ACHEMS - 1992

ABSTRACTS

THE FOURTEENTH ANNUAL MEETING OF THE ASSOCIATION FOR CHEMORECEPTION SCIENCES

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ACHEMS - 1992

Fourteenth Annual Meeting
of the
Association for Chemoreception Sciences

ABSTRACTS

This book contains abstracts of the volunteer papers and posters of ACHEMS 1992. 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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Odorant Identification Testing in the Young Child. JUDITH ANDERSON, (UCSD Medical Center), LISA MAXWELL, (San Diego State University), and Claire Murphy, (San Diego State University and UCSD Medical Center)

The present study continued the development and validation of a clinical test of olfactory function in children. This study was designed to provide validity and reliability measures for a method we have developed for assessment of the young child's olfactory function. We employed a method of odor identification with a response mode which could be used with children who have not yet acquired reading skills. Previous work in this laboratory had identified a battery of odorants which were familiar to the very young child. These were natural odorants presented in opaque odorless jars. Preschool and kindergarten children participated in short sessions conducted individually at school. Re-testing took place in the same setting, but on a different day. Children were excluded from the study if they displayed nasal symptoms as observed by a pediatrician. Each subject was presented a set of 20 visual stimuli in an array. To insure that the visual stimuli were familiar, the subject first identified the visual stimuli before being blindfolded for presentation of odorant stimuli. The subject was directed to sniff the odorant and then remove the blindfold and point to the picture that corresponded to the perceived odorant. No verbal response was necessary. If unsure, the subject was required to guess. A 20 second rest period was given between stimuli. Identification of odorants was near perfect for several items. Percent correct for the odorants presented was as follows: playdough (100), cinnamon (94), bubblegum (90), coffee (90), chocolate (87), mustard (87), peanutbutter (86), baby powder (81), crayons (76), soap (70). There was some tendency for 5-6 year-olds to perform better than 3-4 year olds. These data confirm previous findings regarding the choice of stimuli for an odorant battery for children. T-tests for matched samples showed no significant differences between tests in two sessions, suggesting good test-re-test reliability. Implications for the application of this test in a clinical setting will be discussed.

Suported by NIH grant # AG04085 (CM, JA) and training grant # DC00032 (JA)

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Development and Degeneration of Human Olfactory Epithelium. BARBARA R. TALAMO (Tufts Medical School).
WEN-HUI FENG (Tufts Medical School).
JOHN S. KAUER (Tufts Medical School)

A new whole mount procedure has been developed that reveals morphological and immunocytochemical features of the olfactory epithelium that are difficult to appreciate by histological sectioning. Whole mount preparations were prepared of 24 specimens of human olfactory epithelium (oe) taken at autopsy from patients ranging in age from 2 d to 83 yrs. Surface views of sheets of olfactory knobs, dendrites and cell bodies are generated by treatment with antibody to microtubule associated protein 5 (MAP5 or 1b) using fluorescent ABC techniques. Axons and axon bundles can be seen traveling below the epithelial surface, particularly when the receptor cell sheet is sparse or absent. The interpretation of features viewed from the surface was confirmed by rehydration and sectioning of the stained tissue. Fluorescent staining was preserved in the sections and further immunocytochemical staining could be carried out with additional antibodies. In young individuals, the olfactory receptor neurons (ORNs) are densely packed and evenly distributed across the epithelium. Glandular openings are spaced regularly across the surface. At the transitional zone between sensory and respiratory epithelium, circular patches devoid of ORNs can be seen that may represent areas where sensory stem cells are absent. In young, mature and old specimens, variable numbers of pits or crypts lined with ORNs are observed; these tunnel into the lamina propria where they end in blind pockets. These openings are larger than glandular openings, face in several different directions, and tend to be located on the anterior, lateral wall of the nasal cavity. In mature specimens, sensory and non-sensory areas are interspersed as previously reported for sectioned material; no consistent distribution pattern of ORN loss was observed. In Alzheimer's Disease patients, neuritic fibers immunoreactive (ir) for phosphorylated neurofilament proteins (P-NF) are ectopically located in the oe. With whole mount double staining, the relationship between MAP5-ir ORNs and phenotypically altered cells and fibers was apparent. P-NF ir ORN cell bodies are associated with P-NF ir abnormal fibers and dramatic button-like neuritic masses. Other sensory areas within the same epithelium may appear relatively normal. Occasional alterations in molecular and/or morphological phentoype are seen in very young specimens and somewhat more frequently in older specimens. These phenotypically altered neurons may arise directly from stem cells in the oe or be formed by transdifferentiation from ORNs undergoing neurodegeneration as the result of unknown signals. This shift in molecular phenotype (expression of neurofilament protein or tau) does not appear to be characteristic of the normal turnover process.

3

Olfactory Perception of Androstenone is Related to Male Infertility. JACOB STEINER[1], RONEN GASPAR[1], ALEXANDER SIMON[2] and CHARLES J. WYSOCKI[3] ([1]Dept. Oral Biol., Hebrew Univ. Dental Sch., Jerusalem, Israel, [2]Dept. Gynecol. and Obstetrics, Hadassah Univ. Hospital, Ein-Kerem, Jerusalem, Israel, [3]Monell Chemical Senses Center, 3500 Market St., Phila., PA, 19104)

A few years ago, the Monell Center and the National Geographic Society conducted an International Smell Survey. The test instrument used in this survey was employed to assess possible differences in olfactory-related performance in patients undergoing follow-up at a male-fertility clinic. Their responses were compared to those obtained from healthy males who did not present with problems associated with infertility. The odorant-containing portions of the original survey forms were cut into six parts; their presentation sequence was randomized for each subject. After answering demographic and health-related questions, each participant received each of the six odorants (androstenone, amyl acetate, Galaxolide®, eugenol, mercaptans, and synthetic rose) and answered questions related to each, e.g., Could an odor be detected? After eliminating anosmics, the 59 patients presenting with low sperm counts were divided into the following subgroups: aspermia (having semen without sperm or a count of lower than 5 x 10^6/ml, n=17), severe oligospermia (5-8 x10^6 sperm/ml, n=16), oligospermia (8-20 x 10^6 sperm/ml, n=26) and normal (19 years or older, >20 x 10^6 sperm/ml, n=53). Significant group differences in the ability to detect an odorant in the scratch and sniff samples were noted for one of the odorants that typically is associated with specific anosmia, viz., androstenone; of aspermic, severe oligospermic and oligospermic patients, only 35%, 38% and 62% respectively could smell the odor. This compares with 76% of normals who were able to smell androstenone. Ability to detect the other odors appeared unrelated to sperm count. We are at present unable to generate an adequate hypothesis to account for the results, but additional patient data are being analyzed.

Supported in part by NIH grant DC00298 to CJW.

4

Endoscopic Sinus Surgery: Its Role in Sinusitis and Inflammatory Smell Impairment

TERENCE M. DAVIDSON, M.D. (University of California, San Diego, Medical Center)

Two to 4% of Americans have an impaired sense of smell, 1/3 of which is potentially reversible and is caused by inflammatory nasal disease. Twenty to 30% of Americans suffer from inflammatory nasal disease. Endoscopic sinus surgery is a new approach to chronic sinusitis, an approach which began in Europe and has now spread to the United States. It is clearly in vogue and has become the surgery of choice for sinus disease. Publications are reporting success rates exceeding 90%. The surgery is based on the mucociliary activity in paranasal sinuses and the nasal ostial obstructions which occur with inflammatory nasal disorders. Because this has become the surgery of choice for paranasal sinus disease and because of its role in inflammatory induced smell impairment, scientists with interest in human olfaction should be aware of this therapeutic modality. The presentation will cover the cadaver observations made on mucociliary transportation by Dr. Messerklinger and the conceptual development of endoscopic sinus surgery. The operation as currently practiced in the United States will be described. The published results will be reviewed and then the observations and experience with endoscopic sinus surgery in inflammatory smell impairment will be reviewed and discussed.

5

Endoscopic Biopsy of Human Olfactory Epithelium. DONALD C. LANZA, DAVID A. MORAN, RICHARD L. DOTY, JOHN Q. TROJANOWSKI, DONAH CRAWFORD, & DAVID W. KENNEDY (Smell and Taste Center, Dept. Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA)

Biopsy of human olfactory tissue may be useful for better understanding both normal and abnormal olfactory function. However, tissue sampling from the olfactory cleft carries inherent risks. The most widely employed technique for obtaining specimens from this narrow tissue bed relies upon blind intranasal biopsy sampling using a fine hook-shaped instrument. Another procedure, not previously described in the literature, which has the advantage of not relying upon blind sampling, utilizes endoscopic guidance of the giraffe forceps into the olfactory region. In this study, we compared the efficacy of these two techniques. Biopsy success was examined for these technques in 18 subjects using both morphologic and histochemical criteria. The results of this comparision, along with a discussion of issues of subject safety, tolerance, and specimen usability for electron microscopic and immunohistochemical applications, will be presented.

Supported by National Institute on Deafness and Other Communication Disorders Grant PO1 DC 00161 and National Institute on Aging Grant RO1 AG 08148.

6

Intermediate Voltage Electron Microscopy (IVEM) of Olfactory Epithelia in Patients with Parkinson's Disease and Alzheimer's Disease. D.T. MORAN, J.C. ROWLEY III, D.C. LANZA, I. KRATSKIN, D.W. KENNEDY, & R.L. DOTY (Smell and Taste Center, Dept. Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA)

Patients with Parkinson's Disease (PD) and Alzheimer's disease (AD) experience decreased smell function. In this study, we investigated the ultrastructure of the olfactory mucosae of such patients to detect any histopathologic changes that might be associated with the olfactory dysfunction. After biopsies were obtained from the olfactory cleft by giraffe forceps under endoscopic observation, tissues were prepared for electron microscopy and examined in the JOEL 4000-FX Intermediate Voltage Electron Microscope, which photographs "thick" sections ideal for histopathologic studies. In this study, distinct AD- and PD-related ultrastructural changes were observed. In PD patients, patches of olfactory epithelia often contained few receptors; the number of cilia/receptor was often reduced. In places, the "layering" of nuclei normally seen in healthy olfactory epithelia was disrupted. Receptor cell bodies were often swollen in the supranuclear region; microvillar cells were not observed. The number of axon profiles observed near the basement membrane was greatly increased; the diameters of the axons, which was highly variable, was greater than normal. In AD patients, certain areas of olfactory epithelium showed a greatly reduced number of ciliated olfactory receptors. Some of the bipolar neurons had greatly thickened dendrites. Many dying cells were present; occasional macrophages were observed within the epithelium itself. Near the basement membrane, the olfactory epithelia of AD patients contained increased numbers of swollen axons -- which is consistent with observations of dystrophic neurites detected by other immunohistochemical studies.

Supported by National Institute on Deafness and Other Communication Disorders Grant PO1 DC 00161, National Institute on Aging Grant RO1 AG 08148, and National Institutes of Health Resource Grant RR 2483.

7

Olfactory recognition in Sjogren's Syndrome. JAMES M. WEIFFENBACH and PHILIP C. FOX (National Institute of Dental Research)

Sjogren's syndrome (SS) is an autoimmune exocrinopathy characterized by destruction of salivary and lacrimal glands. It preferentially affects peri- and post-menopausal women. Patients commonly complain of dry mouth and dry eyes and may report changes in their experience of foods. Earlier studies of olfactory involvement is SS have been limited in scope and presented conflicting results. In the present study, olfactory identification performance was assessed in 30 SS patients between the ages of 35 and 72(M= 54.4, SD= 11.65) with the 40 item University of Pennsylvania Smell Identification Test (SIT). These patients had been referred to the National Institute of Dental Research Clinic for complaints of dry mouth and were subsequently found to meet standard criteria for SS. Sixty women between the ages of 33 and 73 (M= 53.8, SD= 13.3) who were generally healthy, community-dwelling volunteers in the National Institute on Aging's Baltimore Longitudinal Study of Aging served as controls. A greater proportion of patients (8/22) than controls (4/56) reported that their sense of smell was decreased or worse (Fisher's exact p=.012). The patients' olfactory identification as measured by the SIT was impaired relative to unaffected controls. The patient's median SIT score (36.6) was significantly lower than that of controls (38)(t=-2.2736, p=.0254). A greater proportion of patients (9/21) than controls (6/54) were classified as microsmic or anosmic (Fisher's exact test, p=.037, one-tailed). Only 1 of the 30 patients correctly identified all 40 SIT odors whereas 19 of the 60 controls did (X^2=9.289, p=.002). These olfactory impairments are much more dramatic than the taste intensity impairments documented in these same patients.

8

Burning Mouth Syndrome and Sjogren's Syndrome: I. Comparison of Clinical Profiles. APRIL E. MOTT, M.D.[1,3], Jonathan M. Clive, Ph.D.[1,2], Leslie Bouvier[1,4] ([1]Connecticut Chemosensory Clinical Research Center, [2]Behavioral Science & Community Health, [3]Dept. Medicine, [4]Univ. of Conn. School of Dental Medicine, Univ. of Conn. Health Center, Farmington, CT 06030)

Burning mouth syndrome (BMS) and Sjogren's syndrome (SS) are two disorders capable of inducing profound changes in a patient's chemosensory system. These disorders are a major public health problem, and the associated taste and smell deficits represent a significant compromise to the quality of life for many patients. This paper reports final results of a comparison of the clinical profiles of 27 BMS patients, 41 SS patients, and 43 age and sex matched controls.* Data were taken from the database maintained by the Connecticut Chemosensory Clinical Research Center. The clinical profiles consisted of the presence of absence of each of 29 symptoms. We found that the BMS and SS groups were homogeneous with respect to subjective decreased, absent, or distorted smell, and decreased or absent taste; the BMS patients showed a significantly higher presence of distorted or absent subjective taste. Both groups showed significantly higher chemosensory dysfunction when compared to the control group on all taste and smell symptoms. The SS patients tended to present significantly higher frequencies compared to the BMS patients on 21 of the remaining 25 symptoms. CCCRC olfactory composite score means were 5.54 (SD=1.57) for SS, 5.02 (2.42) for BMS and 6.30 0.74) for controls. These differences were statistically significant Kruskal-Wallis test, $p<.05$); multiple comparison procedures indicated that the BMS group significantly differed from controls. We observed that 50% of the SS groups and 45.8% of the BMS group showed olfactory loss (anosmia/severe hyposmia, 20.8% (BMS) and SS); moderate to mild hyposmia, 25% (BMS) and 35% (SS). In general, SS is a more active disease than BMS. Although both produce similar subjective disruptions in chemosensory function, the BMS group only showed olfactory losses significantly different from controls.

