increased willingness to try novel foods, however, the mechanism for this effect may not have been specific to novel foods. Odor pleasantness was an important determinant of intake measures for young subjects but was unimportant for elderly subjects, especially those with poor olfactory ability. These findings suggest that there is a decline in finickiness with increasing age and/or with decreasing olfactory ability. One obvious mechanism for the decreased finickiness of subjects with reduced olfactory ability is that they do not notice aversive aspects of food odors as much as do subjects with better olfaction. These findings are based upon acceptance/rejection tasks where factors like availability and cost don't come into play. To get a rough idea about the subjects' usual diets where availability and cost do matter, we administered a questionnaire on their use of approximately 100 commonly eaten foods. Elderly subjects appeared to have less varied diets than young subjects. The finding of decreased finickiness from the laboratory and the finding of decreased dietary variety from the questionnaire are not necessarily contradictory, but may be relevant in different settings.

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Abnormal Olfactory Epithelium in Biopsies of Alzheimer's Patients

BRUCE W. JAFEK, CHRISTOPHER M. FILLEY, PAMELA M. ELLER, MARY M. CHAPMAN, EDWARD W. JOHNSON, DAVID T. MORAN (Rocky Mountain Taste and Smell Center)

The currently accepted method of obtaining biopsy specimens of olfactory epithelium in humans was developed at the Rocky Mountain Taste and Smell Center (RMTSC) and has been used by us to characterize the ultrastructure of the epithelium in the normal subject, Moran, *et al.* (1982), as well as in pathological cases, Jafek, *et al.*(1989, 1990). In conjunction with an extensive project in the Neurology and Psychiatry Departments at the University of Colorado Health Sciences Center (UCHSC) involving patients with Alzheimer's Disease (AD), we have tested and biopsied eight patients and two age-matched controls. Many more patients are available to us and we intend to expand this study based on the results of these initial biopsies. All patients in the study undergo complete physical and neurological examination. The diagnosis of probable AD has been established according to the criteria set forth by the NINCDS-ADRDA, McKhann, et al. (1984), and all patients meet DSM-III criteria for Primary Degenerative Dementia. The severity of the disease has been rated according to the patient's score on the Mini Mental Status Exam with mildly affected patients scoring 19 to 23 , and moderately affected patients scoring 14 to 18 (out of 30). All patients who have participated in the olfactory testing and biopsy have been classified as Stage 4 or 5 on the Global Deterioration Scale (GDS), indicating that they are moderately affected by the disease. The olfactory testing consists of the University of Pennsylvania Picture Identification Test (UPPIT) followed by the University of Pennsylvania Smell Identification Test (UPSIT). In addition, we also administer a butyl alcohol threshold and a seven-item odor identification test, Cain, *et al.* (1983, 1989). We also subject each patient to multiple taste tests. Each patient seen so far has tested as severely hyposmic or anosmic. Biopsies containing olfactory epithelium have been obtained from all but one patient. The results of these biopsies will be reported.

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Olfactory Function in Progressive Supranuclear Palsy: An Index for Differential Diagnosis from Parkinson's Disease.

RICHARD L. DOTY, LARRY I. GOLBE, CHRIS M. LEHRACH, MATTHEW B. STERN, STEVE M. GOLLOMP and HOWARD W. HURTIG (Smell and Taste Center and Department of Neurology, University of Pennsylvania, Philadelphia, PA and Robert Wood Johnson School of Medicine, New Brunswick, NJ)

Olfactory dysfunction is present in nearly all patients with idiopathic Parkinson's disease (PD), including early stage unmedicated ones. It is unknown whether similar dysfunction occurs in Progressive Supranuclear Palsy (PSP). PSP shares with PD a number of clinical and neurochemical features, although, unlike PD, PSP has relatively normal dopaminergic function in mesolimbic and mesocortical pathways. We administered the University of Pennsylvania Smell Identification Test to 20 non-demented PSP patients (11 men, 9 women; respective mean (SD) ages = 67.1 (6.72) and 68.3 (7.57) years. Eighteen were also administered a single staircase odor detection threshold test incorporating the rose-like odorant phenyl ethyl alcohol (a stimulus with comparatively little intranasal CN V activity). The test scores of these groups were compared to analogous test scores obtained from 20 PD patients and 20 neurologically normal controls matched on the basis of age, gender, and ethnic background. The performance of the PSP patients on both olfactory tests was markedly superior to that of the PD patients [e.g., respective mean (SD) UPSIT scores = 31.59 (7.18) and 18.82 (6.94), with about half performing in the normal range. However, on average, the PSP patients still evidenced both odor identification and detection deficits relative to the matched controls [e.g., mean control UPSIT (SD) = 35.60 (4.06)]. These findings suggest that olfactory testing may be useful in the differential diagnosis of PD and PSP. A discussion of possible neurochemical bases for the differences in test scores between the PD and PSP patients will be presented.

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Post-Traumatic Dysosmia: Central VS. Peripheral.

ALAN R. HIRSCH, M.D. (Smell and Taste Treatment and Research Foundation.)
JOSEPH P. WYSE, M.D. (Smell and Taste Treatment and Research Foundation.)

Trauma is one of the most frequent causes of dysosmia. Traditionally it was attributed to pathology of cranial nerve I. To further delineate neural localization, 13 patients with traumatic dysomia were evaluated. The patient's ages ranged from 19-64 with an average of 38. All complained of impaired ability to smell following some form of head trauma and underwent blood testing including B12 level, RBC folate level, FTA, ESR, CBC, glucose, liver function test, electrolytes, total eosinophilic count, IgE level, PT, PTT and platelets. Also, urinalysis and 24 hour urinary MHPG levels were performed. All patients had smell test including Upsit, suprathreshold intensity, suprathreshold adaption, suprathreshold detection, suprathreshold recognition, suprathreshold identification, unilateral pyridine, and unilateral carbinol testing; 10 had unilateral phenol, unilateral phenone, unilateral PD lactone, unilateral cineole, and unilateral thiophane testing; 12 had unilateral Connecticut Home Olfactory Testing. All patients also underwent psychological testing including MMPI, MCMI, and Beck Depression Inventory. Two distinct populations were identified: 62% showed impairment in both threshold and suprathreshold testing, and 38% displayed normal threshold testing with impaired suprathreshold testing. Cranial nerve function is a necessary prerequisite for cortical integration. However, cortical damage can leave the patient unable to interpret smell despite a properly functioning peripheral apparatus. We suggest that threshold testing indicates peripheral nerve function, whereas suprathreshold testing indicates integrity of both peripheral nerve function and cortical integration.

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Comorbidity of Psychiatric and Chemosensory Disorders.

ALAN R. HIRSCH, M.D. (Smell and Taste Treatment and Research Foundation.)
THOMAS J. TRANNEL (University of Illinois.)

The olfactory lobe is part of the limbic system and therefore it would seem that a comorbidity would exist between emotional and chemosensory disorders. In order to investigate this, 46 patients presenting with chemosensory complaints of hyposmia (45), Hyperosmia (1), parosmia (5), phantosmia (16), hypogeusia (44), parageusia (9), and phantageusia (15) underwent psychiatric and neurologic histories and examinations, 67 different olfactory and gustatory tests, and written psychological testing including MMPI-II, MCMI-II, and the Beck Depression Inventory. Thirty-three percent (15) met a total of 30 DSM-IIIR axis one diagnoses; 96% (44) met total of 74 DSM-IIIR axis two diagnoses; and 4.3% (2) were felt to be psychiatrically normal and did not meet a DSM-IIIR axis one or two diagnosis. The most frequent axis one diagnoses were generalized anxiety disorder 22% (10), dysthymia 20% (9), and somatization disorder 11% (5). The most frequent DSM-IIIR axis two diagnoses were obsessive-compulsive personality disorder 35% (16), narcissistic personality disorder 22% (10), personality disorder not otherwise specified 22% (10), and histrionic personality disorder 20% (9). Possible explanations for these findings include: a structural overlap with a common pathogen of the olfactory/limbic system, a common physiologic dysfunction, sensory deprivation with impaired limbic response and/or narcissistic insult in response tc a primary bodily loss. It is also possible that the chemosensory complaint is the somatic manifestation of an underlying psychiatric disease.

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Taste Decisions: No Differences in Speed or Consistency of Sweet or Sour Rejection.

JOHN D. DELCONTE[1], STEVEN T. KELLING[1,2], BRUCE P. HALPERN[1,2,3] [Department of Psychology[1], Field of Physiology[2], and Section of Neurobiology & Behavior[3], Cornell University, Ithaca, NY 14853-7601]

Tastes of national or regional cuisines differ substantially, indicating learning of acceptable and unacceptable tastes of foods, but an aversion to sourness is often considered a fundamental human characteristic. We measured the frequency and speed of spitting or swallowing sour (10 mM citric acid [citric]), sweet (2 mM sodium saccharin [NaSac]) and mixed (mixture containing both [mix]) test liquids, using the perceived taste of one of them (the target taste) as the definition of what was to be spit, for fifteen subjects. Two of 8 test glasses contained citric, NaSac, mix, or distilled water, in random order. Start of contact was detected by a drinkometer; onset of spitting by emg from the lips; swallows by a laryngophone. RESULTS: Frequency of spitting the test liquids if = to a target liquid was >> than if ≠, but frequency of spitting or spitting reaction times did not differ between target liquids and corresponding test liquid. Swallowing reaction times if test liquid = target liquid were > than if ≠, and differed among the target tastes. Our observations fail to support the hypothesis that "natural" human behavior is to rapidly reject sour tasting substances and accept sweet items.

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Effects of metabolic state changes on sweet taste reactivity in rats and humans. HARVEY P. WEINGARTEN & HEATHER LOOY (McMaster University).

The exact relationship between changes in metabolic state and sweet reactivity are unclear. This relationship was first investigated in rats by comparing sham intake of sucrose solutions (ranging from .03-1.3 M) after 6, 12, or 18 hours food deprivation. The maximal level of sham intake increased monotonically with hours of deprivation. These changes are interpreted as a deprivation-induced alteration of sucrose palatability. However, animal experiments do not identify the mechanisms by which palatability shifts occur. To this end, we conducted the analogous experiment in humans. Using a "sip-and-spit" procedure, undergraduates rated the intensity and hedonics of sucrose solutions (range: .03-.83M) when sated or 18 hours food deprived. The amount sipped was also recorded as it reflects the best analogue to the intake measures obtained in rat sham feeding studies. Effects of metabolic state on sweet reactivity in humans depended upon subject's underlying hedonic response to sucrose. Metabolic state changes did not alter intensity or hedonics in "sweet likers" (subjects showing increasing liking with increasing concentration). In contrast, "sweet dislikers" (decreasing liking with increasing concentration) demonstrated a palatability shift; specifically, they increased liking when deprived. Also, sweet dislikers, but not likers, sipped more when deprived. However, within conditions, there was no relationship between intake and hedonics. These data suggest that intake changes observed even in rat sham feeding studies may not indicate changes in palatability and that the relationships among sweet intensity, hedonics, and intake in humans are complex.

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Capsaicin Cross-Desensitization: Psychophysical Evidence of Sensory Complexity in Oral Chemical Irritation. BARRY G. GREEN (Monell Chemical Senses Center)

The ability of capsaicin to desensitize a population of chemosensitive nociceptive fibers [i.e., "capsaicin-sensitive" (CS) neurons] was used to probe the perceptual complexity of oral chemical irritation. Solutions of ethanol (30%), cinnamic aldehyde (2.5%), NaCl (5M) and capsaicin (2ppm) (equated for approximately equal levels of perceived irritation) were applied to the tongue tip on filter paper disks before and after exposure to ten capsaicin (10ppm) stimuli. Subjects rated both the overall intensity and perceptual qualities of sensations of irritation. The results indicated that desensitization was greatest for capsaicin (89.9%) and least for ethanol (51.4%), with the other two chemicals falling between (NaCl = 59.6%, cinnamic aldehyde = 68.8%). Ratings of sensation quality revealed (1) different quality "profiles" for each chemical, (2) a positive correlation between the degree of desensitization and the frequency of "burn" and "sting" ratings, and (3) the sensation quality most often reported for ethanol- "numbness" - tended to persist or be enhanced following desensitization. Experiments are continuing in an attempt to elucidate further the nature of sensations of irritation mediated by CS and non-CS sensory "channels" that innervate the tongue.

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Sensory Evaluation of Acids By Free-Choice Profiling. SONIA M. RUBICO AND MINA R. McDANIEL (Sensory Science Laboratory, Department of Food Science and Technology, Oregon State University, Corvallis, OR 97331).

The technique of Free-Choice Profiling was applied in characterizing the sensory properties of some common organic and inorganic acids. Analysis of panelists' scores by Generalized Procrustes Analysis (GPA) provided information on the relationships among samples and assessors for both the consensus and individual configurations. Results indicated that on a weight basis (w/v or v/v), acids differed in their flavor and taste dynamics. Acids were described differently by individual panelists. The GPA resulted in three important principal axes (PA). The first PA had astringency/ mouthfeel as the most important factor, while bitterness and sourness were the most important for second and third PAs, respectively. At 0.08% (w/v or v/v), the inorganic acids, hydrochloric and phosphoric acids were more astringent than sour. Succinic (S) was a unique acid for its very intense bitterness with mosodium glutamate and brothy characteristics. Adipic and quinic had very weak sensory properties at this concentration. The relationship between astringency and pH was more evident than was the relationship between pH and sourness.

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Taste Intensity Perception in Human Aging: Preliminary Longitudinal Results Confirm Earlier Cross Sectional Findings. JAMES M. WEIFFENBACH (National Institute of Dental Research, NIH Bethesda MD)

Between Nov 1979 and Sept 1981 the taste perception of 170 participants in the Baltimore Longitudinal Study of Aging was assessed through cross modal matches of intensity to distance. The four basic tastes were represented by water solutions of sucrose (Su), sodium chloride, citric acid and quinine sulfate (QS). Comparison of different aged individuals indicated no significant cross sectional age effect on the slope of the psychophysical function for any taste quality. Similar cross sectional analysis was applied to the intra class correlation coefficient (ICC) which reflects the consistence of an individual's judgments on repeated stimulation. The ICC was age stable for Su but declined across age groups for the other tastes. The original participants are now being retested. Exact replications have been obtained for 24 participants (15 men 33 to 72 y/o and 9 women 33 to 71 y/o). Performance of these individuals in two assessments separated by ten years was compared. No significant difference in the slopes of the psychophysical functions obtained in the two assessments of the same persons at different ages was demonstrated for any quality. Repeatability (ICC) declined significantly from the first to second assessment for QS but for no other quality. Age stability for slope was supported by both cross sectional and longitudinal evidence. Moreover, the reliability of intensity judgments was age stable for Su and declined with age for QS regardless of whether the data were cross sectional or longitudinal.

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Taste-odor Similarities Predict Taste Enhancement and Suppression in Taste-odor Mixtures. ROBERT A. FRANK, GREGORY SHAFFER & DAVID V. SMITH (Univ. of Cincinnati)

We have shown previously (Shaffer & Frank, 1990, Chem. Senses, 15:638) that taste-smell interactions are tastant and odorant dependent. The mixing of some tastes and odors results in taste enhancement, while other combinations result in no interaction or taste suppression. The present study examined the possibility that these interactions could be predicted from similarity judgments for the components of a mixture. For example, taste enhancement might be associated with mixtures of tastes and odors that were judged to be similar, while suppression might be seen in mixtures of tastes and odors that were judged to be dissimilar. Human subjects (n=20) judged the similarity of all possible combinations of four tastants (sucrose, sodium chloride, citric acid, quinine sulfate) and six odorants (almond, chocolate, lemon, peanut, strawberry, wintergreen). The stimuli consisted of 2.5 ml aqueous solutions which were swallowed by the subjects. The similarity judgments were made on a nine point category scale. Once similarity judgments had been made for all stimulus pairs, the stimuli were also rated for ten attributes (e.g., sweetness, familiarity, fruitiness, pleasantness) using nine point category scales. The similarity of the stimulus pairs was a good predictor of odor-induced taste enhancement or suppression of the main taste quality of all mixtures except those involving quinine. An examination of the attribute ratings revealed that the fruitiness ratings of the odorants were highly correlated with odor-induced changes in the sweetness and sourness of sucrose and citric acid, respectively. It was concluded that the judged similarity of odorants and tastants is an important determinant of the changes in taste intensity observed in taste-odor mixtures.

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Characterization of Gastropod Salivary Gland Enzymes that Generate Hermit Crab Attractant Peptides.
RENEE LUCAS (Ohio Wesleyan College). DAN RITTSCHOF (Duke University Marine Laboratory and Dept. of Zoology, Duke University).

Hermit crabs use gastropod shells for protection. Shells must be replaced periodically due to growth and fouling. Shells are introduced into hermit crab populations during gastropod predation events. Digestion of flesh proteins by the predator snail's enzymes results in the release of peptides that attract hermit crabs to the potentially available shell. Trypsin and chymotrypsin, two enzymes that are highly conserved in many unrelated organisms, were identified and characterized in the salivary glands of predatory snails. Also, the lysine and arginine carboxy terminal peptides resulting from a tryptic digest of casein were isolated and their attractiveness was measured in the field. Assays using artificial substrates specific to the activity of trypsin and chymotrypsin were used to test for potential activity of those enzymes in the salivary gland homogenate of the tulip snail, Fasciolaria hunteria. Relative activity of trypsin and chymotrypsin was also compared using a casein digestion assay. Lysine and arginine carboxy terminal peptides were purified from a 500 dalton ultrafiltrate by affinity chromatography using immobilized anhydrotrypsin. Field assays to test for attraction of hermit crabs were performed using the purified peptides. In the field, the lysine and arginine carboxy-terminal peptides were significantly attractive to hermit crabs.

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Localization of putative chemosensory cells in the gut of the catfish, *Ictalurus punctatus*. L. E. GOEHLER (Univ. Colorado Health Sciences Center, Denver, CO 80262)

Chemosensitive endocrine cells of the gut have been implicated in the control of digestion and of behavioral states such as satiety. Open-type endocrine (OTE) cells are located in the gut mucosa, and project an apical villous process to the lumen. The basal region of the cell contains vesicles which are secreted into the extracellular space away from the lumen. Several classes of OTE cell exist, each of which secrete a single peptide hormone. Ultrastructural evidence from mammals suggest that some classes of OTE cells are innervated.

The present experiment relied on immunohistochemical techniques to determine the distribution of OTE cells in the gut of the catfish. Cryostat sections of catfish stomach and intestine were incubated with antisera against the secretory vesicle marker SV2, or the peptides calcitonin gene-related peptide (CGRP), L-enkephalin (L-ENK), somatostatin (SS), vasoactive intestinal peptide (VIP), or neuropeptide Y (NPY). Each antibody revealed a unique pattern of immunoreactivity (IR). SV2- and SS-IR were present in numerous slender OTE cells in the stomach, but not in the intestine. CGRP-, L-ENK-, NPY,and VIP-IR were seen in OTE cells only in the intestine. These cells, except for NPY-IR cells, were characterized by a rounder shape than the gastric SV2- and SS-IR cells. CGRP-IR OTE cells were observed only in the proximal intestine, whereas NPY-IR cells were observed primarily in the distal intestine.

The possibility that some OTE cells in the stomach of the catfish are innervated was also investigated by applying to the coelomic vagus the carbocyanine dye, diI, which can cross synapses between nerve fibers and sensory cells. For diI experiments, crystals of diI were placed on the distal cut end of the vagus in catfish perfused with 4% paraformaldehyde. The tissue was incubated at 37° C for several months, then sectioned on a cryostat. In stomach tissue in which diI had been applied to the vagus nerve, labeled cells having the appearance of slender OTE cells were observed in the mucosa. These experiments demonstrate the presence of several types of OTE cells in the catfish gut and suggest that some of these may be innervated.

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TACHYKININS IN THE TASTE PAPILLAE

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Extracts of papillae from swine tongues were analyzed by HPLC separation. Apart from Substance P and neurokinin A identified previously by immunohistochemistry, we also found neurokinin B, neuropeptide K and some minor quantities of oxydized peptides.

Recently, an antibody against neurokinin B has become available. We have compared the distribution of Substance P, neurokinin A and neurokinin B in taste papillae. The localization of these tachykinins in circumvallate and fungiform papillae will be demonstrated. The finding of neurokinin B outside the central nervous system is novel and may be interpreted to be the expression of the cranial nerves.

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Localization of Binding Sites for Epidermal Growth Factor (EGF) in Taste Buds of Neonatal and Adult Rats. ROBERT E. STEWART and DAVID L. HILL (University of Virginia).

Epidermal growth factor (EGF) plays important roles in growth and differentiation, particularly in epithelia. However, many tissues first exhibit sensitivity to EGF postpartum, long after initial appearance of cell surface receptors and ligand availability. Because taste bud and taste response development in rat occurs largely after birth, we hypothesized that the appearance of EGF binding sites (EGF-r) within tongue might coincide with postnatal taste bud formation and taste cell function. In addition, it was hypothesized that an altered time course of EGF-r expression in taste buds might be related to the altered peripheral taste function in rats deprived of sodium throughout development. Fresh or mildly fixed frozen sections of tongue were incubated in biotinylated EGF followed by incubation in streptavidin-Texas Red or streptavidin-peroxidase conjugates. Labelled sections from 2 day-old rat pups exhibited rare EGF-biotin binding, although taste buds were often difficult to localize definitively. By 10 days of age, about 75% of identifiable taste buds showed marked EGF-biotin labelling, a level comparable to that seen in sections of tongue from adult rats. No differences between normal and sodium-restricted rats in either the time course of EGF-r appearance or in the number of labelled taste buds were detected. These findings establish for the first time that cells within taste buds may be targets for EGF, derived from saliva or from tongue tissue. We conclude that EGF could be involved in post-natal taste development and that EGF may be important in the continuous turnover of taste cells prevalent in mammals. We are currently examining the possibility that EGF may be present within cells of the tongue near the lingual epithelium.

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Localization of Endopeptidase-24.11-like Immunoreactivity in Rodent Taste Buds and Olfactory Epithelium.