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Burning Mouth Syndrome and Sjogren's Syndrome: II. Comparison of Clinical Dynamics. JONATHAN M. CLIVE, Ph.D.[1,2], APRIL E. MOTT, M.D.[1,3], LESLIE BOUVIER[1,4] ([1]Connecticut Chemosensory Clinical Research Center, [2]Behavioral Science & Community Health, [3]Dept. Medicine, [4]Univ. of Conn. School of Dental Medicine, of Conn. Health Center, Farmington, CT 06030)

This paper reports results of a study of the chronological development of clinical profiles of a group of 27 Burning mouth syndrome (BMS) patients, 41 Sjogren's syndrome (SS) patients, and a group of 43 age and sex matched controls. Data were taken from the database maintained by the Connecticut Chemosensory Clinical Research Center. The clinical profiles consisted of a presence or absence and date of onset for each of 24 symptoms. SS patients reported an average of 16.8 symptoms, while BMS patients reported an average of 9.6 symptoms ($P<0.05$); control patients averaged 3.8 symptoms. For these three groups, the length of time with disease (onset of earliest symptom) averaged 16.8 years, 11.8 years, and 20.6 years respectively. Although these times are significantly different, the rate of disease development (number of symptoms divided by length of time with disease) was remarkably homogeneous across groups. We found an active subgroup of BMS patients which was more similar on average to SS patients than to the less active BMS patients, in terms of number and type of symptoms developed. This suggests that in some cases, BMS may be a clinical subset of SS. In general, SS is a more active disease than BMS and produces a wider range of symptoms, although both diseases display similar degrees of subjective chemosensory dysfunction.

Supported by NIH Grant No. 5 R01 DC00168-11.

10

Anesthesia of the Chorda Tympani Nerve: Insights into a Source of Dysgeusia. K. YANAGISAWA, L.M. BARTOSHUK, T.A. KARRER, J.F. KVETON, (Yale University School of Medicine), F.A. CATALANOTTO (University of Medicine and Dentistry of New Jersey), C.D. LEHMAN (University of Washington Medical School), and J.M. WEIFFENBACH (National Institute of Dental Research).

Anesthesia of the chorda tympani nerve (a branch of the facial or VIIth cranial nerve) can be accomplished via the ear canal since the chorda tympani crosses the ear drum on its way from the tongue to the CNS. Approximately 0.2 cc of 1% lidocaine with 1:100,000 epinephrine was injected into the posterior ear canal (N=10) to produce the anesthesia. Whole mouth taste tests showed significant increases in the perceived taste intensities of some stimuli. These results are similar to those reported at an earlier AChemS meeting (1985) for anesthesia of the chorda tympani via a mandibular block in the mouth. Spatial testing revealed that the intensifications occurred at the circumvallate papillae (innervated by the glossopharyngeal nerve, the IXth cranial nerve) and that the intensifications were greater on the contralateral side. These results support the existence of inhibitory connections in the CNS between the projection fields of the chorda tympani and the glossopharyngeal nerves. Such inhibitory connections were first proposed by Halpern and Nelson (1965) on the basis of electrophysiological recordings from the rat medulla. One subject spontaneously reported a phantom taste after taste testing was completed. Subsequently, 4 out of 6 subjects experienced phantoms. The qualities of the phantoms were subject specific, persisted for the duration of the anesthetic, and were reproducible. Two subjects experienced phantoms when either chorda tympani was anesthetized and the phantoms were always contralateral. We suggest that phantoms induced by damage to a taste nerve may explain some reports of dysgeusia.

Supported by NIH grant DC00283.

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Mixture Suppression Measured by Thresholds: Implications for Evaluation of Taste Losses in Aging. JOSEPH C. STEVENS and JULIANNE HOFFMAN (John B. Pierce Laboratory, New Haven, CT 06519)

Age elevates threshold for all taste qualities, in various studies, by two to nine-fold between youth and old age. But examination of taste magnitudes well above threshold argues that, except for bitter, loss is limited to very weak levels and accordingly lends the impression that they handicap the aged little. Our recent comparison of the ability of young, middle, and old ages to discriminate the presence-absence of salt in tomato soup gave quite another impression, however, in that the older the subject, the poorer the discrimination; salt thresholds measured in the presence of tomato were several times higher than in water, but the difference between young and old threshold was the same. How general is this phenomenon? To evaluate this question we are measuring detection threshold (forced choice) for one quality (the target) as a function of a wide range of concentrations (from plain water to strong suppressor) of another quality (the suppressor). The elderly's threshold is consistently two or three times higher than the young's, whether in water or in a strong suppressor. Moreover, the way in which the target threshold rises with the concentration of the suppressor is the same for young and old. This information also enlarges knowledge about taste interactions ("taste mixtures"). Thus far we have measured thresholds for NaCl and sucrose as functions of citric acid concentration and the threshold for citric acid as a function of sucrose. Supported by NIH Grant AG04287.

12

Teaching the Chemical Senses: From Elementary School to College.

This workshop will discuss ways of teaching the chemical senses. We will begin with a discussion of teaching the chemical senses to elementary and high school students. This will be followed with a discussion of what should be included in one or two lectures about the chemical senses to college students. We will also examine what might be included in a whole college course devoted to the chemical senses. Finally, we will examine demonstrations and laboratory exercises which could be used to demonstrate chemical senses ideas and concepts. Persons attending the workshop are encouraged to bring their own lecture outlines, syllabi, and laboratory exercises. We plan to put together a booklet containing the material people use to teach the chemical senses. We hope that this booklet could be made available to anyone in the Society who would like a copy.

13

Olfactory Event-Related Potentials: State-of-Art, Opportunities, and Needs.

KOBAL G. (Dept. of Pharmacology and Toxicology, University of Erlangen-Nürnberg, W-8520 Erlangen, Germany)

The first chemosensory event-related potentials (CSERP) to odorous substances have been obtained by Finkenzeller (1966) and Allison and Goff (1967). However, Smith et al. (1971) contested the olfactory nature of these responses, on the grounds that they were not obtainable in patients who had lost trigeminal sensitivity. In 1988 Kobal and Hummel were able to demonstrate that there were indeed olfactory responses to vanillin in man. In patients whose filae olfactoriae had been ruptured stimulation with vanillin evoked no potentials whatsoever, while doing so in normals. On the other hand, non-odorous carbon dioxide was always perceived and elicited chemo-somatosensory event-related potentials (CSSERP) mediated by the trigeminal nerve. Hence, it can be assumed that responses to vanillin are indeed olfactory event-related potentials (OERP). Also, topographical mapping on the skull has permitted the differentiation between these two kinds of CSERPs. To date, only late nearfield OERPs have been recorded in man. Already here, experimenters have found themselves confronted with considerable difficulties, since it is of paramount importance to employ suitable, specially devised olfactometers. They must be able to repeatedly present identical stimuli of steep onsets (rise time < 50 ms) without simultaneously exciting other, non-chemosensory modalities. Rapid habituation, a characteristic feature of olfaction in which it differs from other sensory modalities, precludes high repetition rate which is propitious to improve the signal-to-noise ratio. Besides intensifying the fundamental research, which includes the finding of the cortical generators, the ascertaining of interactions with the trigeminal system, etc., clinical studies are indispensable, in order to determine whether CSERPs will attain the same importance and quality of being useful, a position, auditory, somatosensory, and visual event-related potentials have securely kept now for many years.

14

Differentiating and controlling olfactory and trigeminal stimulation. **JAMES D. PRAH, U. S. EPA, RTP, NC.**

The perception of an odor is a gestalt which, in addition to odor sensation, encompasses cognitive, neuromuscular, and trigeminal events. In order to adequately study the electrophysiology of olfactory sensation and it related neural substrates, these concomitant events must be understood and controlled to obtain responses only from the olfactory substrates. Some of these, notably, the neuromuscular events can be eliminated by using a nasal isolation paradigm consisting of intranasal stimulus delivery while soft palate closure isolates the nasal cavity from the oral cavity. Cognitive events related to voluntary sniffing activity are similarily eliminated using this paradigm. The trigeminal nerve transduces a variety of somatosensory stimuli including thermal, movement, touch, pain, and vibration. Some of these occur during the act of sniffing or stimulus delivery and may confound the interpretation of olfactory-related electrophysiological responses. Knowledge of the sensitivity of the trigeminal nerve to these stimuli will aid in the minimization of the contribution of these stimuli to the olfactory gestalt to reveal a "pure" olfactory response, which, parenthetically, is a different cognitive experience from the experience of an odor within the gestalt of a sniff. In addition, cerebral localization of the electrophysiological response can provide verification of the anatomical locus of the response and thus its putative sensory origin. In an analogous way trigeminal stimulation and response can be studied by intranasal delivery of a nonodorous trigeminal stimulus. These paradigms should not be taken to minimize the importance of the wholistic study of the olfaction.

This is an abstract of a proposed presentation and does not necessarily reflect EPA policy.

15

Olfactory Evoked Potentials in Animals and Humans.

W. JAMES EVANS, SONALI DeFONSEKA, HUNG NGUYEN and ARNOLD STARR (University of California, Irvine)

Olfactory evoked potentials can be reliably recorded from olfactory bulb, piriform cortex and scalp of anesthetized rats in response to brief, intermittent odorant stimuli. The morphology of the evoked potential is similar for different odors with the onset latency increasing with decreasing volatility of the odorant. The evoked potential, recorded from deep within the olfactory bulb, in response to amyl acetate begins as early as 20 ms after stimulus onset (i.e., opening of the solenoid) with the peaks of the three major components occurring at 60 ms (P60), 90 ms (N90) and 140 ms (P140). The latter two components reverse in polarity approximately 0.6 mm below the bulb surface suggesting their origin in the granule cell layer. The amplitude of both the N90 component of the olfactory bulb potential and the corresponding positive component of the scalp-recorded potential increases during the first ten presentations of an odor. The N90 component is obliterated after transection of the olfactory peduncle. Recording from the scalp shows that evoked potential amplitudes are twice as large over the rostral scalp (nose) than over the caudal scalp (parietal regions). By modifying the olfactometer to accommodate larger volumes, we have also been able to record olfactory evoked potentials from the scalp of seven normal human subjects. The human evoked potentials are of greatest amplitude at the nasion with major components at 270 ms (N270), 390 ms (P390) and 560 ms (N560). The amplitude of the P390 component appears to change with stimulus repetition.

Supported by NIH Grant DC-00033.

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Inspiration-Based Averages of Olfactory Event-Related Potentials.[1]

TYLER S. LORIG (Washington and Lee University).
MEG McKERNAN (Washington and Lee University).
GREG HICKS (Washington and Lee University).

Olfactory event-related potentials (ERPs) offer great promise for clinical studies of chemosensory sensitivity and for investigations of olfactory information processing. Kobal and associates have devised a reliable technique for obtaining these potentials which presents the stimuli out-of-phase with subjects' respiratory cycle. Because spontaneous inhalations of odor may prepare the bulb for odor acquisition, olfactory ERPs to inspired odors may provide additional information about the neural events associated with odor perception. Reliable olfactory ERPs have, however, been difficult to obtain using inhaled stimuli. In a previous study, we found that cueing subjects to inhale increased the similarity of nasal inhalations and produced more reliable olfactory ERPs. While an approach such as this is informative, the cognitive demands placed on the subjects could limit the utility of the technique. For this reason, we attempted to improve the method by allowing subjects to breathe freely and control the similarity of inspiration-based ERPs by expanding or contracting the temporal domain of the ERP depending upon the duration of the subject's inhalation. Sixteen subjects participated in the experiment which had three phases. Phase one recorded visual ERPs to abstract polygons. Phase two recorded olfactory ERPs to two different odors (4% butanol, 50% amyl butyrate) and phase three recorded visual ERPs to abstract polygons presented one segment at a time. The rate at which the segments appeared was based on the subject's rate of inspiration. This condition was included to provide a visual analog to the olfactory condition where the rate of neural events is related to the rapidity of inspiration. Comparison of the ERPs obtained in each of these phases indicated that the inhalation-based expansion or contraction of the temporal domain did not improve either olfactory ERPs or visual ERPs related to inspiration (Phase 3). These results suggest that the duration of inhalation has little to do with the similarity of the ERPs and that the ERP waveform appears to be generated as a function of some threshold mechanism.

[1]This research was supported by PHS grant 1-RO3 DC01323-01 awarded by NIDCD.