ROBERT S. LASHER (Dept. of C & S Biology, Univ. Colorado Medical School, Denver, CO 80262)

Recent studies have indicated that both taste buds and olfactory epithelium are innervated by peptidergic nerve fibers, including those containing substance P and CGRP. Therefore, it was of interest to determine if these fibers contained endopeptidase-24.11 (NEP), an enzyme which metabolizes substance P. As a first step, a monoclonal antibody, 172-3:17, which appears to bind to NEP (Lasher, R.S., et al., 1990. *Neurosci. Letts.* 117:43-49.) was reacted with Vibratome sections of either rat or mouse tongue or rat olfactory epithelium fixed with 4% paraformaldehyde. Its localization was visualized using an indirect immunoperoxidase technique. By light microscopy, NEP-like immunoreactivity (IR) was seen in many nerve fibers present both within and between taste buds in adult rat and mouse circumvallate papillae. In taste cells, NEP-like IR was associated primarily with apical cells, and was especially intense in the portion of the cells associated with the taste pore. It was absent in non-taste epithelial cells. The developmental expression of NEP-like IR was examined in circumvallate papillae from tongues of 5, 8 and 13 day rat pups. At 5 days, there were many intensely immunoreactive beaded nerve fibers, especially within developing taste buds, but there were few immunoreactive cells. At 8 days, fibers were still intensely immunoreactive, but there were a few more immunoreactive cells. At 13 days, fibers continued to be intensely immunoreactive, but now many taste cells in all parts of the bud were also intensely immunoreactive. In the olfactory epithelium, intense NEP-like IR was seen in a class of cells having the appearance of receptor cells. This was confirmed by electron microscopy, which also indicated that there was little if any intracellular staining in supporting cells. In addition, intense NEP-like IR was associated with the cilia and their plasma membrane, while weak immunoreactivity was associated with the plasma membrane of microvilli.

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Comparison of High-Pressure Rapid Freezing Fixation and Conventional Chemical Fixation of Catfish Barbel Taste Buds. SUZANNE M. ROYER and JOHN C. KINNAMON (University of Colorado at Boulder and Rocky Mountain Taste and Smell Center, Denver)

We are currently carrying out a study using the glucose analog, ^{3}H-labeled 2-deoxyglucose (2-DG), as a means to identify taste cells that respond to specific chemical stimuli. Because 2-DG and its metabolic products are water soluble, conventional chemical fixation for electron microscopy (EM) is not suitable for preserving the locations of these substances. Hence, we are developing methods for obtaining EM-quality cryo-fixation of the taste buds on the barbels of the catfish, *Ictalurus punctatus*, using high-pressure rapid freezing (HPRF). High-pressure rapid freezing is superior to other methods of freeze-fixation for preserving relatively large structures (such as taste buds) because one can cryofix tissues to depths in excess of 100µm without ice crystal damage.

Small pieces of barbel (1 mm or less in length) were removed from anesthetized catfish, embedded in either yeast paste or hexadecene, and rapidly frozen using a Balzers high-pressure rapid freezing machine. The samples were then freeze-substituted in 1% OsO_4 in acetone at -90°C, gradually brought to 0°C, rinsed in cold acetone, brought to room temperature and embedded in LX-112. For comparison, other barbels were conventionally fixed by perfusing the catfish with a cacodylate-buffered, glutaraldehyde/paraformaldehyde fixative. Samples from these catfish were processed according to standard methods. Some sections from both HPRF and chemically-fixed tissue blocks were post-stained with alcoholic uranyl acetate and lead salts.

Taste buds from the conventionally fixed barbels were similar in appearance to previous descriptions (*e.g.*, Reutter, 1978). The structure of the HPRF taste buds was generally similar to that of the chemically fixed taste buds. Profiles of nerve fibers and their organelles appear to be preserved considerably better with HPRF. The use of HPRF now provides us with the capability to fix entire taste buds for 2DG studies at the EM level without movement of metabolic products and the resultant loss of space resolution.

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Solitary Chemosensory Cells of the Rockling Anterior Dorsal Fin: High Voltage Electron Microscopy, Three-Dimensional Reconstruction, and DiI Labeling of Primary Afferent Nerves. KURT KOTRSCHAL (Konrad-Lorenz-Forschungsstelle für Ethologie, Grünau, Austria), SUZANNE M. ROYER and JOHN C. KINNAMON (University of Colorado at Boulder and Rocky Mountain Taste and Smell Center, Denver)

The anterior dorsal fin (ADF) of the rockling (*Ciliata mustela*) consists of many slender, motile rays that are webbed only at the base. The ray epidermis contains numerous solitary chemosensory cells (SCCs). Solitary chemosensory cells resemble taste bud cells in shape and ultrastructure and are widely distributed among the lower aquatic vertebrates. In rocklings, the several million SCCs of the ADF are innervated by the recurrent facial nerve. We have used high voltage electron microscopy of serial sections and computer-assisted three-dimensional reconstruction to visualize the SCCs of the ADF and to obtain quantitative data regarding the spatial relationships and distribution of these cells and their synaptic contacts with innervating facial nerve fibers. SCCs comprise approximately 15% of the cells in the ADF epidermis and make up about 30% of the epidermal volume. SCCs are often densely packed, and adjacent cells may be intertwined. Although SCCs are usually separated from each other by intervening sheets of epidermal cells, occasionally two SCCs are found to be in direct contact. SCCs are spindle-shaped cells 25-30µm in length and 3-5µm in width. Each extends an apical process toward the epidermal surface; this terminates in a single apical microvillus that penetrates between the surface squamous epidermal cells. The basal processes of SCCs, which may be branched, extend toward the nerve plexus between the basal epidermal cells and the SCC endfeet. Typically, each SCC makes one to three synaptic contacts with facial nerve fibers. These putative synaptic contacts consist of round or oval patches 0.5-1.5µm in diameter with opposed membrane specializations that are more prominent on the postsynaptic side. Presyaptic vesicles may or may not be apparent. Most synapses are found at the level of the basal nerve plexus; only one synapse was found in the nuclear region of an SCC. The carbocyanine dye DiI enabled us to distinguish between facial nerve fibers and spinal nerve fibers in the ADF epidermis. Eventually, the DiI passed across synapses and labeled some SCCs. DiI-labeled facial nerve fibers and SCCs were observed using both conventional and confocal fluorescence microscopy.

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Effects of thyroid hormones on vallate taste buds. RUOYU XIAO, SIWEI WANG and INGLIS J. MILLER, JR. (Dept. Neurobiology & Anatomy, Bowman Gray School of Med. of Wake Forest University, Winston-Salem, NC 27103)

Clinical evidence shows that some diseases of the thyroid gland influence taste function. Anti-thyroid drug therapy, as with PTU, methimazole and methylthiouracil, also have side effects on taste perception. We test the hypothesis that thyroid hormones influence the number of taste buds in the vallate papilla of the mouse. Balb/cBy (Jax) mice, 5 weeks-old, are divided into 8 treatment groups of 10 animals: 1. Pretreatment control, 2. Saline injection, 3. Thyroxine injection (3 µg/day), 4. Thyroxine injection (10 µg/day), 5. TRH injection (1µg/day), 6. Propylthiouracil (PTU) gavage (1mgm/day), 7. Saline gavage and 8. No treatment control. The treatment is performed once a day for 14 days, and mice are weighed once per week. Body temperature is recorded before sacrifice. After treatment, each animal is anesthetized with ether, decapitated, and blood is collected for thyroxine assay. The vallate papilla is removed and fixed in 4% paraformaldehyde and 15% picric acid. The tissue is embedded in paraffin, and serial sections are made and stained with H & E. Growth factors from each group are quantified with anti-NGF and anti-EGF. Taste pores representing individual taste buds are counted in each section under a light microscope. Preliminary results show that the Pretreatment control group had the lowest mean total of 174 ± 19 (N =8) tb/vallate pap. At sacrifice 14 days later, the No injection controls (191 ± 31, N = 5) and the Saline gavage group (191± 59, N =4) contained an equivalent increase over pretreatment controls of about 10%. The Saline injection group (200±24, N=5) and the low dose of Thyroxine (202±21, N =6) showed similar increases over pretreatment controls of about 16%. The PTU gavage group (241±28, N=4) had the largest increase of about 40% over controls, followed by the higher Thyroxine dose (228±13, N =5) group with a 30% increase and the TRH treatment group with an increase of 25%. Preliminary results demonstrate that PTU and thyroid hormone treatment seem to augment the number of taste buds in the mouse vallate papilla.

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Developmental Forms of NCAM on Rat Circumvallate Taste Cells
GINA M. NELSON and THOMAS E. FINGER (University of Colorado Health Sciences Center and Rocky Mountain Taste and Smell Center, Denver, Colorado 80262)

Taste cells are a continuously renewed population of cells thought to be derived from epithelial precursors, but which also posses several neuronal characteristics, including the expression of neural cell adhesion molecule (NCAM). Various forms of NCAM are expressed on the surface of neurons depending on their state of development. All forms of NCAM have a common extracellular protein backbone, but the different forms vary in the length of a cytoplasmic tail and in the structure and composition of the carbohydrate modification.

In order to further understand the maturation and turnover of taste cells, double-label fluorescent immunocytochemistry was used to determine the relationship between the growth-related and mature forms of NCAM present on the taste cells. The general NCAM antibody used here recognizes the protein backbone, whereas the antibody 5B4 recognizes an insert present in the longer NCAM forms with a cytoplasmic tail (the 140 and 180 kDa forms) which are preferentially expressed in neurons during growth. The antibody 735 recognizes the α-2-8-linked polysialic carbohydrate moiety of the embryonic form of NCAM. The olfactory bulb was used as a positive control, since olfactory nerve axons have been shown to possess a form of NCAM reactive to all three antisera.

Only some taste cells were immunoreactive for the NCAM backbone. All of the cells that were immunoreactive for NCAM were also immunoreactive for 5B4. However, none of the taste cells were immunoreactive for the 735 antibody, although the olfactory bulb sections are strongly positive. These results indicate that the NCAMs expressed by the taste cells do not posses the N-acetylneuraminidase structure typical of developing neurons.

In summary, a subpopulation of taste cells express NCAM, but the structural form appears to be different from that seen in developing neurons. While the protein structure of the molecule is the form seen in other neurons undergoing growth (5B4-positive) the molecule lacks the typical developmental carbohydrate moiety (735-negative).

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Reorganization of Gustatory Recipient Zones in the Nucleus of the Solitary Tract (NST) Following Early Postnatal Receptor Damage: Evidence for Competitive Interactions Between Gustatory Axons During Postnatal Development. PHILLIP S. LASITER (*Florida Atlantic University*).

Chorda tympani (CT) axons expand caudally in the NST during the first 20 days of rat's postnatal life. Anterior tongue receptor damage at P2 attenuates caudal expansion of the CT terminal field and reduces CT terminal field volume by approximately 30%. Because caudal regions of the CT terminal field merge with the glossopharyngeal (N.IX) terminal field in normal rats, and development of CT axons in this region is affected by P2 receptor damage, we wished to examine whether topographical convergence between CT and N.IX axons is affected by P2 receptor damage. Fluorescent tracing studies were conducted to simultaneously label CT axons (lucifer yellow CH), N.IX axons (propidium iodide), and parabrachial projection neurons (cascade blue) in the NST of normal animals and receptor-damaged animals. Following P2 damage N.IX terminal endings invade more rostrally-situated regions of CT terminal fields and innervation patterns of PBN projection neurons are altered significantly. In normal animals N.IX axons invade 22% of CT terminal fields; In receptor damaged animals N.IX axons invade 53% of CT terminal fields. The ***total*** number of PBN projection neurons enveloped by CT and N.IX axons is not affected by receptor damage, however, receptor damage alters innervation patterns of these neurons; In normal animals 36% of projection neurons are enveloped by CT axons, 31% of neurons are enveloped by both CT axons and N.IX axons, and 33% of neurons are enveloped by N.IX axons. In receptor damaged animals 15% of neurons are enveloped by CT axons, 47% of neurons are enveloped by both CT axons and N.IX axons, and 38% of neurons are enveloped by N. IX axons. These results suggest that during normal development gustatory activity propogated by the CT nerve specifies innervation patterns of CT and N. IX terminal fields, perhaps by establishing competitive interactions between migrating axons and their respective postsynaptic targets.

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Glutamate is NOT the neurotransmitter of primary gustatory afferent fibers. K.C. DOCKSTADER, T.V. DUNWIDDIE, & T.E. FINGER (Univ. Colorado Health Sciences Center, Denver, CO)

Glutamate is an amino acid that can act as an excitatory neurotransmitter and which occurs in high concentrations throughout the central nervous system. This excitatory amino acid has been proposed as the neurotransmitter utilized by primary afferent fibers in various sensory systems and glutamate-like immunoreactivity is high in the nucleus of the solitary tract. We used immunocytochemical and pharmacological experiments to test whether glutamate is the neurotransmitter in the primary gustatory afferent fibers. The vagal gustatory lobe of goldfish presents a unique preparation in which to study this question. The primary gustatory fibers terminate in a distinct laminar pattern obvious at light microscopic levels. Our immunocytochemical preparations employed a monoclonal antibody which reacts with fixed glutamate but not with aspartate. These studies show that the pattern of glutamate-like immunoreactivity within the lobe does not correspond to the layers of termination of the primary gustatory fibers. Many intrinsic neurons and their fibers within the vagal lobe are immunoreactive, as are fibers in the ascending secondary gustatory tract. These findings are consistent with results from pharmacological manipulations of in vitro slices. In these experiments a bimodal population response with negative peaks at 3 and 5 msec is evoked following stimulation of fascicles of incoming gustatory fibers. Several lines of evidence indicate that the 3 msec peak is attributable to a synaptic potential associated with the primary gustatory fibers, while the 5 msec potential appears to be due to synaptic activation of second- or higher-order neurons in the lobe. Application of kynurenic acid (a relatively nonspecific glutamate antagonist) to the slice preparation has relatively little effect on the primary response but does reduce or eliminate the later potential. These results are consistent with the hypothesis that while the primary afferent fibers are not glutaminergic, higher-order gustatory neurons, including those giving rise to the ascending secondary tract, do use glutamate as their neurotransmitter.

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Cortical Projections to the Rostral Pole of the Hamster NTS Exhibit Bilateral Differences in Strength and Area of Origin. THEODORE S. DONTA and JILL A. LONDON (Center for Neurological Sciences, Dept. BioStructure and Function, Univ. CT Health Center)

This laboratory has previously described a robust contralateral and weaker ipsilateral cortical projection to the rostral pole of the hamster nucleus tractus solitarius (NTS). In the present work, the organization of these projecting cells was compared using fluorescent, retrograde tract-tracers. Multi-unit activity was recorded in the rostral pole of the NTS in response to application of a search stimulus (0.03 M NaCl, 0.1 M KCl and 0.1 M sucrose) to the anterior tongue. The boundaries of a taste active region were delimited. Latex microspheres (Lumafluor, Inc.) filled with either rhodamine or fluorescein were pressure injected into the center of the taste delimited region; one tracer was injected into one NTS and the other was injected into the NTS of the opposite hemisphere. After 4-5 days, subjects were perfused and brains were postfixed and cryoprotected. Results were consistent with previous experiments using WGA-HRP (wheat germ agglutinin horseradish peroxidase) as the label. Labeled cells were found in layer 5 in the agranular and granular insular cortex. The rostral-caudal extent of labeled cells was 5 mm, greater than previously found in experiments using WGA-HRP. The number of cells projecting contralaterally was 2-3 times greater than cells projecting ipsilaterally. Less than 1% of the cells were double labeled. Particularly interesting was the observation that the distributions of contralateral and ipsilateral cells were not homogenous. Cells projecting ipsilaterally were contained within the boundaries of the contralateral projection area. Ipsilateral cell density appeared greatest ventral to the majority of contralateral cells. Injections into NTS areas outside the taste delimited region are being made in order to determine the overlap of gustatory and visceral cortical projections.

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ABSTRACT WITHDRAWN

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Morphological Characteristics of Neurons in Areas of the Solitary Nucleus Receiving Chemosensory and Mechanical Information from the Epiglottis. ROBERT D. SWEAZEY (Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor).

Information from receptors on the lamb epiglottis is relayed via the superior laryngeal nerve to the nucleus tractus solitarius (NTS). Previous investigations have described the response characteristics of lamb NTS neurons to stimulation of the epiglottis with chemical and mechanical stimuli. The present study was undertaken to determine the morphological characteristics of these neurons. NTS regions responsive to stimulation of the epiglottis were defined using electrophysiological recordings. Small lesions were placed at the edges of the responsive regions and the tissue was subsequently processed for neuron reconstruction using the Golgi-Cox method. Epiglottis-evoked responses were located in the intermediate NTS from 3.5 to 1.5 mm rostral to obex. These mapping studies found that the area of the NTS responsive to chemical stimulation of the epiglottis was embedded in a larger mechanosensitive region. Neurons within the epiglottal-responsive areas of the NTS could generally be classified as multipolar or elongate. At NTS locations responsive only to mechanical stimuli large multipolar neurons with extensive dendritic trees predominated. At locations where chemical as well as mechanical activity could be elicited both multipolar and elongate neurons were present in equal numbers. Multipolar neurons in this latter area generally had smaller somas with less extensive dendritic trees. Elongate neurons exhibited a wide range of sizes and were usually orientated either parallel or perpendicular to the solitary tract. No preferred orientation was evident for multipolar neurons. Neurons reconstructed to date exhibited a wide variety in the numbers and types of spines. These preliminary results indicate that neurons in NTS regions responsive to stimulation of the epiglottis are similar to more rostral NTS neurons which process sensory information from receptors in the oral cavity.

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Distribution of Synapses on HRP-Filled NST-PBN Projection Neurons in the Golden Hamster. MARK C. WHITEHEAD and DANE E. BOWEN (Ohio State University).

Two morphological types of cells in the gustatory subdivisions of the nucleus of the solitary tract (NST) send axons to the parabrachial nucleus (PBN) (Whitehead, JCN 301:554, '90). One type, the elongate cell, is characterized by an oval cell body and two proximal dendrites, one oriented medially, the other laterally. The other type, the stellate cell, has a multipolar cell body and 3-5 primary dendrites radiating in various directions. In this study, elongate and stellate cells are compared in terms of the types and frequencies of synaptic endings that contact cell bodies, proximal dendrites, distal dendrites, and spines. In 8 animals, cells in the rostral NST were labelled by HRP injections centered in the medial PBN. Elongate cells (n=7) and stellate cells (n=3) showing Golgi-like filling were traced through a drawing tube and then serially sectioned and examined with electron microscopy. The stellate cells received a greater number of synapses on their cell bodies than elongate cells. These synapses consisted predominantly of endings containing pleomorphic vesicles engaged in symmetrical junctions. Primary-like endings with round vesicles and asymmetrical junctions (Whitehead, JCN 244:72, '86) were infrequent and tended to contact stellate rather than elongate cell bodies. Proximal dendritic inputs were similar for the two cell types with equal proportions of the two ending types. Distal dendrites of stellate cells received more synapses than those of elongate cells; for both cell types primary-like endings predominated. Dendritic spines, more numerous for stellate cells, received both types of synapses in equal numbers. All cells examined contained invaginated nuclei, although in some serial sections the nuclei were non-invaginated (compare Davis and Jang, Soc. Neurosci. Abstr. '85). The results suggest that the two types of cells projecting from the NST to the PBN may have different roles in gustation. Their differences in synaptic inputs could relate to differences in response properties (Bradley and Sweazey, Brain Res., 508:168 '90).

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Taste responses in units in the nucleus of the solitary tract that do and do not relay information to the parabrachial pons. S. MONROE & P.M. DI LORENZO (Dept. of Psychology, SUNY at Binghamton, Binghamton, N.Y. 13901)

Several reports have investigated the taste-responsive units in the nucleus of the solitary tract (NTS) that project rostrally to the parabrachial nucleus of the pons (PbN). The present study was designed to clarify some discrepancies in those previous reports and to extend the analysis of taste coding in those NTS units that are monosynaptically connected to the PbN (relay units) compared with those NTS units that do not appear to project directly to the PbN (non-relay units). Taste responses to sapid solutions of NaCl (.1 M), HCl (.01 M), quinineHCl (.01 M), sucrose (.5 M) and N-saccharin (.004 M) were recorded in single units in the NTS of anesthetized rats. Following gustatory stimulation, electrophysiological responses to electrical stimulation of the taste-responsive part of the ipsilateral PbN were recorded. A .2 msec pulse was delivered at 75-250 μA at rates of 1, 25, 50 and 100 pps through a bipolar stainless steel electrode. Criteria for antidromic responses were: time-locked spikes that occurred at a fixed latency following PbN stimulation, following at high stimulation frequencies, and collision. Of 37 taste-responsive NTS units that have been recorded thus far, 18 (49%) were antidromically activated by electrical stimulation of the PbN. Compared with non-relay NTS units these relay units generally produced more robust responses to all taste stimuli that were tested and produced significantly elevated responses to sucrose and Na-saccharin. 50% of the relay units responded best to HCl (9 of 18) and another 22% of these (4 of 18) responded best to NaCl. In contrast, 63% of the non-relay units responded best to NaCl and only 21% responded best to HCl. Three NTS units were orthodromically activated by PbN stimulation; one of these also showed an antidromic response.

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Interspike Interval Patterns Recorded from Taste Neurons in the Hamster Solitary Nucleus. SARAH C. NUDING and MARION E. FRANK (Center for Neurological Sciences and Dept. of BioStructure and Function, Univ. of Connecticut Health Center).

Single unit taste-responsive neurons in the hamster solitary nucleus (NST) can be described using interspike interval (ISI) response patterns (Nuding et al., 1989). We have subsequently recorded from additional taste neurons, quantified ISI pattern properties, and compared actual and computer-generated ISI patterns. A unit generates one of two types of ISI patterns during either spontaneous or evoked activity (stimulation of anterior tongue with NaCl, KCl, and sucrose). Unimodal ISI patterns have a single broad peak at 284.7 ± 70.4 ms spontaneous and 78.8 ± 12.8 ms stimulated. Multimodal ISI patterns have a distinct sharp peak at 11.3 ± 0.4 ms spontaneous, 9.3 ± 0.5 ms stimulated, and a second, broader peak at 273.9 ± 45.9 ms spontaneous, 71.5 ± 14.6 ms stimulated. As rate of firing increases, all peaks move towards lower intervals, but the ISI pattern-type does not change. This constancy of ISI pattern holds for responses of a unit across all stimuli, occurs in phasic and tonic response components, and is not related to the stimulus specificity or the location of the unit within the NST. Simulation of both the unimodal and the broad multimodal peak can be accounted for by a single random spike generator set at one rate, but the multimodal's short interval peak can not. Amiloride, a sodium-channel blocker known to suppress response rates of certain rat NST neurons to NaCl (Scott and Giza, 1990), has similar rate effects in hamster NST units but does not affect their ISI pattern. Therefore, ISI patterns may be an intrinsic property of sensory neurons and related to the way they process information.

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Ascending efferents of the parabrachial nucleus in the golden hamster. C.B. HALSELL and M.E. FRANK (Center for Neurological Science and Dept. of Biostructure and Function) University of Connecticut Health Center.