Expression of Urinary H-2 Odortypes by Infant Mice. KUNIO YAMAZAKI[1],GARY K. BEAUCHAMP[1], YOSHIHISA IMAI[1], JUDITH BARD[2] AND EDWARD A.BOYSE[2] ([1]Monell Chemical Senses Center, Phila., PA [2]University of Arizona, Tucson, AZ)

Odortypes, defined as genetically-determined body scents that enable individuals of species to distinguish one another by scent, are specified by polymorphic genes of the Major Histocompatibility Complex (MHC), called H-2 in the mouse. Perception of MHC odortypes causes preferential mating and also affects the maintenance of early pregnancy, thereby favoring the propagation of particular MHC genotypes in the mouse. Several studies have shown that a mother can discriminate her own pups from alien pups by odor but no specific genetic basis for this has heretofore been identified. So far, all of our studies of MHC-determined odortypes have involved odors of adult mice. The age of onset of MHC-determined odortype was thus examined. We tested the ability of trained mice to discriminate odors of pups differing only at the MHC in a Y-maze. When whole pups were used as odor sources, MHC-determined odors were not detected by trained mice until the pups reach 11-14 day of age. However, female rodents tend to lick and consume urine from their young pups and young pups may not urinate spontaneously. This could serve to minimize the ability of the trained mice to detect MHC-determined odortypes of pups since urine is the most potent source of these odors in adult. Consequently, urine was next used as the odor source. The results clearly demonstrated that urine of pups as young as one day of age express MHC-determined odor. MHC-determined odortypes may play a prominent role in the ability of the mother mouse to discriminate her own pups from alien pups beginning at least as early as day 1 of age.

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

Genetic Control and Expression of the Major Urinary Proteins (MUPs) in the Laboratory Mice: MUPs as a Potential Pheromone Messengers?
GENNADY A. CHURAKOV, ANATOLY A. PHILIMONENKO (Dept. of Genetics, St. Petersburg State Univ.), and SERGEJ N. NOVIKOV (Pavlov Inst. of Physiology, St. Petersburg 199034, Russia)

The activity of androgen-dependent pheromones in laboratory mice may be associated with protein fraction of the voided urine, especially with major urinary proteins (MUPs) [Vandenbergh et al., J. Reprod. Fert. 43: 515-523 (1975); Marchlewska-Koj, Biol. Reprod. 17: 729-732 (1977)]. Thus the interstrain differences in pheromone activity may be due to the total MUPs amount and/or to different MUPs variants in the lines to be compared. We report here the first results on the electrophoretic variants of MUPs and on the total protein content in the urine collected from mature male and female mice of CBA/LacSto and C57BL/6Sto strains: the males of the latter is known to be described as a pheromone-deficient (Novikov, Pheromones and Reproduction in Mammals: Physiological Aspects, 1988, Nauka Publ. House). The analysis of the MUPs profiles by PAGE from intact, castrate, and castrates treated with testosterone permits us to distinguish several bands which can be regarded as proteins associated with pheromone activity. The presented data is considered in the scope of intriguing homology of MUPs and several olfactory and taste ligand binding proteins with presumed carrier function (Schmale et al., Nature, 343: 366-369 (1990); Singer, J. Steroid Biochem. Molec. Biol. 39B: 627-632 (1991).

Human Axillary Odors and Their Precursors. George PRETI[1], Xiao-Nong ZENG[1], James J. LEYDEN[2], Kenneth J. McGINLEY[2] and Andrew I. SPIELMAN[3] ([1]Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA; [2]Dept. of Dermatology, Univ. of PA; [3]Dept. of Oral Medicine and Pathology, NYU).

Numerous studies have suggested that axillary secretions and their odors can convey information between individuals or groups of people. These odors allow individuals to identify their own odor as well as those of their spouse and close genetic kin. Extracts made from axillary secretions can change the length and timing of the female menstrual cycle. In addition, hundreds of millions of dollars are spent each year on products to suppress, mask or eliminate our most characteristic odors. Our recent studies have determined the nature of the characteristic axillary odors in male samples; they are C_6-C_{11} saturated, unsaturated and branched acids with the largest component being (E)-3-methyl-2-hexenoic acid (3M2H). The precursors to these odors are found in the apocrine secretion which is odorless until it interacts with the resident axillary microflora. We can separate the apocrine secretion into aqueous and organic phases and generate 3M3H and other C_6-C_{11} acids from the water phase by hydrolysis using NaOH and interaction with axillary bacteria. The aqueous phase is rich in proteins which we have separated and hydrolyzed individually. This has shown that two proteins of apparent molecular weight of 26,000 and 45,000 carry 3M2H. The fact that a characteristic human odor is secreted onto the skin bound to proteins is a novel finding. It suggests (a) that if the odors which characterize the axillae are responsible for the primer pheromone activity reported for axillary extracts (e.g., menstrual cycle alteration), the chemistry involved may be similar to other mammalian pheromone systems; and (b) heretofore untried methods for suppressing axillary odor.

An Unusual Compound and Further Characterization of a Pre-ovulatory Pheromone of Asian Elephants, Elephas maximus.
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A compound isolated from pre-ovulatory urine of Asian elephants as apparently a single entity (as assessed by a single band on TLC, a single peak by HPLC and a single dominant mass by field desorption mass spectrometry {FDMS}), was consistently active during bioassay and exhibited a reproducible dose response curve. Once pure (apparently), this compound was rapidly identified using a combination of spectral and mass spectral techniques. The principal component of the active fraction exhibited a molecular ion (m/z) at 248. An exact mass measurement on the molecular ion was obtained by electron ionization (EI) mass spectrometry (MS) analysis. From the mass of 248.056, the composition $C_{15}H_8N_2O_2$ was established. The isotope distribution of the molecular ion calculated from this composition was consistent with that observed in the mass spectrum. UV spectral data indicated an extended, complex chromophore, probably a nitrogen heterocyclic. Fragmentation information by collision-activated, EIMS demonstrated ions at 220 and 192. The fragment ions in the EI spectrum (m/z 220 and 192) were consistent with the sequential loss of carbonyl groups; Fourier transform infrared (FTIR) spectra also indicated carbonyls. Definitive NMR data indicated 8 aromatic hydrogens, assigned on the basis of their coupling characteristics observed in the 2-D spectrum that were individually assigned to two different benzene rings. All of these spectral data and comparison with an authentic sample established unambiguously the structure as **indolo[2,1-b]-quinazoline-6,12-dione, (tryptanthrine).** Subsequent bioassays of the synthetic, authentic compound exhibited an initial high, novel substance response, followed by sustained low-level response which gradually diminished to zero during a 6th month test period. Several hundred bioassays of wide ranges of concentrations and conditions were conducted such that we are reasonably sure that tryptanthrine is **not** the active pheromone. Re-evaluation of the active elephant preparation by HPLC and UV spectrometry demonstrated an earlier eluting, UV distinctive peak that, when isolated and bioassayed by itself, was active. Preliminary data on this compound are reported.

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Antecedents and Consequences of Mating in Mongolian Gerbils.

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It focuses on the preferential, monogamous life-long bonding of a male to a female in cages with a male and two females, with the subsequent infertility of the rejected females; and on cases in which no mating takes place or double-mating occurs -- the former being more common. The hypotheses are 1) that the hierarchy for selection would be governed by a preference for the younger female: non-kin over kin; dominant agouti color over black; submissive over aggressive; 2) that rejected females would not enter estrous; 3) that this is produced by priming pheromones sprayed by the male or the bonded female or both; 4) inducing estrous would not result in mating for rejected females -- since the reasons for her being rejected are in the perception of her own chemosignals (odor print) by the rejecting male; 5) further more, estrous cycling would only be temporarily induced by injection of bromocriptine mesylate (Sandoz Laboratories, a dopamine agonist), and the rejected female would return to her infertile condition. In short: her infertility is a result of rejection and not the opposite. Groups of three animals are made with variations of age, kinship and color. Status of each is established by observation, weight, and vaginal smears. Rejected females are injected with bromocriptine mesylate, or sham or untreated for control. To date data confirm postulated hypotheses.

This research is supported, in part, by ADAMHA-MARC Grant MH-17138 and by a HOFFMAN/LA ROCHE/SUNY equipment grant which made the research possible.

Effects of Contact with Males on Metabolic Rate and Food Intake in Female Voles.

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JOHN J. LEPRI & ROBERT E. GATTEN, JR. (Department of Biology, The University of North Carolina at Greensboro)

Female prairie-voles (*Microtus ochrogaster*) do not express spontaneous estrous cycles, but instead require contact with males to go through a process called reproductive activation. Chemosensory and physical stimuli from males have been identified as critical cues for inducing estrus in females. We examined the effects of physical and chemosensory contact with males on the metabolic rates and food intake of female prairie voles. An earlier study in this lab demonstrated that exposure of female voles to males resulted in increased metabolic rates. We are presently working to extend those findings by comparing the food intake and metabolic rate of females during activation with females not exposed to males. We will also measure the fat content in the carcasses of the activated females to try and determine the source of the increased metabolic rate.

SUPPORTED BY THE UNIVERSITY OF NORTH CAROLINA INSTITUTE OF NUTRITION.

Do Large, Frequently-Changed Cages Increase Reproduction at the Expense of Pup Mortality in Prairie Voles?

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Social odors and environmental complexity can influence the development and expression of social behavior and reproductive biology. Disruption of the social-odor environment, as typified in standard lab practices of cage-changing, might affect social behavior and reproductive biology. To test this, we produced empirical measures of reproduction and behavior while experimentally manipulating the cage environment, using a captive population of prairie voles. Reproductive output was monitored over 4 months for paired voles maintained in one of 4 treatment groups: i) frequently-changed [every 3 days], small [27Lx21Wx14H cm] cages; ii) infrequently-changed [every 2 weeks] small cages; iii) frequently-changed, large [36Lx24Wx19H cm] cages; and iv) infrequently-changed, large cages. The greatest number of births occurred in the large cages, and frequently-changed cages were associated with higher levels of pup mortality. Every 2 weeks, the males of each breeding pair underwent a behavioral test examining their investigative responses to odors from other males, in order to determine whether the "social responsiveness" of the males was affected by the cage conditions. The males were tested in a circular arena divided into 4 quarters, with one quarter containing the odor stimulus. Voles housed in the frequently-changed cages were significantly more active, and groomed and scratched more, than those housed in the infrequently-changed cages. Also, voles housed in large cages approached the odor stimulus more rapidly, and spent more time near it, than did voles housed in small cages. Cautiously generalizing from this single set of results, we conclude that optimum reproduction in our vole colony is best achieved by housing the breeding pairs in large cages. While frequent changes of cages may permit a higher birth rate, they may also increase pup mortality and affect common measures of odor-guided behavioral responses.

Discrimination of Sex Pheromones by the Cabbage Looper Moth, Trichoplusia ni (Hübner)

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Several compounds often are found in the volatile emission of an insect engaged in sexual signalling. Conventional thought is that a mixture of all of the compounds confers specificity. Inherent to specificity, however, is an underlying presumption of signal discrimination. Do all of the compounds play a part in signal discrimination, however? This question may be answered in part by determining which of the compounds are detected through specialized receptor neurons on the antenna. Further insight is gained by determining which stimuli elicit behaviors that may be associated with signal discrimination. Pheromone-elicited behavior results from complex CNS integration of olfactory stimuli, hence behavioral response differences would signify signal discrimination. By definition, discrimination is the ability to distinguish two or more stimuli. Therefore, any appropriate assay of pheromone discrimination must measure response differences in the presence of more than one stimulus. It follows that responses in the presence of a single stimulus might measure a perceptual quality, but do not measure discrimination. Furthermore, because the character of an aroma depends upon both its composition and concentration, it is obligatory to assay at normally-encountered pheromone concentrations. Otherwise, the assay may lose reference with what happens in nature. Neurophysiological examinations have demonstrated that only three of six compounds found in the volatile emission of female cabbage loopers are detected by a male's antennal specialist neurons at relevant concentrations. The discrimination process of male cabbage looper moths was assessed by measuring their behavioral response differences to two emission sources within a wind tunnel. Statistically different behavioral responses that signify discrimination occurred between (1) different concentrations of Z7-12:Ac; and (2) Z7-12:Ac alone and the volatile emission from excised female sex pheromone glands. More equivocal statistical differences demonstrated that the moths had difficulty discriminating between (3) Z7-12:Ac and the emission from a mixture of six synthetic pheromone components that mimics the volatile emissions of a female gland; and (4) mixtures containing slightly different ratios of three components. The evidence suggests that a mixture of components may confer a small quantitative augmentation of discriminability.

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Olfactory Modulation of Pheromone-Mediated Flight in Moths

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The response of males to pheromone that results in sustained upwind flight and source location is, for at least some moths, a reiterative process, as recently proposed by our laboratory. We previously hypothesized that each iteration would involve a rapid, phasic, anemotactic response to the onset and offset of a single filament of pheromone in the finally structured plume, the response consisting of an upwind surge. It would also involve a tonic, long-lasting behavioral response to the large parcels of pheromone-free air during a large-scale wind shift, which consists of casting flight, which we only recently recognized as being pheromone-mediated and involves a program of counterturning. In an attempt to understand the behavior of male moths during each iterational response to pheromone ON and OFF, we experimentally manipulated the filament frequencies of *Heliothis virescens* pheromone, and determined the reaction times of *H. virescens* males in response to a single filament of the natural blend of pheromone components. These experiments demonstrated for the first time that moths intersecting a single filament of odor respond by exhibiting a surge of upwind movement. The latency of the response to onset of odor seems to be somewhat variable, but in most cases the moths reacted in less than 0.30 sec. The response consisted of an upwind surge lasting ca. 0.30 sec., and a decrease of the course angles (and resultant track angles) to point the moth more upwind. The single pheromone filament did not extinguish counterturning, and thus the surge retained a zig-zag shape. The surge was also comprised of a slight slowing of the airspeed (and resultant groundspeed), and altitude was much better regulated during the surge as opposed to during casting, before and after the surge. The 0.30 sec. reaction time to a filament was especially interesting because we also showed that male H. virescens did not respond with upwind flight in significant numbers to a plume comprised of fewer than 4 pulses per second. The result from the single-filament study that each upwind surge lasts ca. 0.30 sec predicts that the male must contact, on average, at least 3-4 filaments per second in order to maintain an upwind flight track.