The second central synapse in the ascending gustatory pathway is the pontine parabrachial nucleus (PBN). The PBN can regulate the flow of ascending gustatory information since PBN efferents project to multiple brain regions with diverse functions. In the present study, ascending PBN projections are described and the location of the efferents are related to anatomically defined PBN subdivisions in the hamster. Wheat-germ agglutinin horseradish peroxidase (WGA-HRP) was injected iontophoretically into the PBN to determine the extent of efferent terminal fields. Anterograde label was seen in the lateral hypothalamus (LH), ventral posteromedial-parvicellular nucleus of the thalamus (VPMpc), central nucleus of the amygdala (CNA), bed nucleus of the stria terminalis (BST) and insular cortex. Label was heavier ipsilateral to the injection site, with no contralateral label visible in CNA, BST, and cortex. WGA-HRP injections were made into two of these terminal fields, the VPMpc and CNA. The VPMpc injection retrogradely labelled cells in the PBN and insular cortex bilaterally, with more cells labelled ipsilaterally. The CNA injection labelled cells in the PBN and insular cortex bilaterally and VPMpc ipsilaterally. Although scattered throughout in both cases, labelled cells were more concentrated in the PBN central medial subdivision with VPMpc injections and more concentrated in the external lateral and ventral lateral subdivisions with the CNA injections. The central medial and ventral lateral subdivisions are anterior-tongue mediated taste-responsive regions in the hamster (Halsell and Frank, '91) and the external lateral is considered to be part of the general visceral system. Thus, ascending PBN efferents are organized with respect to PBN subdivisions, which have been anatomically and functionally defined. This organization may prove to be useful in understanding the diverse central ascending gustatory pathways.

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Taste Mixture Interactions Revealed by Concentration-Response Functions in Single Hamster Parabrachial Neurons. MARK B. VOGT and DAVID V. SMITH (University of Cincinnati College of Medicine).

Previous neurophysiological studies of the mammalian central gustatory system have provided few data on responses to taste mixtures. Taste stimuli exhibit widely varying concentration-response functions. Therefore, to determine whether the mixture response reflects a simple additive process or a more complex interaction indicative of mixture suppression or enhancement, it is necessary to measure the response to several concentrations of a stimulus presented alone and in mixture. In order to characterize CNS mixture interactions, we are recording responses of single neurons in the hamster PbN. A taste stimulus is presented in two concentration series, first alone and then in a binary mixture with a single strong concentration of another stimulus. Initial recordings have employed mixtures of taste stimuli of different qualities for which there is some evidence of mixture interactions: sucrose with NaCl, and sucrose with QHCl. Responses to sucrose-NaCl mixtures vary according to the neuron's best stimulus. For NaCl-best neurons, the responses to the mixture concentration series are a simple additive function of the response to each of the components. However, some sucrose-best neurons display a mixture response function equal to that of the more effective component. Other sucrose-best neurons show mixture response functions greater than the concentration-response function of either component, but less than predicted by simple additive processes. For sucrose-QHCl mixtures, QHCl-best neurons show simple additive response functions, while one sucrose-best neuron displayed a mixture response function that was markedly less than that to either component alone. These concentration-response data allow us to evaluate the effectiveness of several mixture models in predicting the responses of central gustatory neurons to binary taste mixtures.

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Morphology and Cytoarchitecture of the Elasmobranch Olfactory Bulb. LAURENCE DRYER, PASQUALE P.C. GRAZIADEI, (Florida State University)

The olfactory bulb of many Elasmobranch species is an elongated structure at the convex margin of the crescent-shaped olfactory organ. It can be macroscopically separated into several distinct subdivisions. Gross observations reveal these subdivisions as a succession of swellings or "sub-bulbs" along the olfactory organ. The cytoarchitecture of the olfactory bulb of species such as the Bonnethead shark (Sphyrna tiburo), the Sharp-nosed shark (Rhizoprionodon terranovae), and the Atlantic Stingray (Dasyatis sabina) have been examined using conventional histological techniques as well as Golgi method. Although less distinct than in higher Vertebrates, several cell layers are present, from the periphery to the olfactory tract: A large nervous layer, a glomerular layer, a mitral layer, and a granular layer. Mitral and glomerular layers slightly overlap. Several specific features of the elasmobranchs olfactory bulb are noticeable: first, in contrast to higher vertebrates, granule cells have distinct axons. Second, some triangular cells, the function of which is at present unspecified, are present in the vicinity of the granule cells. Third, the bulb exhibits at least two different sizes of glomeruli. Injection of Biocytin into the medial part of the olfactory epithelium results in labeling restricted to the medial portion of the bulb. This preliminary result suggests that olfactory information coming from a single lamella is processed initially by neurons located within a single olfactory bulb subdivision. Further experiments are in progress to determine if the macroscopically recognized subdivisions of the olfactory bulb reflect a functional compartmentation of the second order center.

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Projection Patterns of Goldfish Olfactory System Structures.
JEANINE S. STEWART AND PETER C. BRUNJES (University of Virginia).

Goldfish exhibit continual growth of peripheral and central neural structures throughout life. A recent study from our laboratory provided a descriptive developmental analysis of olfactory structures in the goldfish. Both the bulb and the olfactory rosette, which contains the sensory epithelium, grow in proportion to the rest of the animal (Stewart & Brunjes, *Devel. Brain Res., 54*, 1990). During growth cells are added preferentially to the medial aspect of the rosette while few cells are added to the bulb. Currently topographical relationships between rosettes and bulbs are being examined in aldehyde-fixed tissue with the lipophilic tracer DiI. Crystals of the tracer are applied to a discrete region of each rosette. After 3 to 4 weeks at 37°, the tracer diffuses anterogradely from the application site to bulbar terminations. Vibratome sections of rosettes and bulbs are analyzed to elucidate projection patterns from various rosette regions to corresponding bulbar termination sites. Preliminary results suggest the presence of a very gross spatial organization: receptors from anterior and lateral aspects of the rosette project to lateral bulb; while cells from posterior and medial epithelial regions terminate in antero-medial bulbar sites. We are also investigating whether reorganization of mucosa to bulb projections results from discrete ablations of individual rosette regions. Related studies examine other ways in which olfactory structures may be able to reorganize projections following insult.

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Localization of Growth Factor mRNA Expression in the Rat Olfactory System
KATHLEEN M. GUTHRIE and CHRISTINE M. GALL (Dept. of Anatomy and Neurobiology, University of California at Irvine, CA 92717)

Blot hybridization studies have indicated the rat olfactory bulb contains relatively large amounts of mRNA for nerve growth factor (NGF), however, the cellular localization of this message is not known. We have examined the cellular distribution of mRNAs for NGF and the partially homologous brain derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3) in the adult rat olfactory system using *in situ* hybridization with ^{35}S-labeled cRNAs. Hybridization with rat NGF cRNA labels specific neuronal populations in several olfactory structures. Neurons were labeled with moderate density throughout superficial piriform cortex and the anterior olfactory nucleus (AON), with lighter labeling apparent in the juxtaglomerular region of the main olfactory bulb. Hybridization was largely confined to middle tufted cells lying immediately beneath the glomeruli, although a few periglomerular cells also labeled. Hybridization with BDNF cRNA also labeled cells in piriform cortex and the AON. In the main olfactory bulb, differential labeling was seen in the granule cell layer, with superficial granule cells more densely labeled than deeper granule cells. Subpopulations of periglomerular and tufted cells were also labeled. Cell labeling for NT3 mRNA was robust in the tenia tecta, but no specific hybridization ocurred in the olfactory bulb, AON or piriform cortex. Surprisingly, none of our ribroprobes revealed significant cell labeling for growth factors in the accessory olfactory bulb. The expression of moderate levels of NGF and BDNF by cortical regions which receive afferents from the olfactory bulb suggests that cells in these target areas may provide trophic support for bulb output neurons. Similarly, the expression of NGF and BDNF by juxtaglomerular neurons may indicate a trophic influence on peripheral sensory afferents, although the comparatively low levels of expression detected in these neurons suggests that these compounds play a minor role in olfactory receptor support. The olfactory bulb may contain unique, as yet unidentified trophic factors which influence the development and maintenance of olfactory receptor afferents.
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Olfactory System Organization in the Grey, Short-Tailed Opossum *Monodelphis domestica*.
PETER C. BRUNJES, MARK J. SUTHERLAND AND AMIR A. JAZAERI (University of Virginia)

Marsupials have become popular for studies of mammalian brain development because their short gestations allow access to quite early developmental stages. For example, *Monodelphis* gives birth after a 12 day gestation to pups which are approximately 8 mm in length. At this time the prosencephalic region is quite immature, with few post-mitotic neurons present. Importantly, *Monodelphis* is a "pouchless" opossum: the young attach to nipples and hang exposed on the mother's ventrum, allowing easy access and the possibility of experimental manipulation. We have been examining the olfactory bulbs and higher central structures in adult *Monodelphis* with several techniques in order to gather baseline information. Nissl stains reveal a highly laminated olfactory bulb with a widely patent olfactory vesicle in caudal regions, and densely packed cells in the deep portions of the periglomerular layer (PGM). ACHe staining was found to be heaviest in the PGM and in a band beginning in the deep third of the external plexiform layer (EPL) and becoming densest in the wide internal plexiform zone. Timm staining was found to be heaviest in cellular regions. Tyrosine-hydroxylase immunostaining was heaviest in the glomerular zone, with small to large cells exhibiting stained somata. Large, presumably tufted cells in the deep PGM and superficial EPL were also stained, as well as a varied population of cells scattered throughout the EPL which had thin, highly varicose fibers. Finally, Golgi staining revealed all standard cell classes which appear to have a similar degree of dendritic complexity as seen in the rat. Occasional mitral cells were observed to have spiny processes on their primary dendritic shafts. Overall, bulb morphology seems to follow the general mammalian pattern.

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The Olfactory Thalamocortical System: Functional Analysis Using A Split-Brain Preparation.
SHARON A. McBRIDE AND BURTON M. SLOTNICK (The American University)

Rats with lesions of the olfactory thalamocortical system have specific deficits in olfactory discrimination tasks. Perhaps the most reliable is an increased error score in an olfactory reversal-learning task for rats with bilateral lesions of the mediodorsal thalamic nucleus. Typically, this deficit has been obtained with relatively large lesions of the dorsomedial thalamus. However, only a small segment of MD receives olfactory input and the deficit may, in part, stem from disruption of non-olfactory thalamocortical connections. To better define the anatomical basis of the learning deficit, rats were tested on a discrimination reversal task after receiving bilateral MD lesions (n=9) or unilateral MD lesions + removal of the contralateral orbital (OB) and agranular insular (AI) cortex (n=11). Rats with sham lesions and those with lesions in the same cerebral hemisphere served as controls (n=16). Experimental rats had no deficits in simple odor detection or in a 2-odor discrimination task. However, the 2 experimental groups performed more poorly than controls on the reversal task and bilateral MD lesions and unilateral MD + contralateral OB/AI lesions were equally effective. These results demonstrate that the deficits resulting from bilateral MD lesions stem from disruption of the olfactory projections to MD. Non-olfactory components of MD and surrounding areas make little or no contribution to this olfactory task.

This 'split brain' design may also provide a model for examining the functional role of the anterior commissure (AC). Thus, if rats with unilateral MD lesions + contralateral bulb removal perform as poorly as those with bilateral MD lesions, this would suggest that the relevant information is not transferred via the AC. On the other hand, if such rats have no deficits this would indicate complete transfer of information by the AC. Our preliminary results suggest that the former holds: In 3 rats tested thus far, performance was as poor or nearly as poor as those with bilateral MD lesions.