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Biologically Based Repellents Reduce Food Consumption In A Forest Pest, The Mountain Beaver.

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The mountain beaver (Aplodontia rufa), a primitive herbivorous rodent from the Pacific Northwest of the U.S. and Canada, causes considerable forest damage. It feeds on several tree species, particularly conifers. This study was performed to evaluate the effects of scents from predators as feeding deterrants. Choice experiments were conducted during which the beavers were presented with 2 bowls, each containing 20 gm of apple. Scent from a predator was applied to the rim of one bowl, control scent to the rim of the second bowl. After each 2 hr test period, the amount of apple consumed from each bowl was determined. The following experiments were conducted, presenting predator and control scents in a number of combinations: Exp. 1 Unscented bowls versus bowls scented with 50 µl of 2.5% butyric acid in mineral oil. Exp. 2 Unscented bowls versus bowls scented with 500 µl of guinea pig urine. Exp. 3 Bowls scented with 50 µl of 2.5% butyric acid versus bowls scented with 50 µl of anal gland secretion from male mink. Exp. 4 Bowls scented with 500 µl of urine from either mink, bobcat, coyote or domestic dog versus bowls scented with 500 µl of urine from prairie voles. In Exp. 1 and 2 the beavers consumed equal amounts of apple from unscented bowls and from bowls scented with the control odors. These results show that novel odors such as butyric acid or guinea pig urine do not inhibit feeding. However, scenting bowls with mink anal gland secretion or with the urine from any of the predator species significantly reduced feeding from this bowl. These results show that mountain beavers respond to scents from a number of natural predators, as well as from domestic dogs, with a reduction in feeding from the sent source. This may represent an innate generalized response to chemical cues derived from carnivores and could be of value in the development of biologically based repellents.

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The Olfactory Sensitivity of Sea Lamprey to Amino Acids is Specifically Restricted to Arginine

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The sea lamprey (*Petromyzon marinus*) is a modern representative of the most primitive group of vertebrates and it is thus a good model for comparative studies of olfactory function. This study used electro-olfactogram (EOG) recording to examine the olfactory specificity and sensitivity of sea lamprey to α-amino acids, a group of highly stimulatory odorants for teleost fish. Lamprey EOG responsiveness to amino acids is unique in four ways: (1) Of 40 amino acids tested, only L-arginine is a potent olfactory stimulant (threshold 10^{-10} Molar (M)). Other basic amino acids have thresholds ranging from 10^{-7} to 10^{-5} M, and acidic and neutral amino acids have thresholds ranging from 10^{-4} to 10^{-3} M; (2) D-amino acids are either as potent as, or only slightly less potent than their L-isomers; (3) The guanidinium side chain appears to be responsible for the potency of arginine -- arginine-related compounds lacking guanidinium groups are not as potent as L-arginine, while those with guanidinium groups are equally potent. Also, altering non-guanidinium components of arginine-related compounds does not alter their olfactory potency; (4) Generally, altering the primary carboxyl group or α-amino group increases the olfactory activity of amino acids. In addition, removing these groups (to make amines or organic acids) often increases the potency of these compounds. In conclusion, it appears likely that the specific olfactory sensitivity of sea lamprey to arginine is mainly attributable to the nitrogen-rich side chain attached to the anomeric carbon atom. This unique pattern of olfactory sensitivity may be related to the parasitic feeding behavior of sea lamprey.

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Run or Play: Chemically Stimulated Behaviors Related to Shell Fit.

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Hermit crabs have complex behaviors for assessing, trading and obtaining the gastropod shells that they occupy. Crabs specifically chemically detect potential shells from a distance. Although hermit crab populations are often shell limited, not all crabs are in poor fitting shells. Here we report two major findings: 1) Hermit crab responses to chemical shell availability cues are dependent upon shell fit. Crabs in relatively large shells withdraw into existing shells. Crabs in relatively small shells are attracted to the source of chemicals and investigate every potential shell. Crabs in shells that are not too large and not too small flee the area. We show that each behavior can be stimulated in crabs by changing their shells. 2) A new source of specific chemical cues eliciting this behavior has been discovered. The source of the cue is hemolymph of a conspecific. Molecules from hemolymph responsible for the behaviors are <500 Da and can be purified by affinity chromatography using anhydrotrypsin. These chemically-mediated behaviors are thus directly related to increasing or decreasing the potential for shell exchange. They appear to be unrelated to the potential for death by predation.

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Effects of Flow Regime and Speed on the Structure of an Odor Plume: Implications for Orientation Strategies of Aquatic Animals.

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Many animals can chemically orient to odor sources in a variety of flow regimes and flow speeds. Since the dispersal of chemicals (and hence stimulus structure) is due to the fluid dynamics of a particular environment, we wanted to quantify the changes in the fine structure of an odor plume under different dispersal conditions, including different flow speeds and physical dispersal processes. We measured a turbulent odor plume at 10 and 200 Hz using a microchemical sensor under two flow speeds; 3.8 and 14 cm/s in three different flow regimes: a viscous sublayer, boundary layer, and free flow region, respectively. A 3-min record was sampled at 6 down-current distances (range 10 - 100 cm). The high flow velocity exhibited a greater level of turbulence which resulted in more odor pulses than the low flow velocity. The most intense odor fluctuations occurred in different layers for the two flow speeds. Preliminary data obtained using a new probe design showed that patch sizes may be as small as 200 microns. Plume parameters such as pulse height and pulse slope had spatial distributions that could provide directional or distance information about the odor source to an orienting animal. Different probability distributions for pulse height and slope were seen for the two flow speeds and different flow regimes. From these results, we hypothesize that animals orienting to chemical signals under different flow conditions will have different search strategies and thus different search behaviors under different flow conditions. In addition, those animals that sample from different flow regimes receive different information from each of these regimes that may help them orient to food sources.

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Chemosensory Orientation In Fully Defined Turbulent Flow.

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Physiological studies demonstrate many marine predators possess chemosensory receptors which respond to prey odors. Chemical sensing is invoked as a mechanism regulating predator-prey interactions, yet there is little direct evidence from behavioral and ecological studies to support this conclusion. To provide meaningful information, chemical and hydrodynamical measurements of odorant distributions are required on spatial and temporal scales relevant to chemosensing, combined with non-invasive observations on predator behavior. We have examined predatory abilities of the blue crab, Callinectes sapidus, in field and laboratory flume studies using infrared video imaging, and computer-video motion analysis. Flow regimes are fully characterized by determining boundary layer velocity profiles, boundary shear velocities, and roughness Reynolds number (a parameter relating to the degree of turbulence in the boundary layer). We have also employed microvoltametric measurements of a conservative chemical tracer in characterizing physical properties of odor plumes. Crabs use chemoreception to locate a variety of prey, tacking upstream in a way resembling moth orientation in pheromone plumes. Changes in crab orientation behavior, and ability to locate prey, are associated predictably with changes in boundary layer flow. Our presentation will describe the relative contributions of advective and eddy diffusive processes in odor transport, as they influence orientation and prey localization by crabs.

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Peptides are the natural inducers to settlement in oyster larvae.

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Adults of most marine animals reproduce sexually by shedding gametes into the surrounding seawater where external fertilization occurs. The embryos then develop into larvae which spend hours to months in the water column, before becoming competent for metamorphosis to the adult form. At the time competence is gained, the larvae search for suitable habitats to colonize. The site of colonization is critically important, especially to animals having adult forms which are sessile and attached to the seafloor. Some examples of marine animals with free-swimming larval but sessile adult forms are barnacles, oysters, worms and corals. In these cases, the larvae respond to environmental chemical cues triggering settlement on the seafloor and metamorphosis. Since the 1940's, investigators have worked to identify the chemical inducers to settlement in oyster larvae but only recently have we succeeded in characterizing these compounds. We employed computer - video motion analysis in measuring the behavioral responses to applied chemical agents by oyster larvae (*Crassostrea virginica*) swimming freely in the water columns of large test chambers. Substances released by juvenile and adult conspecifics caused oyster larvae to swim downward in the water column and attach to the bottom. Pressure ultrafiltration, hallow fiber dialysis, selective enzyme degradations, and high-performance liquid chromatography, all were employed to isolate the inducers. The inducers were found to be peptides having three to five amino acid residues with arginine at the C-terminal, effective at the picomolar level. We are presently focusing research on biosynthetic pathways for the inducers, and we are working to determine the relative influences of chemical and hydrodynamical factors in mediating settlement by oyster larvae in nature.

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A Possible Olfactory Component mediating Prey Search in a Carnivorous Leech.

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How do selective forces driving evolution operate on the nervous system? To answer this question, one requires a model system of two species with similar nervous systems yet different behavior. Prey search in the medicinal leech is mediated by mechanoreception or photoreception whereas we believe that prey search in a closely related species, the carnivorous green horse leech, *Haemopis marmorata*, is at least partially mediated by olfaction. Using a Y-maze, we examined the leeches' choice of four prey items vs. the other empty arm of the maze. The four prey items were (1) a live earthworm; (2) a clean foam rubber "worm" the size and shape of a live earthworm; (3) a foam rubber "worm" soaked in shock-induced earthworm secretion (SIEWS); and (4) a foam rubber "worm" soaked in earthworm washings (EWW). Leeches chose the arm of the maze containing a live earthworm in 65% of trials (34/52, $G=5.004$, $p<0.05$). When the prey was a clean foam rubber "worm," leeches displayed no preference (3/11, $p>0.2$) and moved in only 50% (9/18) of trials. When the foam rubber "worm" was soaked in SIEWS, the leeches displayed no clear preference (14/28). When the foam rubber "worm" was soaked with EWW, the leeches tended to choose the prey arm more often (18/30), but not significantly. The leeches' searching behavior consisted of extending the prostomium and turning from side to side with the rear sucker planted. We quantified searching behavior as the number of prostomial turns per 10 seconds. When the prey was a live earthworm, searching behavior was significantly increased over the other three types of prey (t-test, $p<0.01$). We are currently performing more experiments with EWW to determine if the leeches do indeed show a preference. We are also planning to test anosmic leeches to obtain further evidence that their preference for earthworms is mediated by olfaction.

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Role of Olfaction in Recognition of Dominance in the American Lobster (*Homarus americanus*)

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Communally held American lobsters (*Homarus americanus*) will initially engage in agonistic encounters to determine dominance relationships. These relationships will then remain stable with little aggression expressed. Several factors could maintain this stability such as behavioral acts and postures as well as chemical signals. Since dominance in correlated with sex, size and molt state, a dominant lobster could have a certain hormonal state that is broadcast as an unique dominance odor. To test how chemical signals may influence dominance, we paired stranger adult male lobsters of equal size in artificial boxing matches. On four consecutive nights, each pair was fought for 20 minutes while being kept visually and chemically separate between fights. On the second night, 10 pairs were randomly chosen as treatment pairs. Before the fights, both members of these pairs had their antennules dipped in distilled water for 5 minutes which effectively destroys their olfactory abilities for at least 24 hours. Ten control pairs received sham lesions with seawater. The treatment and control lesions were repeated for the same pairs on nights 3 and 4. In all fights, dominance was established in the first fight. This dominance relationship was maintained for all 4 fights. The time it took to determine a winner however was significantly shorter in the control fights when compared to the treatment fights (Karavanich and Atema, 1991). The control pairs seemed to "remember" the outcome of the first fight, and losers would immediately concede after little or no fighting. Treatment pairs however continued to fight to re-establish dominance. These results suggest that some type of olfactory signalling is occurring to maintain dominance relationships. This chemical signal may be a dominance odor or, alternatively, a chemical individual recognition odor. Experiments are in progress to distinguish between these hypotheses.

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The sources of sex recognition odors in meadow voles: effects of daylength and gonadal hormones

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Attraction to the odors of the opposite-sex by free-living meadow voles varies seasonally. Voles emit odors that are attractive to the opposite-sex during the spring\summer breeding season, but not during the fall\winter period of reproductive quiescence. This seasonal effect was induced in voles kept in long and short daylengths, respectively. The opposite-sex attractant cue was extinguished by gonadectomy, but restored after therapy with the gonadal hormones. Odor cues may be transmitted by the release or deposition of excretory products, glandular exudates, the mouth, and various areas of the integument. Yet, odors from different glands may have different functions; and different small mammals may use different scents and glands for the same functions. Thus, it is important to examine all the odor secretions that are deposited by an animal and the function of these secretions. Using a preference test we identified the urine, feces, mouth, anogenital region and flanks as the specific sources of the odor cues that meadow voles, Microtus pennsylvanicus, employ to transmit information about sexual identity and condition. Areas on the back and chest, head and ears, and feet did not. Currently, we are determining if the salience of these specific cues are mediated by photoperiodically-induced changes in gonadal hormones.

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Social Organization in a Colony of Olfactory Bulbectomized Male Mice

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We studied the social organization of 6 sham lesioned and 5 bilaterally bulbectomized male CF-1 strain mice. After surgery, each group was maintained for 35 days in separate 123 cm x 61cm x 82 cm Calhoun type enclosures with food, water and nesting stations. As expected, normal mice established a dynamic partial dominance hierarchy, in which the alpha male vigorously defended the floor territory against frequent intrusions by other males who, in turn, were forced to inhabit the second level of the enclosure. Most aggressive behavior was initiated by the alpha mouse, but there was fighting among the remaining 5 mice. The subdominant animals had body scaring and a scruffy appearance after only 5 days of co-habitation, but remained active and apparently healthy. In sharp contrast, the group of bulbectomized mice were less active, showed almost no aggression or territorial behavior, roamed freely through the enclosure, engaged in little social behavior and, unlike subdominant controls, tended to sleep alone in nest boxes. None had body scars and each appeared well-groomed. There was no difference in weight between the experimental and control groups.

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Age Related Differences in Olfactory Sensitivity in Wistar Rats after Low-Level Formaldehyde Gas Exposure

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Experimentally induced long-term exposure to formaldehyde gas at high concentrations has been associated with the development of nasal cancer in rats. However, there is no experimental evidence for noxius effects of low-level exposure. Therefore, to investigate possible harmful effects of low-level formaldehyde concentrations, the olfactory sensitivity of Wistar rats of two different ages was checked in a psychophysical study. Five juvenile/subadult male rats (group I) were exposed to 0.25 ppm formaldehyde gas from postnatal day 30 until 160, while another five adult males (group II) were exposed to 0.5 ppm formaldehyde gas for 90 days. In the psychophysical study an air-dilution olfactometer was used. The olfactory threshold was defined as the lowest concentration in which criterion performance was achieved. In the experimental group I thresholds were significantly higher ($5x10^{-2}$ - $5x10^{-4}$ Vol%) than in a control group ($3x10^{-5}$ - $7x10^{-6}$ Vol%) of the same age ($p<0.002$; Mann-Whitney U-test). In the experimental group II no differences in the threshold values could be detected before ($3x10^{-5}$ - $1x10^{-5}$ Vol%) and after ($2x10^{-5}$ - $1x10^{-5}$ Vol%) exposure. These results indicate, that there are age related differences in the vulnerability of the olfactory system.

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The Effect of Methyl Bromide on Measures of Olfactory Function. JAMES E. EVANS and LLOYD HASTINGS (Dept. of Environmental Health, University of Cincinnati, Cincinnati, OH).

Methyl bromide is a widely used fumigant and insecticide. Animal studies have shown a specific and severe, but recoverable lesion in the nasal olfactory epithelium after inhalation exposure. It was hypothesized that this methyl bromide induced lesion would produce a functional olfactory deficit. In a previous study involving exposure to methyl bromide, latency to recover a buried food pellet was used as a measure of olfactory function (Hastings, *et al., Chem. Senses*, 1991). While an exposure-induced increase in latency was shown, the need for a more sensitive measure of olfactory function was apparent. Two approaches were taken to address this need. First, olfactory threshold to ethyl acetate was measured in six rats using a conditioned suppression behavioral paradigm. Three out of the six subjects showed a recoverable increase in absolute threshold or threshold response variability after a single, six hour exposure to 200 ppm methyl bromide. In the second experiment, four rats were trained to perform a go-no-go discrimination task and were then exposed to methyl bromide as described previously. This paradigm makes it possible to measure both olfactory threshold as well as discriminatory ability in the same subject. While there was no change in the ability to discriminate between ethyl and methyl acetate, all four rats showed an increase in their threshold to ethyl acetate. Threshold returned to pre-exposure levels within four days after exposure for three out of four rats. Generally, these results support the hypothesis of a definable exposure induced functional deficit with subsequent recovery of function. More specifically, while conditioned suppression may be more sensitive, the discrimination paradigm is a more versatile and reliable method to measure olfactory function.

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The Role of Conditioned Odor Aversions in the "Sick Building Syndrome". DOUGLAS G. KOHLRIESER, JAMES E. EVANS and LLOYD HASTINGS (Dept. of Environmental Health, University of Cincinnati, Cincinnati, OH).

Frequently the causative agent precipitating an incidence of the "sick building syndrome" (SBS) is never identified. However, the occurrence of a novel or unique odor is often associated with such incidences. We set out to determine the relationship between conditioned odor aversions and the neurological symptoms (headaches, nausea, etc.) often seen with the SBS. We examined whether the odor of xylene could produce alterations in behavior previously elicited by high-level exposures. Sixteen adult, male rats were trained to perform a visual discrimination with delay paradigm and tested during 90 minute exposures to various concentrations of xylene. The experimental apparatus was designed to allow simultaneous exposure and behavioral testing. Therefore, discrimination performance served as a measure of the direct, as opposed to residual, effects of exposure on neurobehavioral functioning. All subjects showed significant reductions in discrimination performance at the highest exposure level (800 ppm). However, the same deficits were not observed during 100 ppm exposures of xylene. To facilitate the development of the conditioned aversion, exposure to 800 ppm xylene was paired with lithium chloride injections. Neurobehavioral functioning was reassessed during exposure to the odor alone (15 ppm). No difference in discrimination performance was detectable using this test. As an alternative test, a two chambered apparatus was constructed to track the subjects location while xylene (15 ppm) or clean air was randomly presented to either of the two chambers. Subject preference to clean air or xylene odor was assessed by measuring the amount of time spent in each air type. Subjects previously exposed to xylene showed a significant aversion to the xylene while non-exposed controls did not. Thus, exposure to xylene at levels which do not affect neurobehavioral function can still have aversive effects.

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Taste Characteristics of ß-D-Fructose Derivatives in Rats CHARLES N. STEWART, MARCUS W. THOMSEN, SUSAN M. PACANA, KAREN M. L. CIANCI (Departments of Psychology and Chemistry, Franklin & Marshall College, Lancaster, PA 17604 USA), PANKAJ S. SHAH and JOSEPH G. BRAND (Monell Chemical Senses Center, 3500 Market Street, and the University of Pennsylvania, Philadelphia, PA 19104 USA)

The original theory of sweet taste as proposed by Schallenberger requires a hydrogen donating moiety, AH, and a hydrogen accepting moiety, B, separated by 2.5 - 4.0 Å. It was originally proposed that positions 1 & 2 of fructose (in the pyranose form) corresponded to this critical pair. However, Birch has stated that either positions 3 & 4 or 4 & 5 represent the critical pair in fructose. We have prepared three derivatives of ß-D-fructose in order to assess the role of the hydroxyl groups at positions 1 and 3 in the taste characteristics of fructose. The three compounds are 1-chloro-1-deoxy-D-fructose, 1-fluoro-1-deoxy-D-fructose, and 3-O-methyl-D-fructose. In the chloro derivative the hydrogen bonding at position 1 is virtually eliminated. In the fluoro derivative position 1 may still accept a hydrogen but may not donate one. Similarly, in the 3-O-methyl derivative, position 3 may accept a hydrogen but may not donate one in a hydrogen bond. The taste characteristics of the derivatives in *Rattus norwegicus* were examined by establishing a conditioned taste aversion (CTA) to these derivatives and then testing for the generalization of the CTA to standard taste stimuli (i.e. NaCl, HCl, quinine hydrochloride and sucrose). The rats exhibited a generalization of the fluoro-fructose and the 3-O-methylfructose to sucrose, but none from the chloro-fructose to sucrose. The results suggest that the hydroxyl groups at positions 1 and 3 are not essential for the sucrose-like taste of fructose; however, the results are consistent with a hypothesis that hydrogen bonding acceptors at these positions may be necessary for the sucrose-like taste of fructose.

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Anterior Oral Cavity Gustatory Denervations Produce Minimal Effects on Acquisition of Aversions to NaCl or Sucrose LISA S. RASKIN, LISA AKEY AND SUSAN TRAVERS (Ohio State University).

Neurophysiological studies suggest that lingual and palatal taste bud subpopulations in the anterior oral cavity are differentially responsive to the 4 standard taste stimuli (e.g., Travers et al., '86). Specifically, NaCl (N) is most effective for anterior tongue (AT) stimulation, sucrose (S) for the nasoincisor ducts (NID). We investigated whether selective denervation of these receptor groups had differential effects on conditioned taste aversions (CTA) given to S or N. Three groups of 8 male, Sprague-Dawley rats were anesthetized and bilateral section of the chorda tympani nerve (CT), cautery of the NID, or no surgery was performed. After 1 wk for recovery, rats were placed on a 23.5 hr H_2O deprivation schedule and trained to drink H_2O in an operant chamber. After training, rats were a given a CTA to 1.0M S or 0.3M N (conditioned stimuli [CS]; unconditioned stimulus=LiCl, 167 mg/kg, i.p.), followed by testing with 4 concentrations of the CS and H_2O (0.1M, 0.3M, 1.0M, 1.5M S or 0.05M, 0.1M, 0.3M, 1.0M N). The following wk, a 2nd CTA was given to the other CS. In summary, neither type of lesion produced marked effects on CTAs given to either CS. Following N aversion, rats with CT lesions did drink more salt at each concentration than control or NID-lesioned rats, but the effect was not significant (ANOVA). Following S aversion, all 3 groups drank nearly equal amounts of S. An earlier study demonstrated that reducing CS intensity produces an attenuated CTA (Nowlis, '74). Apparently, removal of input from the AT or NID did not affect CS intensity enough to observe such an effect. Results were also analyzed by considering the number of licks that occurred before the 1st pause in licking (a 200 ms inter-lick interval [Halpern & Tapper, '73]). Regardless of lesion condition, rats paused quickly after tasting the CS (4.2 ± 2.1 for 1.0M S & 6.4 ± 6.8 for 0.3M N). Thus, at mid-range concentrations, neither input from the NID nor AT is critical for rapid detection of S or N, although NID-lesioned rats did lick more before pausing when 0.1M S was presented (p=.052). The present experiments thus provide little evidence for differential roles of the AT and NID in behavioral responsiveness to S and N. Previous denervation experiments, however, have shown that the AT plays a special role in sodium discrimination (Nitabach et al.,'89; Spector et al.,'90). The present study helps delimit that role and emphasizes the need to assess behavioral deficits with multiple procedures. Pilot data suggest that a more sensitive test of our hypothesis would be interposing the lesion between conditioning and testing. With this paradigm, a CTA to N appears greatly attenuated by CT lesions.

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Prior Exposure to Physiologic Levels of Estrogen Mitigates Estrogen-Induced Conditioned Taste Aversions.
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Estrogen can serve as a potent unconditioned stimulus in producing a conditioned taste aversion to saccharin in rats, with its effects being greater in males than in females. To determine whether this sex difference might reflect the female's prior experience with low doses of estrogen, we pretreated three groups of prepubertally-ovariectomized females with low (physiological) doses (0.3, 0.75 and 1.50 ug, respectively) of estradiol benzoate (EB) for eight days. A control group received sesame oil alone during this period. During each of two subsequent conditioning sessions, a 0.2% saccharine solution was presented prior to a 100 ug injeciton of EB. In tests performed during the following week, the EB-pretreated animals evidenced significantly weaker taste aversions and extinguished them more rapidly than did the oil-pretreated controls, even at the lowest pretreatment dose. These findings suggest that prior experience with physiological levels of estrogen can significantly mitigate the magnitude of a conditioned taste aversion produced by a high dose of estrogen.

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A study of sweet taste behavior in old naive Fischer-344 rats.
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Sixteen Fischer-344 rats (22 months old) were tested using 24 hour measurements to examine sweet taste behavior in old rats with no prior exposure to tastants. The 16 naive healthy rats were selected from a larger group of rats that had been individually housed with ad. lib. water and Purina Rat Chow 5001 pellets for 20 months. The rats were divided into two groups with half receiving sucrose solutions in an ascending series and the other half receiving a descending series. The groups were tested for two days each on: .0312M, .0625M, .125M, .25M, .5M, and 1M concentrations of sucrose. A water bottle was always present.

The groups were tested in an apparatus which continually monitored eating and licking behavior. Standard Hoeltge cages were modified to accommodate a food jar on the front of the cage and two slots located in the back of the cage behind which lick tubes were located. In front of each lick tube and the food jar was a photobeam. When the beam was broken it was recorded by a microcomputer as a lick (lick tube beam broken) or as time spent eating (food jar beam broken). These data were stored on floppy disks every 23 hours for later analysis. The food and solution bottles were refilled and weighed each day.

Five days of baseline food and water were recorded. Then the first solution was presented for two days. The position of the water and solution were switched on the second day of presentation to observe any side preferences. A day of food and water only followed the two days of solution. After all of the solutions had been tested the presentation series was reversed i.e. the group starting with an ascending series now received a descending and vice versa.

The amount of food and fluid consumed, number of eating and drinking bouts, bout duration, and interaction of sucrose drinking with eating chow were analyzed and compared with data from earlier studies in this laboratory in which older rats had received a lifetime of experience with sucrose.

Photoperiodic Control of Diet Self-Selection by Siberian Hamsters.
JACQUELINE B. FINE-LEVY AND TIMOTHY J. BARTNESS. (Departments of Psychology and of Biology, Georgia State University, Atlanta, GA 30303).