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A Longitudinal study of EEG responses to odours using Brain Electrical Activity Mapping (B.E.A.M).

SUE HOTSON (Warwick Olfaction Research Group)
STEVE VAN TOLLER (Warwick Olfaction Research Group)

Using the technique of Brain Electrical Activity Mapping (BEAM) it is possible to monitor ongoing, 'real-time' EEG activity. Recent work at the Warwick Olfaction Research Laboratory has been concerned with the cortical processing of odours. A longitudinal study has been untertaken to systematically explore the cortical responses of a group of adult subjects to a variety of odorants over time. These odorants include a group of fine fragrances, androstenone, linalyl acetate, a musk, and iso-valeric acid. Testing of the subjects took place in a specially designed low odour room. Subjects wore headphones and goggles to reduce perceptual cuing. EEG data was collected from 28 scalp electrodes located in a modification of the International 10/20 placement system. Cortical activity was recorded for a total of forty seconds per trial, the presentation of the odour/blank occupying ten of these. The collected data underwent discriminant analysis. The results so far, using this technique, suggest that it is possible to discern common patterns of cortical response to various odours in the 12-15 Hz (Alpha) waveband.

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Odor Representation on the Olfactory Bulb Surface: Theoretical Analysis of Alternate Models. WILLIAM T. NICKELL (University of Cincinnati).

If it is assumed, in analogy with color vision and taste, that odors can be decomposed into a small number of "primary odors" a fundamental question is the manner in which these "primary odors" are represented on the surface of the olfactory bulb. A basic distinction is between models in which the entire bulb is needed for representation of an odor and models in which small parts of the bulb are sufficient to represent odor quality. For example, if, as suggested by metabolic mapping studies, each "primary odor" maps into a single, relatively large, region of the bulb surface, then each part of the bulb is unique and necessary for complete representation of odor quality. If, on the other hand, each glomerulus receives olfactory nerve fibers representing a single "primary odor" then each portion of bulb surface large enough to contain one of each kind of glomerulus could completely represent the odor.

It would first appear that these two contrasting hypotheses could be readily distinguished by lesions of the olfactory bulb. If each portion of the bulb is unique, ablation of portions of the bulb surface should alter perceived odor quality and prevent recognition of a previously learned odor; but, if small portions of the bulb surface are equipotent, remnant bulb fragments should correctly represent the stimulus odor and a previously learned discrimination should survive. Limited experimental data support the second hypothesis. Interpretation is complicated, however, by the probable ability of deeper olfactory structures to reconstruct previously learned odors from partial inputs. In this case after removal of bulb tissue representing some of the "primary odors" the remaining system might still reconstruct the original neural representation of the odor.

To illustrate, assume there are 20 "primary odors" and that the relative intensity of the "primary odors" comprising each learned odor is added as a row to a matrix. The "primary odor" profile of a stimulus odor could be compared with each of the stored rows. In the case of a sufficiently close match, the contents of the table entry could replace the actual input. After ablation of bulb tissue representing some fraction of the "primary odors" an odor might still be correctly identified from remaining information.

Analysis and numerical simulation of the effects of hypothetical lesions on this simple model, using different numbers of "primary odors," can provide insight into the ability of lesion experiments to distinguish between the two mapping models.

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Spatial Distribution of Inhibition During Olfactory Bulb Response to Odor: Computer Simulation MICHAEL MEREDITH (Dept. Biological Science, Florida State University, Tallahassee, FL 32306 USA)

Inhibition is a prominent feature of olfactory bulb output cell response to odor stimulation in many species, including fish, amphibia and mammals. Responses containing inhibitory episodes increase with intensity of stimulation and predominantly inhibitory responses appear to increase with bulbar "imprinting" during development. Sources of inhibition intrinsic to the bulb include the well known granule cell dendrodendritic reciprocal synapses, similar synapses between periglomerular (PG) cells and output cell local dendrites, and synapses between Pg cell axons and distant output cells. Each class of cells has its own characteristic spatial distribution of processes and connections. Some predictions about the spatial distribution of output-cell inhibition around a restricted area of excitatory input can be made using the known spatial organization of the circuit network. A computer simulation indicates a region of moderate inhibition surrounding isolated areas of excitatory input but profound inhibition between neighboring areas of excitation and, under some circumstances, within areas of excitatory input. The simulation includes a 30x30 array of (900) glomeruli and their associated output cells (3 classes) and interneurons (2 classes). The type and distribution of synaptic connections modeled are derived from the known connections in the mammalian bulb. The effect of the inhibitory circuits, in addition to confining diffuse excitation to a smaller area, include the generation of complex spatial and temporal patterns of activation, that vary with intensity and odor quality (modeled as differing spatial patterns of input) in ways similar to those observed in single-cell recordings from the bulb. The glomerular-level inhibitory circuits (via PG cells) are potentially more potent for producing inhibition in output cells than are the local circuits through granule cells. The feedback nature of the granule-output cell connection makes it difficult for this circuit to drive output cell activity to zero unless granule cells are driven from non-local sources. The feedforward part of the PG circuit is not so limited. The granule cell circuit is more effective than the PG circuit at controlling spontaneous activity.

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A Large-Scale Computer Model of the Salamander Olfactory Bulb: Responses to Simulated Electrical and Odor Stimulation. J. WHITE, S.N. NEFF, A. CINELLI, and J.S. KAUER. (Neurosci. Program, Tufts/NEMC, Boston, MA 02111).

Anatomical and physiological data of sufficient detail have accumulated to allow computer modelling of the olfactory bulb (OB). We are incorporating these data into a large-scale model of the salamander OB to investigate its odor information processing properties. The model was written in the C programming language and runs on a desk-top PC and math co-processor. In the model, the membrane potentials of various OB cell types are represented as numerical arrays in computer memory. The potential of each modelled cell is then based on its previous potential and the summed influences of all its inputs, calculated by a series of linked differential equations. The membrane and synaptic properties of modelled cells and the connectivities between them are based on anatomical and electrophysiological data from the salamander and other animals. Initial tests showed that modelled mitral/tufted cell responses to simulated orthodromic and antidromic electrical stimulation were similar to actual salamander intracellular recordings, and the ensemble activity of the entire model was similar to that seen in voltage-sensitive dye recordings. Model parameters were further constrained so that responses closely resembled actual salamander data. The model was then tested with an "odor" input, which was simulated as a spatially distributed pattern of activity on the olfactory epithelium. Modelled mitral/tufted cells display a variety of responses depending on the stimulus pattern and the stimulus intensity (i.e., odor concentration). These responses compare well with those observed in intracellular recordings and typically consist of complex periods of depolarization and hyperpolarization during the stimulus, followed by a long period of hypolarization.

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Spatial and Temporal Properties of Facilitation in Optical Responses from the Salamander Olfactory Bulb Evoked by Orthodromic Electric Stimulation A.R. CINELLI and J.S. KAUER (Tufts Medical School and New England Medical Center, Boston, MA)

Previous studies using paired condition/test (c/t) electrical stimuli have shown response suppression following orthodromic activation of the olfactory bulb. Under certain circumstances this suppression is reduced and a period facilitation can appear. In the present study this phenomenon was further investigated by video imaging voltage-sensitive dye responses in slices of water phase, salamander olfactory bulb. The spatio/temporal properties of the signals were studied after orthodromic electric stimulation of the olfactory nerve trunk or of peripheral nerve fascicles. Facilitated test responses were evoked when electric shock intensities to the nerve trunk were close to the threshold for the optical signals and at c/t intervals of 50-150 msec. In general, facilitation was enhanced by reducing stimulus intensity, by decreasing the temperature of the bathing medium and by applying both conditioning and test shocks to distal nerve fascicles. The c/t intervals were also critical; for each combination of other parameters there was an optimal c/t interval that evoked the largest facilitation. At shock intensities 3-4x threshold, facilitation disappeared and typical suppression ensued, in some cases abolishing all test responses and lasting for more than 2 secs. Shocks applied to peripheral fascicles, in contrast to the nerve trunk, evoked larger and more reliably facilitated responses. Fascicle stimulation evoked activity that varied in different sectors of the bulb having different latencies, degrees of depolarization, and time courses for each sector. Whether activity in a particular bulbar sector was facilitated or suppressed depended on the c/t interval and stimulus intensity. This spatial segregation suggests that there are different spatio-temporal sequences for activation of the layers in different regions and that conditioning stimuli can alter this spatio-temporal pattern by suppressing and/or enhancing particular components of the activation in different sectors.

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Patch Clamp Recordings in the Salamander Olfactory Bulb. DAVID P. WELLIS and JOHN S. KAUER (Tufts-New England Medical Center, Boston, MA 02111)

The recent application of patch clamp techniques to *in vitro* slices of brain offers a powerful technique for studying the physiologic and pharmacologic organization of a synaptic circuit. For this reason, we have initiated a series of whole cell recordings from tiger salamander olfactory bulb neurons in an *in vitro* hemibrain preparation.

Tight seals (up to 20GΩ) have been obtained with unpolished, uncoated electrodes and no special cleaning procedures. Upon obtaining the whole cell configuration, spontaneous synaptic currents ranging from <10 pA to >100 pA were commonly observed in symmetrical Cl^- conditions at a holding potential of -70 mV. These currents reversed near 0 mV and were presumed to produce the spontaneous depolarizations from rest (-45 to -72 mV) observed in current clamp mode. A large multicomponent, long-lasting (up to 3 sec) synaptic current was evoked following olfactory nerve stimulation, much of which appeared to be composed of events similar to those found spontaneously. These events could be blocked by bath application of bicuculline ($GABA_a$ antagonist), leaving behind a rapid inward current and a smaller, long-lasting current following nerve stimulation. When E_{Cl} was adjusted to -56 mV, a long hyperpolarization (similar to that observed from *in vivo* mitral/tufted cells (Hamilton and Kauer, 1988)) was produced following nerve stimulation in a slightly depolarized cell, and the component assumed to be associated with bicuculline sensitivity reversed near rest. Therefore, we believe that these spontaneous and nerve driven bicuculline-sensitive synaptic currents may represent the activation of a GABA-mediated Cl^- conductance. Finally, neurons were identified following the perfusion of the cell with a 1% solution of Lucifer yellow or biocytin in the patch electrode.

This salamander hemibrain preparation can potentially be used for the simultaneous application of both whole cell and voltage sensitive dye recordings. The ability to monitor a single neuronal element within a synaptic network in combination with ensemble recordings will provide an opportunity for studying the synaptic relationships between single cells and their associated circuitry and the spread of potentials within single neurons.

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Coding Reliability of Hydroxy-L-proline Sensitive Chemoreceptor Cells of the Lobster's Lateral Antennule.
Carl L. Merrill, Rainer Voigt and Jelle Atema (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543, USA)

Quality and intensity coding were studied in narrowly tuned Hydroxy-L-proline chemoreceptor cells (Hyp cells) from the lobster's lateral antennule. First, we found that Mean Response Magnitudes (MRMs) of small populations of Hyp cells were reliably reproduced with repetition of identical Hyp stimuli and yielded stimulus-response (S-R) functions that increased monotonically. Single cell responses were much less reliable. However, Across Fiber Patterns (AFPs) of these same cells were more similar for repetitions than for changes in intensity, thus allowing for AFP intensity coding.
Second, reliability of MRMs and AFPs in coding of stimulus concentration was evaluated with Hyp-mixtures in non-Hyp backgrounds. Again, individual cell responses were less reliable than MRMs in reporting stimulus intensity. A high organic background of 14 compounds (14C) reduced MRM to Hyp stimuli and thus altered the apparent MRM code for intensity. Although Gly was a weak stimulant of Hyp cells, a binary Hyp-Gly mixture gave smaller MRM than Hyp alone. This Gly effect was reliably reproduced with repetition. However, Hyp and Hyp-Gly caused similar AFPs. Therefore Gly altered the MRM code for Hyp intensity, but the AFP code was not affected. In conclusion, both AFP and MRM of small cell populations contain information for coding intensity and quality.