Most Siberian hamsters (*Phodopus sungorus*) exhibit a constellation of seasonal adjustments when exposed to short 'winter-like' days (SDs). These photoperiod-sensitive SD responders (R) show decreases in body weight and fat, testes weight and food intake compared with long, 'summer-like' day (LD)-exposed hamsters. A sub-population of SD-exposed hamsters are SD-insensitive (non-responders [NR]). We have begun to investigate possible gustatory influences underlying the SD-induced decreases in food intake seen in R, but not NR hamsters. In this initial study, we examined diet self-selection patterns in adult male Siberian hamsters exposed to either LDs (light:dark 16:8) or SDs (light:dark 8:16). Hamsters were given *ad libitum* access to three powdered diets: sunflower (SF) and millet (M) seeds, or rabbit chow (RC). LD-housed hamsters had increased food intake compared with SD-R, but not SD-NR hamsters. The diet self-selection pattern of the LD-housed hamsters was SF > M > RC, which was reflected as macronutrient caloric intake percentages of fat > carbohydrate > protein. SD-R had decreases in body, epididymal fat pad, and testes weights compared with the LD controls, whereas SD-NR were similar to the LD-housed hamsters for these measures. SD-R, but not SD-NR, had decreases in food intake compared with LD-housed hamsters. However, regardless their responsiveness to SDs, both SD-R and -NR had similar diet self-selection patterns and macronutrient caloric intake percentages (M > SF > RC and carbohydrate > fat > protein, respectively) that were different from their LD-housed counterparts. These are the first data to show photoperiodic control of seasonal changes in diet and macronutrient selection. Since both SD-R and -NR had similar patterns of diet and macronutrient selection, but gonadal regression was seen only in SD-R, then these changes would appear to be primary effects of the daylength, rather than secondary effects of SD-induced decreases in serum testosterone. The contribution of gustatory versus post-ingestive metabolic factors to the selection patterns observed remain to be resolved.

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The effect of K^+ channel blockers on mudpuppy feeding behavior.
ANDREW G. BOWERMAN and SUE C. KINNAMON (Colorado State University and the Rocky Mountain Taste and Smell Center).

It is known that acids and other taste stimuli depolarize taste cells in the mudpuppy (*Necturus maculosus*) by blocking potassium channels localized in the apical membrane of the taste cells (Kinnamon & Roper, J. Gen. Physiol. 91:351-371, 1988; Kinnamon et al., Proc. Natl. Acad. Sci. 85:7023-7027, 1988). Yet the animal's ability to detect and discriminate these taste stimuli has not been studied. Groups of mudpuppies were used in blind tests to determine the effect of particular taste stimuli on feeding behavior. Colored gelatin cubes containing either pond water, NaCl (1M), citric acid (.01-.1M), $CaCl_2$ (1M), quinine (.1M) or TEA (.1M) were attached to strings and presented to each animal (cf., Takeuchi et al., Int. Comp. Biochem. Physiol. 3:101, 1991). Minnow extract (.5 ml) was added to the aquarium as a feeding primer if the mudpuppy failed to show a feeding response. Approximately 70% of the total animals tested exhibited a feeding response, i.e., initial ingestion of the gelatin cube. Results were expressed as the percentage of animals that rejected the cube within 5 min of ingestion. Gelatin cubes containing either water or NaCl were rejected by less than 20% of the animals tested. In contrast, gelatin cubes containing the K^+ channel blockers citric acid, quinine, $CaCl_2$ or TEA were rejected by 50-90% of the animals. Thus, K^+ channel block appears to trigger an aversive response in mudpuppies. This response could have developed as a defensive mechanism against harmful toxins present in food sources of their aquatic environment.

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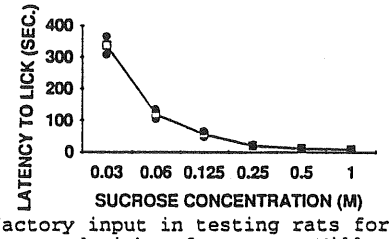
The Odor of Sucrose in Short-Term Taste Tests in Rats. JAMES C. SMITH, JULIANNE SCHUMM AND JODI R. DOTY (Florida State University)

A reliable brief taste test for measuring the behavioral response to sweet tasting substances was developed by Smith, O'Keefe and Davis where rats were trained to lick on a tube when the shutter in front of it was opened. As many as six concentrations of sucrose (or other sugars) were presented for 30 seconds each in a daily testing session. It was shown that the number of licks made in a 30 sec period was a direct function of the concentration of the sugar. The different concentrations of sugar were presented in a random order which differed each day. As can be seen below, the latency to take the first lick after the shutter opened was much longer for the low than the high sucrose concentrations. These data are the means from 14 rats tested over 20 days. The sucrose was commercial grade mixed in tap water. Similar results were obtained when reagent grade sucrose was mixed with distilled water.



Implications of olfactory input in testing rats for sweet tastes are discussed with reference to Miller and Erickson's work indicating rats' olfactory perception of chloride salts (1960) and Henkin's report that humans detect salts, sucrose, urea and HCl by smell.

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Gustatory and Olfactory Detection Performance in Hypothyroid Rats.

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The effects of hypothyroidism on the rat's gustatory (Study 1) and olfactory (Study 2) detection performance were determined using high-precision gustometry and olfactometry and signal detection measures of sensitivity (SI) and responsivity (RI). Rats were tested prior to, during, and after being made hypothyroid by the addition of propylthiouracil (PTU, 0.1% w/v) to drinking water. In Study 1, experimental rats demonstrated significant decreases in serum T_3 and T_4 but no changes in gustatory SI or RI values for any tastant. Significant alterations in preference behavior were observed for salty, sour, and bitter tastants and, while attenuated during the post-treatment period, were still present after the return of thyroid hormones to near-baseline levels. No changes in SI, RI, or preference behavior were observed in control animals. Analogous changes in taste preferences but not gustatory SI and RI values were observed in rats following thyroidectomy with no changes in taste behavior observed for thyroidectomized animals maintained on thyroxin. In Study 2, neither experimental nor control rats demonstrated any change in olfactory SI or RI values for the odorant ethyl acetate during or after treatment with PTU. The role of thyroid hormones in modulating rodent and human gustatory and olfactory function are discussed.

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Isolation of Hawaiian *Drosophila* Variants Which Prefer Glucose To Fructose At Equipotent Suprathreshold Concentrations. JASON E. POSKANZER[1,3], LYNNE RUDNICK[2,3], CHENGTAO HER[1,3] and LINDA M. KENNEDY[1,2,3] (Depts. Biol.[1] and Psychol.[2] and Neurosci.Program[3], Clark Univ., Worcester, MA 01610)

Behavioral and physiological data indicate different receptor cell mechanisms for pyranose and furanose sugars in flies. Although variants for glucose, pyranose sugars, and trehalose are known in *D. melanogaster*, variants for fructose and furanose sugars have not been described. For the Hawaiian, *D. adiastola*, our data support separate receptor cell mechanisms for fructose and glucose, and our screening tests have isolated apparent fructose variants. In behavioral tests, groups of food-deprived, water-satiated flies chose between, and fed on red or blue solutions (agar or sugars in agar). They were counted by abdominal coloring, and Preference Indices (PI) were calculated. Reversal tests showed that color did not affect the flies's choice (Her & Kennedy, Chem. Senses 16, 1991, 533). In tests with 1.5-250 mM sugars vs. agar, thresholds (PI=0.5), sensitivity ranges, dose-response curves, slopes, half-maximal and maximal response concentrations were similar for sucrose and fructose, but different for glucose. In neurophysiology tests, action potential responses of proboscis sensillar cells to 5-160 mM sugars suggested that the feeding depends on those cells: relationships between firing rates and behavioral PI values were linear, and dose-response curves for firing and PI values were similar, for each sugar. In screening tests, flies chose between, and fed on red and blue fructose and glucose at concentrations for which the PI had been 0.6. Flies excreting only one color during 2 post-test days with colorless sucrose ad lib were retested with reversed colors. To date, several 'fructose nontasters', but no 'glucose nontasters' have passed the second test.

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Are Basal Cells in Taste Buds Identical to Cutaneous Merkel Cells? RANDY TAYLOR , RONA DELAY and STEPHEN ROPER (Colorado State University and the Rocky Mt. Taste and Smell Center)

In 1875, the German histologist F. Merkel identified a specialized cell in cutaneous epidermis that he inferred was involved in tactile sensitivity. This cell has become known as the cutaneous Merkel cell. Several researchers, including ourselves, have noted similarities between the ultrastructure of basal cells in fish and amphibian taste buds and Merkel cells. In *Necturus*, the most striking features of basal taste cells are the presence of vesicles with dense cores, tiny cell processes ("spines") and their small, ovoid shape (cf. Delay & Roper, J. Comp. Neurol. 277: 268-280. 1988). These features are shared by cutaneous Merkel cells. Further, an important phenotype of cutaneous Merkel cells is that they contain serotonin (5HT). Coincidentally, taste buds contain serotonergic cells. Esakov, Grazeidei, Hirata, Nada, Reutter, Takeda and Toyoshima, among others, have all suggested that basal cells are the monoamine-containing structures in fish and amphibian taste buds, but until now a critical correlation has not been established. We report here that there is a 1-to-1 identity between Merkel-like basal cells and 5HT-containing cells in *Necturus* taste buds. ❶Lingual tissue was dissected, fixed in 2% paraformaldehyde with 0.1% glutaraldehyde, frozen, and sectioned at 10 µm on a cryostat. Frozen fixed sections were processed for 5HT immunoreactivity (cf. Jain & Roper, J. Comp. Neurol. 307: 675-682. 1991; Delay et al., submitted). 5HT immunopositive cells were situated at the base of the taste bud, consistent with them being basal cells (Welton, et al., submitted). These sections were photographed. The 10 µm cryostat sections were then embedded in plastic and thin-sectioned for electron microscopy. Low magnification electron micrographs of the sections were aligned with light micrographs to map the exact positions of 5HT immunopositive cells. High magnification electron micrographs of these cells showed that in every case (N=11) the cells had the characteristic features of cutaneous Merkel cells, especially vesicles with dense cores. ❷Other cells in the immediate vicinity were neither immunopositive for 5HT, nor possessed ultrastructural features of Merkel cells, although some had the general shape and position for basal cells. The cytoplasm of these latter cells lacked any identifying features and instead gave the appearance of undifferentiated stem cells. ❸We conclude, therefore, that *Necturus* taste buds contain basal cells that are very similar, if not identical, to cutaneous Merkel cells. The role, if any, that these Merkel-like cells play in taste transduction is not understood.

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Effects of Denervation on Foliate Taste Buds in the Golden Hamster. L.D. SAVOY and M.A. BARRY. Univ. of Connecticut Health Center, Farmington, CT 06032

Taste buds are a classic example of neurotrophically dependent receptor cells. When deprived of their afferent (gustatory) nerve supply, taste buds atrophy and often disappear. The relative persistence of denervated taste buds appears to be species and taste field dependent. Denervated vallate and foliate taste buds disappear by about 2 weeks in all species examined. Most denervated fungiform taste buds persist for indefinite periods in adult hamsters, to a lesser extent in other rodents, and possibly among anamniotes. This study was designed to test whether the other taste fields in the hamster would also show long term persisting taste buds following denervation. Either nerve IX or both IX and CT (IX-CT) were cut and devitalized unilaterally in adult male golden hamsters. After survival times of 1 to 3 weeks, sections through the foliate taste buds were stained for calcium dependent ATPase (Ca-ATPase), which stains taste cells much darker than the surrounding epithelium. In contrast to the rat, it appeared that the CT has little or no role in innervating hamster foliate taste buds, because there was very little difference between the results of cuts of IX-CT versus IX alone. By 2 to 3 weeks survival, almost all taste buds had disappeared in the IX-CT cut cases. However, there were always a few small, but recognizable taste buds. The persisting buds were always narrower than normal, but often spanned the depth of the epithelium. Thus the foliate taste buds are much more neurotrophically dependent than fungiform taste buds, but there are some taste bud cell remnants persisting 3 weeks after surgery. However, the persistence of most denervated fungiform taste buds is not related to a generalized propensity of all lingual taste buds to survive denervation in hamsters.

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Behavioral and Histological Changes in Post-Irradiation Gustatory Dysfunction.
GINA M. NELSON and THOMAS E. FINGER (Rocky Mtn. Taste and Smell Ctr. and Univ. Colorado Health Sci. Ctr, Denver, CO)

Post-irradiation gustatory dysfunction is the inability to taste following therapeutic doses of radiation to the head and neck, including the oral cavity. In humans, taste loss is gradual, varies with different types of tastants, and has a recovery period of 60-120 days. It is unknown whether the damage resulting in taste loss involves the receptor cells or the nerve fibers innervating them. We developed a rat model of gustatory dysfunction. A two-bottle preference test was used to assess behavioral changes and immunocytochemical methods were used to assess structural changes to nerve fibers and taste cells. A radiation dose of 1700cGy was delivered to the oral cavity and anterior esophagus of rats without exposing the CNS or the rest of the body. The animals' tendency to avoid 1.8% NaCl decreased by 7 days post-irradiation, with eventual recovery around 11 days. The change in drinking behavior includes an increase in salt consumed as well as a decrease in total volume consumed. Tests with .01% quinine-HCl indicate no loss of ability to taste this stimulus following the radiation dose used. Initially, intragemmal fibers appear normal, even at locations where taste buds do not. Changes in fiber morphology were not apparent until about 20 days post-irradiation. Changes in taste bud morphology begin at 4 days post-irradiation and peak at 8-10 days. Taste cells initially loose their spindle shape, become shriveled, and then only remnants of cells are present. This degeneration is a continuum in the circumvallate papilla, from most severe for taste buds at the top of the crypts where no taste buds are visible, to nearly normal at the base of the crypts. Also, most fungiform taste buds degenerate, although some remain intact. These results indicate that the initial taste loss occurs at approximately the same time as the initial changes seen in the morphology of the taste cells, while changes in nerve fiber morphology are not seen until later, and that the taste buds which remain provide for the recovery of normal gustatory behavior.

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Strain Differences in Glucuronidase Activity in Mouse Taste Buds
LESLIE M. STONE AND THOMAS E. FINGER (Univ. of Colorado Health Sci. Ctr., Denver, CO).

Various strains of mice are used to study the genetics of taste. Many of these strains also are known to have significant differences in the activity of enzymes such as ß-glucuronidase in many tissues. Accordingly, preliminary investigations were undertaken to examine taste buds in 2 strains (C57BL/6J and C3H/He) that are known to exhibit both taste and enzyme differences. Previous studies have shown that alleles of the structural gene for glucuronidase, Gus, differ between C57BL/6J mice and C3H/He mice resulting in a ten fold difference in the liver enzyme activity in the two strains. ß-glucuronidase, an acid hydrolase, is concentrated within lysosomes and in association with endoplasmic reticulum. The enzyme is concentrated within the liver, kidney and spleen and is present in significant levels in some large neurons such as cerebellar Purkinje cells. The present experiments show that glucuronidase is also present in mouse fungiform, foliate and circumvallate taste buds. Enzyme activity was detected using Naphthol-AS-BI-b-D-glucuronic acid and pararosaniline; the enzyme cleaves the substrate, releasing Naphthol-AS-BI which then combines with pararosaniline to produce a red pigment. In the tongue, this reaction product is the most dense in the taste buds and the ducts of salivary glands. Lesser reaction product appeared in the dermis and at the base of the lingual epithelium, extending 10-20 µm above the basal lamina. Salivary glands were also moderately reactive.
The lingual striated muscle was virtually devoid of reactivity. Although the general distribution and relative density of reactivity is similar in the two mouse strains, glucuronidase reactivity is far lower in all tissues from C3H/He mice relative to the same tissues in C57BL/6J. In summary, taste bud cells contain concentrated glucuronidase activity, and this activity differs between C57BL/6J mice and C3H/He mice. Whether this difference relates to the differences in taste behaviors of the two animals remains to be tested.

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Polysialic Acid and NCAM Expression in the Nerve Fibers and Taste Buds of the Rat Vallate Papilla. PIROSCHKA HORVATH, RAISA KLEVITSKY, RICHARD A. AKESON, MICHAEL T. SHIPLEY and DAVID V. SMITH (University of Cincinnati College of Medicine).

Taste bud cells in both the fungiform and vallate papillae of the rat turn over continuously throughout life. We and others have shown that the neuronal cell adhesion molecule (NCAM) and other developmentally related molecules are expressed by taste cells and/or their nerve fibers. In early development, NCAM molecules contain high levels of polysialic acid (PSA); there is evidence that PSA regulates the degree of cell-cell interaction mediated by NCAM. It has been shown that NCAM is involved in axon fasciculation and nerve fiber/target recognition and that the presence of PSA reduces axon fasciculation by interfering with NCAM binding. The present investigation employs immunocytochemical methods to demonstrate the distribution of both NCAM polypeptides and PSA on rat vallate taste bud cells and their innervating IXth nerve fibers. Monoclonal antibodies (mAbs) to NCAM polypeptides (mAb 3F4) and to PSA (mAb 5A5) were applied to sections of adult rat vallate papillae. All tissues were examined by light microscopy using either 40 µm frozen or 1 µm plastic sections. NCAM polypeptides were distributed on the surface of a subset of cells within the taste buds and on the nerve fibers innervating the gustatory epithelium. By contrast, PSA immunoreactivity was seen only on nerve fibers, including a small number of fibers entering the vallate taste buds. There was no PSA expression on the taste bud cells. Based on the ability of PSA to regulate NCAM binding during development, the present results suggest that IXth nerve fibers express a polysialylated form of NCAM until the fibers reach their target cells within the taste epithelium. At that point, downregulation of PSA in the nerve fibers would allow NCAM to mediate recognition between IXth nerve fibers and those gustatory epithelial cells that have differentiated sufficiently to form synaptic contacts.
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Light and Electron Microscopic Demonstration of Lectin Binding in the Anuran Taste Disc

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The cells of anuran taste discs contain glycoproteins which, apart from their protective functions, probably play an important role for the modulation of the chemoreceptive processes. O-glycosylated *mucous glycoproteins* occur as major constituents of mucous (associate) cells. These secretory cells contribute to the mucous layer above the receptor field. Furthermore, N-glycosylated *plasma glycoproteins* are constituents of structural (e.g. membranes) or functional (possibly immunoglobulins or hormone-like substances) elements of all the cells of the taste disc including the sensory cells. In order to characterize the composition and distribution of carbohydrate residues of mucous secretions and cells, we applied a panel of gold-labeled, peroxidase-conjugated and biotinylated lectins to paraplast and ultrathin sections cut from the tongues of *Rana pipiens* and *Bombina orientalis*. The gustatory epithelium of anurans is formed by mucous cells, the wing cells and the sensory cells. The mucous cells are relatively large and situated apically. Wing cells and sensory cells lie in the more basal parts of the epithelium and reach with slender processes to the surface. Most of the lectins specific to galactosamine residues bind to the mucous cells. Incubations on transverse sections show that apart from single mucous cells, also both slender cell types, the wing cells and the sensory cells, are reactive to the lectin from *Helix pomatia* (HPA). Not all of the mucous cells possess HPA binding sites, indicating that the large receptor field is subdivided into several separate functional areas (microenvironments). The glucosamine-specific lectin from *Triticum vulgare* (WGA) reacts with both types of slender cells. There is no reaction in any cell type with the sialic acid-specific *Maackia amurensis* lectin (MAA). The positive reaction of the *Canavalia ensiformis* lectin (Con A) with all three cell types, in particular with sensory cells, indicates their relatively high amounts of mannosyl residues. This sugar is not a typical constituent of mucous glycoproteins, but characterizes *plasma glycoproteins*. Therefore it is suggested that some particular taste disc cells fulfil additional specialized, e.g. hormone-like functions.

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Expression of Blood Group Antigens by Cells in the Vallate Papilla of the Rat.

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Cell-surface carbohydrates are thought to be important in the development and differentiation of mammalian cells. Distinct lactoseries and globoseries oligosaccharides are expressed on the surfaces of dorsal root ganglion cells that project to different lamina of the spinal cord. Based on lectin binding and immunocytochemistry, cells in the gustatory and olfactory epithelia have been shown to express a number of cell-surface carbohydrates. Blood group antigens H and B are expressed by olfactory receptor cells differentially during development. A subset of taste cells expresses the Lewis b blood group antigen. The present investigation used immunocytochemical techniques to localize several blood group antigens in the rat vallate papilla. Monoclonal antibodies (mAbs) against the A, B and H blood group determinants (Dako Corporation) and against the Lewis b blood group antigen (American Type Culture Collection) were applied to 40 μm frozen sections of adult rat vallate papillae and examined by light microscopy. As shown previously, the Lewis b antigen was localized to a subset of cells in the taste buds of the vallate papilla. In contrast, the H antigen was expressed on the surfaces of squamous epithelial cells throughout the tongue surface and a majority of or all of the cells within the taste buds. The B antigen, on the other hand, was restricted to cells within the taste bud. The A antigen was expressed by some taste cells and also by cells within von Ebner's and Weber's glands. Seven days after bilateral crush of the IXth nerve, expression of all but the H antigen disappeared from the gustatory epithelium; H was still expressed on the squamous epithelium of the denervated trenches of the vallate papilla. Since taste cells disappear following deafferentation, the loss of expression of the Lewis b and the A, H and B antigens following nerve crush confirms that these antigens are expressed on differentiated taste cells.

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Laminin during development of lingual gustatory organs in the rat.

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Mammalian lingual taste buds are located within gustatory papillae in a distinctive distribution on the tongue. The role of innervation in taste organ development and maintenance has been extensively studied, but little is known about potential regulatory roles of extracellular matrix factors. We have begun to study a basement membrane component, laminin (LM), known to have important roles in neurite outgrowth and epithelial cell differentiation in other systems. Initially, we have focused on development of foliate papillae in rat, as an organ that has a complex morphology and contains hundreds of taste buds. Immunocytochemistry with a rabbit anti-human polyclonal antibody (Telios) was used to determine the distribution of LM in embryonic (E) and postnatal (P) rat. At E14 and E15, LM immunoreactivity (LM-IR) was apparent in a ramified network throughout the deep tissue of the tongue, but not within the subepithelial dermal mesenchyme. LM-IR was intensely associated with the entire lingual epithelial basement membrane, however. At E18, LM-IR remained in the epithelial basement membrane throughout the dorsal tongue. In the P0 foliate, LM-IR outlined the basement membrane region of the entire papilla epithelium and adjacent nongustatory epithelium, and was found diffusely within the foliate papilla dermal cores. However, at P5 and P14, LM-IR was associated with basement membrane only at apical papilla regions and more weakly at the papilla base or trough, not in lateral walls. LM-IR was also in bands within the dermal papilla cores, but not in adjacent nonpapillary dermis. At P21, intense LM-IR was distributed in association with the basement membrane in apical portions of the foliate papillae only, not in lateral (taste bud - bearing regions). Therefore, in foliate papillae, LM-IR in association with epithelial basement membranes becomes progressively restricted to apparently nongustatory regions, not to papilla regions where active taste bud multiplication is occurring.

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Calbindin-like Immunoreactivity in a Putative Human Vomeronasal Organ

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There has been a recent resurgence of speculation about the existence of a functional vomeronasal organ (VNO) in humans (see J.Steroid Biochemistry and Molecular Biology 39 [4B], 1991). Two reports have presented ultrastructural evidence that such a structure does exist in human adults (Moran et al., 1991; Stensaas et al., 1991). It has not been established if this putative VNO is functional. We have begun to address this issue by determining if cells in this tissue contain neuron-specific proteins found in the VNO of other mammals. We have obtained biopsied nasal tissue from a region inferior to the olfactory epithelium of an adult patient; this tissue included the vomeronasal pit. We tested this tissue for immunoreactivity to anti-calbindin (generously provided by A.W. Norman), since we have observed that the rat VNO neuroepithelium is immunoreactive to this antiserum while the olfactory receptor cells are not (Johnson et al., Brain Res.: in press [1992]). Our preliminary results show small lumenal profiles opening onto the surface of the epithelium. The lumena are bordered by columnar cells which have microvilli at their apices. These cells are morphologically similar to the cells identified within human vomeronasal organs by two independent laboratories (Moran et al., 1991; Stensaas et al., 1991). Furthermore, some of these cells were labelled after incubating the tissue with anti-calbindin. The significance and applications of these observations will be presented.

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CONNECTIONS OF THE ACCESSORY OLFACTORY BULB IN THE RAT: EVIDENCE FOR AFFERENTS FROM THE MEDIAL DIVISION OF THE ANTERIOR OLFACTORY NUCLEUS
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The accessory olfactory system provides chemosensory regulation of rodent social and sexual behavior. Previous degeneration and HRP studies have shown some of the afferent and efferent connections of the accessory olfactory bulb (AOB), but its connections have not been comprehensively examined with discrete injections of tracer confined to AOB. We have investigated the organization of AOB connections in the rat with injections of the highly sensitive tracer, WGA-HRP, restricted to AOB. Our results confirm several previous studies and describe some novel features of AOB circuitry.

Single, discrete injections of WGA-HRP restricted to AOB produced intense fiber labeling in lateral olfactory tract (lot), olfactory tubercle (Tu), bed nucleus of stria terminalis (BNST), nucleus of accessory olfactory tract (NAOT), posterior division of amygdaloid nucleus (MeP), anterior cortical amygdaloid nucleus (ACo), optic tract (opt). Intense anterograde labeling was also seen in the region ventral to the posteromedial cortical amygdaloid nucleus (PMCo).

Discrete injection of WGA-HRP in AOB, retrogradely labeled numerous neurons in the anterior olfactory nucleus (AON). The densest labeling was evident in medial AON (AONm) and, more caudally, in the medial part of the posterior division of AON. Scattered cells were lightly labeled in the dorsal division of AON; the lateral and ventral divisions of AON were devoid of retrograde labeling. Because AON projections to AOB have not been reported previously, discrete injections of WGA-HRP were made in AONm. These injections anterogradely labeled a dense fiber projection to the granule cell layers, of both AOB and MOB. The projection from AONm to AOB is currently being analyzed by PHA-L tracing.

The same injection also produced intense retrograde labeling of neurons in the horizontal limb of diagonal band (HDB), the lateral preoptic region, lateral hypothalamus (LH), anterior division of medial amygdaloid nucleus (MeA), posterior division of amygdaloid nucleus (MeP), ACo, PMCo and the nucleus of the accessory olfactory tract. Locus coeruleus had numerous labeled cells. By contrast, the dorsal and median raphe nuclei had very few or no retrogradely labeled cells. Consistent with this, 5-HT immunostaining confirmed our earlier report of dense 5-HT innervation of MOB but revealed only scattered 5-HT fibers in AOB.

The projection from AONm to AOB is novel. If individual neurons in AONm project to the granule cell layer in *both* AOB and MOB, then AONm could *coordinately* regulate activity in the main and accessory olfactory systems.

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The vomeronasal organ of the domestic pig (*Sus scrofa*)
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Although the domestic pig is one of the very few mammalian species for which an interspecific chemical signal (androstenone) has been identified, there has previously been no detailed description of the pig's vomeronasal organ (VNO) and associated ducts. The odor of androstenone and related 16-androstenes in boars' saliva is attractive to sows in estrus, and also facilitates expression of an immobile mating stance in estrous sows. It is not yet known whether these behaviors are mediated by the VNO. Information gained from a detailed description of the VNO will be important for the future study of the role of the VNO in proceptive and receptive behavior of the female pig. In order to further understand the olfactory processing of androstenone in the pig, we have begun an examination of the VNO. In the pig, the VNO is a paired organ located along the nasal septum just dorsal to the floor of the nasal cavity. It consists of two epithelium lined pouches, each approximately 2 cm in length and .5 cm in height, encased in cartilage, one on either side of the nasal septum. The vomeronasal ducts leave the organ at the anterior end of each pouch, runs ventrally approximately .25 cm and joins the nasopalatine ducts, which connect the oral and nasal cavities. Thus stimuli entering either the nose or the mouth could have access to the organ. The opening of the nasopalatine ducts in the oral cavity lie in the caudal portion of the hard palate, along the lateral edges of the incisive papilla. In addition to examining the structure of the VNO and its ducts, we have devised a possible method for blocking stimulus access to the organ, that we have performed on semi-intact pig heads (with tongues removed). Entering through the oral opening of the nasopalatine duct after reflection of the jaw, a syringe needle can be inserted into the VNO. Veterinary surgical adhesive can then be injected into the VNO, completely filling the organ and the vomeronasal and nasopalatine ducts.