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Properties of olfactory bulb mitral, tufted and periglomerular neurons studied by electrical stimulation of the olfactory nerve layer. J.W. Scott, D.P. Wellis* and B. Priddy (Emory Univ. School of Medicine, Atlanta, GA 30322 and *Tufts-N.E.M.C., Boston, MA 02111)

We report data from intracellular recordings of mitral cells, tufted cells and periglomerular (PG) cells in the rat olfactory bulb. The mitral and tufted cells were identified by their pattern of antidromic stimulation and in most cases by their morphology following HRP or biocytin fills. The PG cells were identified by the presence of a long lasting depolarization evoked by olfactory nerve layer (ONL) stimulation. The average spike response latencies after ONL stimulation were 6.8 msec for mitral cells (this excludes mitral cells that failed to spike), 5.0 msec for tufted cells and 4.2 msec for PG cells. In each case, the standard deviation was approximately 1 msec. indicating considerable variability in the response. These results are consistent with anatomic observations that PG cells receive direct olfactory nerve terminals and are not exclusively activated by mitral/tufted cell apical dendrites. This order of activation is consistent with very early IPSPs evoked in some mitral cells by nerve stimulation. A rapid inhibition of mitral cell by PG cells or by a pathway involving tufted cell axon inputs to granule cells may be responsible for their poor response to ONL stimulation. In our population of 20 marked mitral cells, 5 of 16 type I mitral cells failed to respond to ONL stimulation, but all 4 type II mitral cells responded (2 with multiple spikes). This indicates that the relative inexcitability of mitral cells to ONL stimulation may correlate with the position of their basal dendrites.

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Metabolic Activity in the Rat and Mouse Olfactory Epithelium and Olfactory Bulb following Odour Stimulation

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Stimulation of the rat olfactory system with controlled dilutions of pure chemical odorants produced odour-specific patterns of metabolic activity, detected by the radioactive 2-deoxyglucose method, in the glomerular layer of the main olfactory bulb. Relating these patterns to activation zones which occur at the olfactory epithelium has proved a difficult task owing to the complex 3-dimensional geometry of the epithelial surface within the nasal structure. Applying these methods to the mouse olfactory system, we have found similar patterns of metabolic activity in the olfactory bulb. This suggests that the function served by these patterns are common to both mammalian species. We are now attempting to make sense of the mouse epithelial activity by making autoradiographs of flat-mounted septal tissue, thereby removing the need for 3-dimensional reconstruction and allowing more robust data sets.

1. Associate of DITAC National Teaching Company Scheme

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Odor Induction of c-fos Expression Reveals Functional Topography in the Rat Main Olfactory Bulb

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Odor responsive regions of the main olfactory bulb can revealed with ^{14}C 2-deoxyglucose (2-DG) autoradiography. This technique is limited in some aspects, particularly in its lack of cellular resolution. Additionally, while odor activation in regions of the glomerular layer can be easily visualized, differential activity at later stages of bulb processing are usually not evident in 2-DG autoradiograms. The expression of the immediate early gene (IEG) *c-fos*, which encodes a transcription regulatory protein, has been shown to respond to neural activation in a number of different neuronal systems. We report here the mapping of odor responsive regions of the rat olfactory bulb using cellular localization of mRNA for *c-fos*. Young rats (PN20-22) were removed from their home cage and placed in an open glass jar in a laboratory fume hood for 30 min, after which they were injected with ^{14}C-2DG (20 uCi/100g, s.c.) and exposed to either air, peppermint odor or isoamyl acetate odor (1:10 dilution in air; flow rate = 2L/min) for 30 min. Animals were then decapitated and the brains dissected out and frozen in methyl butane (-50°C). Tissue was sectioned on a cryostat and alternate sections were processed for 2-DG autoradiography and *in situ* hybridization with *c-fos* ^{35}S-cRNA. Periglomerular, tufted and granule cells were labeled with *c-fos* ribroprobe. In air-exposed animals, low levels of hybridization occurred throughout the glomerular layer (GL), while in odor-exposed animals, dense cell labeling occurred in discrete regions of the GL. Analysis of adjacent sections indicated that these glomerular areas corresponded to regions of high 2-DG uptake. These data suggest that odor stimulation activates *c-fos* expression in cells of the olfactory bulb. Differential cRNA hybridization also occurred in the granule cell layer of odor-exposed animals, with those areas underlying the activated glomeruli exhibiting the heaviest cell labeling. Significant changes in the granule cell layer with odor stimulation have not been observed using the 2-DG autoradiographic technique, even though the organization of bulb circuitry predicts activation of granule cells. This suggests that in situ hybridization with cRNA probes for IEG's may provide a more sensitive and precise means of identifying odor-responsive regions of the olfactory bulb.

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Development of an N100 Component in the Odorant Evoked Potential of Rats After Repetitive Stimulation.

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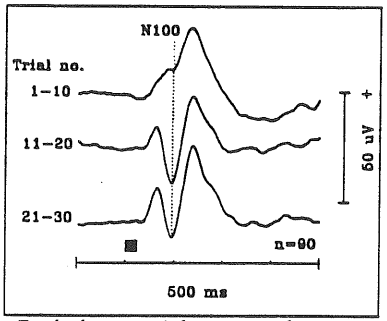
Evoked potentials were recorded from the scalp and intracerebral electrodes in anesthetized rats in response to odorants presented through a nasal cannula by means of a constant flow olfactometer. Stimuli consisted of 1% amyl-acetate in diethyl-phthalate at 20 ms duration, 500 ml/min flow rate and interstimulus intervals greater than two seconds. Odorant evoked potentials from olfactory bulb consisted of a triphasic waveform with major components at 70 ms (P70), 100 ms (N100) and 140 ms (P140). Analysis of single trials from nine animals revealed that the N100 component appeared only after the first few stimuli were presented, reaching maximum amplitude at the seventh stimulus. The development of a sensory evoked potential component within the first few repetitions of a stimulus suggest that odorant evoked potentials reflect dynamic aspects of the encoding of olfactory information.



Evoked potentials to amyl-acetate from olfactory bulb: grand means of nine rats (bar shows stimulus).

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Sub-cellular localization of sialylated glycoconjugates in secretory cells of the salamander olfactory mucosa.

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An indirect gold-labeling method was used to characterize the sub-cellular distribution of sialic acid in secretory products of sustentacular cells (SC) and acinar cells of Bowman's glands (BG). Thin and semi-thin sections of olfactory mucosa were incubated with the lectin from Limax flavus (LFA) that binds to terminal sialic acid residues. The sub-cellular localization of LFA binding sites was identified by a second incubation with gold-labeled fetuin, which is a sialylated glycoprotein. Location of LFA binding was detected at the light microscopic level by a silver intensification technique. The supranuclear region of SC was intensely stained. BG were lightly stained while the deep respiratory glands (RG) of the ventral olfactory mucosa were not stained. Observations at the electron microscopic level showed that labeling was heaviest in secretory vesicles of SC (32.2 $\pm$ 10.5 gold particles/um^2). This indicates that the contents of secretory vesicles are sialylated. The density of gold particles in secretory granules of BG (9.4 $\pm$ 1.6 gold particles/um^2) was less than that of SC. Labeling of BG was confined primarily to electron-lucent regions of secretory granules. Gold labeling in secretory granules of RG was substantially less (1.2 $\pm$ 0.8 gold particles/um^2). Treatment of sections with neuraminidase, pre-absorption of LFA with sialic acid, omission of LFA or incubation with unconjugated fetuin prior to incubation with fetuin-gold all resulted in the absence of staining, which indicates that binding was specific for sialic acid residues. These results demonstrate that SC, BG and RG differ in the amount of sialic acid residues contained in their secretory products. These findings most likely reflect differences in concentration, activities or expression of sialyltransferases in these cell populations.

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Olfactory Nerves Contain Two Types of Glial Cells, Based on GFAP Immunostaining in Culture and In Situ. S. K. PIXLEY (Univ. of Cincinnati, Cincinnati, OH).

Olfactory nerve glial cells may be important in axonal regeneration of olfactory receptor neurons. These glial cells resemble Schwann cells, but also contain glial fibrillary acidic protein (GFAP), like astrocytes. They are similar, but not identical, to non-myelinating Schwann cells. In dissociated cell cultures of newborn Spraque-Dawley rat olfactory mucosal tissues, most GFAP-positive cells resembled Schwann cells (Pixley, (1990) Neurosci. Abs. 16:364.11). However a subpopulation, described here, resembled astrocytes. The presence of two types of glial cells in olfactory mucosal cultures has been reported previously (Barber and Lindsay, 1982, Neurosci. 7:3077). Astrocyte-like olfactory nerve glial cells (ALONGs) were more densely immunostained by anti-GFAP than Schwann cell-like glia and they were only about 1-5% of all cells present. To rule out that they might be a contaminant from the olfactory bulb, cultures were prepared either including the cribriform plate in the dissociation, as usual, or prepared without the cribriform plate (using littermates). Since not all GFAP-positive ALONGs were eliminated when the cribriform plate was excluded, and because some ALONGs were tightly attached to clumps of undissociated olfactory epithelium, a peripheral location was suspected. To explore this, GFAP immunostaining was done on paraformaldehyde-perfused, cryostat-cut, sections of newborn rat pup nasal tissues. Olfactory nerve bundles were fairly uniformly stained at a 1/500 dilution of anti-GFAP but only a minor subset of spindle-shaped cells remained immunostained at a dilution of 1/10,000. These ran parallel with axonal processes, as would be expected of Schwann cells, and were evenly and apparently randomly spaced. These data show the existence of two types of olfactory nerve glial cells but they do not, at present, suggest any possible function or significance.

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Morphological observations on the skate olfactory system. SHIGERU TAKAMI (Department of Biological Science, Florida State University, Tallahassee, FL 32306-3050), CARL A. LUER* (Mote Marine Laboratory, Sarasota, FL 34236) & PASQUALE P. C. GRAZIADEI (Florida State U.).

Recently one of us (C.A.L.) has succeeded in hatching and growing the clearnose skate, *Raja eglanteria* in the laboratory. We are planning now to do study, under strictly controlled laboratory conditions, the development of the connections between the olfactory organ (OO) and the olfactory bulb (OB) as a preliminary study to experimental embryology studies aiming at understanding the role of the nose in the development of the telencephalon. We report here the structure of the OO and OB in young adult animals.

The OO is a dome-shaped bilateral structure limited by a basal lamina containing numerous pigmented cells. The organ is composed of numerous convoluted lamellae covered by sensory and respiratory epithelia. Bowman's glands are absent, however numerous mucous cells are seen in the non-receptor part of the mucosa. The olfactory receptor bundles form glomeruli in the adjacent OB. The olfactory glomeruli lack a well defined periglomerular cell layer. The putative output neurons which are not arranged in a distinct layer are often in close proximity of the glomeruli. The basic anatomy of the olfactory system will in the future be explored in its developmental dynamics. (Supported by Grant No. 20699 to P.P.C.G. from the NIH).

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Calbindin-like immunoreactivity in the Receptor Cells of the Rat Vomeronasal Organ and in Isolated Cells of the Olfactory Epithelium EDWARD W. JOHNSON, PAMELA M. ELLER, BRUCE W. JAFEK (Rocky Mountain Taste and Smell Center)

Although the rat olfactory epithelium (OE) and vomeronasal organ (VNO) share the characteristics of containing peripheral chemosensory cells which project to the CNS (to the main olfactory bulb and accessory olfactory bulb respectively), the chemosensory cells are structurally different. While the receptor cells in the OE have ciliated vesicles which protude into the nasal cavity, the VNO receptor cells have apical microvilli which extend into the cavity of the vomeronasal organ. Although morphologically different, these two cell types both contain olfactory marker protein. This feature may be shared by a third cell type, found in the OE, the microvillar cell (see Moran et al., J. Neurocytol. 11:721 [1981])). The microvillar cell has short apical microvillar processes which extend into the nasal cavity. It has been suggested by others that the olfactory microvillar cell in the guinea pig and man contains at least one class of calcium binding protein (Iwanaga et al., Biomed. Res. 6:329 [1985]; Lomri et al., Gene 80:87 [1989]). By using an antiserum directed against the calcium binding protein, calbindin (generously supplied by A.W. Norman, U. of CA, Riverside), we have found labeling in VNO receptors and isolated cells in the otherwise unlabeled OE. At the light microscopic level these isolated cells resemble microvillar cells; we are presently extending our examination to the electron microscopic level to clearly identify the cell type. The results obtained thus far demonstrate that calbindin is found in receptor cells in one peripheral chemosensory organ (the VNO), but not in the known chemoreceptor cells in a related structure (the OE). Nevertheless, isolated cells (putative microvillar cells) within the OE do contain this calcium binding protein. The role of calbindin in these peripheral chemosensory structures is not yet known.

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Phylogenetic Distribution of the Vomeronasal System in Aquatic Salamanders. HEATHER L. EISTHEN, DALE R. SENGELAUB, and DOLORES M. SCHROEDER (Program in Neural Science, Indiana University).

Jurgens (1971) and Bertmar (1981) suggest that the olfactory system is plesiomorphic in vertebrates and that the vomeronasal system arose as a terrestrial adaptation; however, Broman (1920) and Parsons (1971) have suggested the reverse. Amphibians represent the transition from aquatic to terrestrial vertebrates, so information regarding the presence of vomeronasal and olfactory systems in amphibians may contribute to an understanding of vomeronasal evolution. We have examined the anatomy of olfactory and vomeronasal systems in salamanders from four diverse families at different stages in the lifecycle. Light-microscopic examination of sections through the nasal cavity and forebrain revealed the presence of separate olfactory and vomeronasal epithelia and of discrete main and accessory olfactory bulbs in aquatic adult salamandrids (red-spotted newt, *Notophthalmus viridescens*), larval and adult ambystomids (tiger salamander, *Ambystoma tigrinum*; Jefferson's salamander, *A. jeffersonianum*), neotenic ambystomids (axolotl, *A. mexicanum*), and neotenic amphiumids (three-toed amphiuma, *Amphiuma tridactylum*), indicating that members of these families possess both olfactory and vomeronasal systems throughout the lifecycle. In contrast, members of the proteid family, all of which are neotenic, have long been suspected to lack a vomeronasal system (Seydel, 1895; Bruner, 1914; Farbman & Gesteland, 1974). On the basis of light-microscopic examinations of the nasal cavity and forebrain and electron microscopic examination of the nasal epithelia in mudpuppies (*Necturus maculosus*), we agree that mudpuppies lack a vomeronasal system. Given the presence of the vomeronasal system in the earlier-diverging amphiuma, the absence of a vomeronasal system in mudpuppies appears to be a derived condition. We conclude that presence of both olfactory and vomeronasal systems is a plesiomorphic condition in aquatic larval salamanders, indicating that the vomeronasal system may not have arisen as a terrestrial adaptation.

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Appetite and Taste Preference For Amino Acid In Growing Rats With Protein Nutrition

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Appetite and taste preference with protein nutrition was studied using male Sprague-Dawley strain rats, fed a diet containing purified egg protein(PEP) varied and 15 kinds of taste solutions in a choice paradigm. Rats under protein deficiency(Def) showed anorexia, concurrently with preference for NaCl and/or amino acid(AA), glycine and L-threonine to be sweet, but began to ingest L-glutamate(Glu) and L-glutamine(Gln), umami taste along with NaCl intake declined, when their nutritional state became normal. When rats ingested a 5%, 15% or 45%PEP diet, or both 5% and 45%PEP ones in a choice paradigm, plasma AAs in each group except 5%PEP ever essentially altered regardless of AAs in solution available or water alone, but plasma ammonia decreased as half as water alone. The dietary protein in a choice paradigm was a little higher than expected for the maximum growth, and preference for AA was Glu and Gln. Anorexia was also observed when rats were fed a diet with some essential AA Def regardless of flavoring. L-lysine(Lys) alone in plasma and brain declined postprandially and normalized until meal, but plasma glucose and ammonia were unchanged. Rats began to select Lys among AAs in solution after Lys Def, and then they started to grow normally and prefer for Glu. Rats, fed normal diet again, stopped to ingest Lys but still sustained Glu intake in order to suppress plasma ammonia increase 6h after meal, suggesting that anorexia and Lys hunger under Lys Def could be coupled with the homeostasis peripherally and centrally. In addition, single unit activity of neurons in the lateral hypothalamic area(LHA) was recorded during iontophoretic application centrally as well as ingestion of AA. LHA neurons became much more sensitive to the deficient AA rather than others and prompted to ingest deficient one. Preference for Glu and Gln was only observed whenever protein nutrition was normal, such as Lys Def with sufficient Lys intake for body needs. These data suggested that LHA could be an candidate to regulate the preference for nutrients improving malnutrition and that the plasticity of neurons response to AA should be necessary for the AA homeostasis.

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Sucrose Reduces the Salt Intake of Sodium Deficient Rats. SANDRA P. FRANKMANN, JOHN H. DOKKO, JAMES GIBBS, GERARD P. SMITH & DEBRA A. VELTUNG. (Bourne Lab, New York Hospital/Cornell University Medical Center).

Sodium depletion produces a salt appetite and increases the acceptability of sodium solutions. Recent work by Jacobs et al. (J. Physiology, 1988) has shown that during sodium depletion the major responsibility for encoding sodium in the nucleus of the solitary tract is shifted from salt-profile neurons to sugar-profile neurons. This result predicts two alternative behavioral changes in the response to NaCl when in the sodium deplete state: 1) NaCl now tastes sweet or 2) the palatability of NaCl is now closer to the palatability of sweet solutions. The following experiment tested the specific predictions that sodium deplete rats can distinguish NaCl from a sucrose solution and prefer the NaCl to the sucrose solution. Sodium deficient rats (10 mg furosemide and sodium deficient diet overnight, n=6/condition) were offered 0.3M NaCl alone or in choice with 0.6M sucrose (equi-osmotic) to drink in a 1h appetite test. Rats drank 64% less NaCl when given in choice with sucrose than when given alone ($F(1,10) = 5.26$, $p<.05$; Table 1). In the choice condition, sucrose intake was significantly larger than NaCl intake ($F(1,10) = 4.77$, $p=.05$; Table 1).

TABLE 1. CUMULATIVE FLUID INTAKE, ml ($\bar{X}$ ± SEM)

Condition		5 min	15 min	60 min
1. Only:	NaCl	1.6 (±0.9)	4.0 (±1.1)	5.5 (±1.5)
2. Choice:	NaCl	0.7 (±0.3)	0.9 (±0.4)	2.0 (±1.0)
	Suc	2.8 (±1.3)	5.1 (±2.1)	7.0 (±1.7)

When oral factors were minimized by giving the sucrose by gastric preload (5 ml at 10 min prior to access to 0.3M NaCl), there was no supression of NaCl intake. Thus, the effectiveness of sucrose in suppressing NaCl intake is likely to depend on the taste of sucrose rather than postingestive osmotic effects. These data suggest that under conditions of sodium depletion: 1) the ingestive potency of sucrose is still stronger than NaCl and 2) sucrose intake can substitute in part for the short-term satiating effect of NaCl. Thus, the preference for sweet taste prevails, even when in a state of sodium need.

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Rats Like Calcium Salts

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The preferences of adult male Sprague Dawley rats for calcium solutions was investigated. Groups of 10 rats received an ascending series of 48-hr two-bottle tests with a choice between water and calcium chloride (0.2 - 300 mM), phosphate (0.2 - 70 mM), hydroxide (0.2 - 4.0 mM), gluconate (0.2 - 70 mM) or lactate (0.2 - 300 mM). The results for each calcium salt were similar: Relative to water, rats preferred concentrations between ~0.4 and 5 mM, were indifferent to concentrations between 5 and 12 mM, and avoided higher concentrations. Direct comparisons using two-bottle tests (plus water) of pairs of calcium salts at a single 2-mM concentration indicated that the various anions were preferred more-or-less equally. These results question the validity of previous behavioral and electrophysiological studies using calcium because the concentrations used were far above the preferred range. They reopen the issue of whether an innate appetite for calcium exists. It is intriguing that, in parallel to the case for sodium, the most preferred calcium concentration is similar to those of plasma and saliva.

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Ontogeny vs phylogeny in determining patterns of chemosensory mediated behavior. MARC WEISSBURG. (Marine Environmental Sciences Consortium, Dauphin Island, Ala, 36528).

Among the forces which mold the characteristics of organisms two may be particularly important: developmental constraints and shared ecological requirements of closely related species. Developmental constraints may be particularly important in organisms possessing disparate life history stages, and therefore experience different environments during the course of maturation. Closely related species may experience similar environments, leading to character convergence. To investigate the importance of each factor, I assayed chemosensory mediated behavior in 2 day old larvae, and adults, of the fiddler crab Uca longisignalis. Larvae are confined to oceanic waters, whereas the adults are semi-terrestrial, and feed on estuarine intertidal sediments. Larval crabs were placed in solutions containing amino acids or sugars, and their swimming behavior analyzed by computer-aided video motion analysis. Larvae responded by changing swimming speed and rate of directional change, relative to behavior in sea water. Ten amino acids and 6 sugars were tested. Alanine, valine, and all the sugars (galactose, glucose, maltose, sorbose, sucrose, trehalose) provoked responses. Dose-response trials indicated larvae detect substances at micromolar concentrations, whereas adult thresholds typically occur at 10^{-2} M. Adult crab feeding behavior is mediated by chemoreception, so feeding assays were used to determine chemosensory properties of adults. Test compounds were those that stimulated larvae, or were known to elicit feeding in other Uca species. Using an analysis of percent of animals feeding, and feeding time, adult U. longisignalis are more similar to other Uca, than to larval U. longisignalis. Adults responded only to glucose, maltose, and sucrose, and response pattern was quantitatively similar to patterns observed in U. pugnax. I speculate that behaviors observed in larvae fall into two distinct classes. Amino acids elicit feeding in other marine invertebrates, and probably represent larval responses to feeding cues. Responses to amino acids are necessary in the larval environment in which amino acids, but not sugars, are predominant chemical cues. In contrast, the observed response to sugars arises as a consequence of development. Sugars are not common constituents in sea water, and thus larval chemosensitivity to sugars is probably not functionally important. Sensitivity to sugars is necessary only in the adult habitat, where sugars are common constituents of the primary food source, and serve to indicate food quantity and quality.

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