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Sulfated Sex Steroids: A New Class of Olfactory Stimulants with Pheromonal Actions in Teleost Fish
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It is now firmly established that the unmodified sex steroid hormone, 17α,20β-dihydroxyprogesterone (17,20βP), is a potent olfactory stimulant with pheromonal actions in the goldfish. However, recent studies of sex steroid release by ovulatory goldfish have discovered that in addition to releasing unmodified 17,20βP, goldfish also excrete considerable quantities of 17,20βP conjugated with either sulfate (17,20βP-S) or glucuronic acid (17,20βP-G) at the 20 position. To test whether these conjugated steroids might also have pheromonal function they were synthesized and tested for olfactory potency by electro-olfactogram (EOG) recording from the olfactory epithelium of mature male goldfish. Here we report that the novel steroid 17,20βP-S is an excellent olfactory stimulant with a detection threshold of 10^{-12}Molar. Furthermore, cross-adaptation experiments indicate that 17,20βP-S is detected by a different receptor mechanism than 17,20βP suggesting that it may function as a pheromonal cue complimenting 17,20βP. Whole-animal bioassays of hormonal responsiveness lend credence to this possibility. 17,20βP-G, on the other hand, is a poor olfactory stimulant as are forms of 17,20βP sulfated at positions other than the 20. It is now apparent that olfactory receptor mechanisms can be highly sensitive to the precise manner in which steroids are conjugated suggesting that mixtures of hormonal metabolites may be used as species-specific pheromones.

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Odorant Binding to Olfactory Receptor Molecules in the Spiny Lobster: Mixture Interactions and G Proteins. KIRBY OLSON, CHARLES DERBY, AND WILLERT LYNN (Georgia State Univer.)

We are examining the role that inhibition of binding of odorants to receptor molecules may play in the reception of mixtures, and characterizing G proteins in the olfactory system of the spiny lobster *Panulirus argus*. An enriched dendritic preparation of olfactory membrane was incubated with combinations of radiolabeled and unlabeled odorants in a radiolabel filtration assay to determine the potential for inhibition of binding at the receptor sites. Unlabeled forms of AMP, cysteine, glutamate, succinate, and taurine were tested as inhibitors of binding of [^{3}H]-AMP and [^{3}H]-taurine. Neither cysteine, glutamate, succinate, nor taurine acted as competitive inhibitors for [^{3}H]-AMP, although each caused a noncompetitive, predominantly concentration-independent inhibition of 10-20% of the specific binding. Cysteine and AMP caused a similar degree of noncompetitive inhibition of [^{3}H]-taurine binding. Glutamate and succinate, however, acted as apparent competitive inhibitors of the taurine-labeled sites: 1 mM glutamate inhibited binding at 42% of the sites, and 1 mM succinate inhibited binding at 33% of the sites. One explanation of these results is that a proportion of the sites designated as taurine sites (because they were labeled with taurine) actually were glutamate and succinate sites that have a lower affinity for taurine. From extracellular recordings of spiking responses of olfactory receptor cells, we find that glutamate or succinate suppresses responses to AMP, and cysteine suppresses responses to taurine. Thus, inhibition of binding at the receptor sites cannot explain all examples of mixture interactions seen at the cellular level. Additionally, we are investigating the nature of G proteins and their coupling to receptor molecules in the olfactory cells of spiny lobsters. Functional coupling of receptor molecules to G proteins is indicated by the fact that 100 μM $G_{pp}NH_p$ substantially inhibited specific binding of [^{3}H]-AMP to the lobster olfactory membrane preparation, without affecting nonspecific binding. We are currently examining the effects of other analogs of GTP and inhibitors of G proteins.

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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

Several lines of evidence suggest that the chemo-electrical signal transduction process in olfactory receptor cells involves second messenger cascades. To demonstrate that upon odorant stimulation the levels of second messengers actually changes in a time range relevant for primary processes, a rapid kinetic methodology was applied monitoring the odorant-induced formation of second messengers in the subsecond time range. A very rapid increase in the second messenger (cAMP or IP_3) concentration was detected after about 50 msec; thereafter the signal rapidly attenuated to the basal level within 100-200 msec. Thus, the induced molecular signal clearly preceeds the generator currents of the receptor cells. The transient nature of the signal is apparently due to rapid desensitization phenomena mediated by specific protein kinases. Experiments using specific inhibitors have indicated, that the cAMP pathway in terminated by protein kinase A, whereas the IP_3 cascade is attenuated via protein kinase C activity. It is presently unclear if receptor proteins are phosphorylated. The observation that odorant-induced second messenger formation requires active G-proteins suggests that the reaction cascade is initiated by an interaction of odorants with receptor proteins belonging to the superfamily of G-protein-coupled receptors. Based on recent work by Buck and Axel and using PCR-amplification procedures a number of putative odorant receptors from rat and salamander have been cloned. For some of the receptor subtypes the sites of expression in the olfactory epithelium were revealed using in situ hybridisation techniques. The pattern of expression corresponds to groups of olfactory neurons topographically distributed within the nasal neuroepithelium.

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Mucosal Inherent Activity Patterns in the Rat: Evidence from Voltage Sensitive Dyes.
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Kent & Mozell (Chem. Sense 13:704, 1988) has demonstrated that particular voltage sensitive fluorescent dyes can monitor the same mucosal events in response to odorants as does the EOG. We have recently monitored the fluorescent changes in one dye, di-4-Anepps, at 100 contiguous sites in a 10 x 10 pixel array on two relatively flat expanses of the rat's olfactory mucosa (septum and medial surface of the turbinates) every 15 ms in response to odorant stimuli. The odorants were 2-propanol, citral, carvone, ethyl acetoacetate, and propyl acetate, each presented in randomized sequence at two concentrations in a 3:1 ratio. Each odorant was humidified and puffed uniformly upon the entire olfactory mucosa of either the septum or medial surface of the turbinates for a duration of 700 msec at a flowrate of 300 cc/min. In Experiment 1 we formally evaluated whether inherent activity patterns exist for the rat nasal septum (N=10), whereas in Experiment 2 this was done for the medial surface of the turbinates (N=10). The five odorants for each of the ten animals produced fifty recorded spatial activity patterns from the 10 x 10 pixel arrays. A measure of dissimilarity for each pair of animal-odorant patterns was obtained and was entered into a 50 x 50 matrix. The dissimilarity data were then entered into an MDS analysis (ALSCAL) and the resulting fifty sets of coordinates from a two dimensional solution formed the basis for an Analysis of Multidimensional Variance (ANOMVA). The results of these studies demonstrated that, for each of the two mucosal surfaces evaluated, there do inceed exist different spatial activity patterns for the different odorants tested. Furthermore, graphic representation of the MDS coordinates illustrates the relative "hot spots" of the five odorants on each mucosal surface.

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Modulation of Odor Transduction by Membrane-permeable Analogs of cAMP. T.D. BAHNSON and V.E. DIONNE (University of California San Diego)

Previous studies of olfactory receptor neurons from the mudpuppy, *Necturus maculosus*, have suggested that odorants can both stimulate and inhibit cell firing with mechanisms that appear to be mediated by second messengers (V.E. Dionne, J. Gen. Physiol. in press). However, neither the second messenger system(s) activated by odorants nor the specific membrane conductances that are their targets have been identified. We show here that membrane-permeable analogs of cyclic AMP can mimic some but not all of the odor-elicited responses in these cells. Olfactory receptor neurons were dissociated from the mudpuppy using enzyme-free techniques and studied using the nystatin whole-cell recording method. Odorants (100 μM L-ala, or a mixture containing 100 μM each of L-ala, L-arg, L-thr, L-ser, and L-his) and cyclic nucleotides (8-Bromo-cyclic AMP, 300 μM, or 8-chlorophenylthio cAMP, 500 μM) were applied separately by microperfusion. In 34 of 72 cells studied, one or both of the test stimuli altered the membrane conductance or firing pattern of the cell. Different types of responses occurred in different cells. These responses included hyperpolarizing (n=3) and depolarizing (n=11) receptor potentials, inhibition (n=16) and stimulation (n=1) of spontaneous or evoked action potentials, reduction of total membrane conductance without a change in resting potential (n=10), and changes in voltage-stimulated potassium (n=4) and sodium (n=2) currents. Of the cells tested with both types of stimuli (n=34), 7 responded to amino acids only, while 10 gave similar responses to the amino acids and to the cAMP analog. Three cells responded to the cAMP analog but not to the odorants, and one cell showed different responses to the odorant and the cAMP analog. The responses mimicked by the cAMP analog were of different types, but not all responses of a type were mimicked. Since membrane-permeable cAMP analogs accurately mimic the odor-elicited responses in only a subset of mudpuppy olfactory neurons, it suggests that other second messenger systems may also be important for odor transduction.

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Rp-cAMPS: A Membrane-Permeant Antagonist of Cyclic Nucleotide-Gated Cation Channels from Olfactory Receptor Neurons.
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GARETH R. TIBBS
STEVEN A. SIEGELBAUM

Cyclic nucleotide-gated (CNG) ion channels generate the primary electrical signal in olfactory neurons during sensory transduction. Cyclic nucleotides are thought to bind to a site on the channel which is highly homologous to the cyclic nucleotide-binding domain of cAMP-dependent protein kinase. Therefore, we have investigated the effect on CNG channels of Rp-cAMPS, a thioate cAMP derivative which is a known competitive antagonist of cAMP-dependent protein kinase. We have studied CNG channels in large excised patches from acutely dissociated catfish olfactory neurons. We have found that Rp-cAMPS is a partial agonist on CNG channels: when applied alone it weakly activates the channels, but it strongly antagonizes activation of the channels by co-applied cAMP. Saturating concentrations of Rp-cAMPS (500-1000 μM) activates 10-20% of the maximal current generated by saturating cAMP (10-50 μM). Nonetheless, 500 μM Rp-cAMPS completely blocks the current elicited by 1 μM cAMP. The inhibition is competitive, with a large effect at low cAMP levels (<5 μM) and no effect at saturating cAMP. In contrast, Sp-cAMPS, the diastereoisomer of Rp-cAMPS, as well as cGMP and other cAMP analogs (8-Cl cAMP, 8-Br cAMP, 8-PCPT cAMP) are all full agonists of CNG channels. Rp-cAMPS also inhibits activation of the cloned catfish CNG channel expressed in Xenopus oocytes. Single channel analysis reveals that Rp-cAMPS reduces channel open probability without affecting single channel conductance. Rp-cAMPS and related membrane-permeant analogs should be useful tools for investigating the role of the CNG channels in mediating the electrical response of olfactory neurons to odorants.

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Photolysis of caged cyclic nucleotides used to study the olfactory transduction mechanism. GRAEME LOWE and GEOFFREY H. GOLD (Monell Chemical Senses Center, Philadelphia, PA).

We used photolysis of caged cyclic AMP and caged cyclic GMP to produce rapid and repeatable increments in cyclic nucleotide concentration within isolated salamander olfactory receptor cells. Brief (20 ms) illumination of cells loaded with 100 μM caged cyclic AMP (Calbiochem) induced a large transient inward current (peak amplitude 355 $\pm$ 200 pA at -50 mV (n=11); the caged compounds were introduced by whole-cell dialysis). The photolysis response began with a latency of 4-11 ms indicating that the photo-released cyclic nucleotides acted directly on the cyclic nucleotide-gated conductance. In contrast, an odorant response of identical amplitude, recorded in the same cell, had a latency of several hundred ms. Photolysis responses were observed in all cells tested (n=60), supporting the generality of the cyclic nucleotide pathway across odorants. The amplitudes of photolysis and odorant responses, recorded in the same cells, exhibited almost identical voltage dependence between -50 mV and +25 mV, with both reversing at *ca.* 0 mV. Photolysis of 100 μM caged cyclic GMP produced currents which were similar in amplitude and timecourse to those produced using caged cyclic AMP, consistent with the similar affinities of the cyclic nucleotide-gated conductance for cyclic AMP and cyclic GMP. When the flash was spatially limited to the cilia, the amplitude of the photolysis response increased with the length of the cilia illuminated (for cilia shorter than 30-40 μm) while the latency remained constant at 4-1[illegible] ms. Limiting the flash to the soma or basal dendrite increased the latency to several hundred ms, indicating that the cyclic nucleotide-gated conductance is localized primarily in the cilia. Summation of simultaneous odorant and photolysis responses was nonlinear, the flash-induced current being enhanced during a small odorant response and attenuated during a large odorant response, indicating that photolysis and odorant responses are mediated by a common cyclic nucleotide-gated conductance which exhibits positive cooperativity. Summation of two photolysis responses was similar. The above results provide evidence that the ciliary cyclic nucleotide-gated conductance mediates olfactory transduction.

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Two types of mechanisms for the regulation of the activity of the olfactory cyclic nucleotide-gated ion channel by divalent cations. FRANK ZUFALL (Physiological Inst. TU, 8000-Munich 40, FRG) and STUART FIRESTEIN (Section of Neurobiology, Yale Medical School, New Haven, CT 06510).

We have previously described an odor-sensitive ion channel from salamander olfactory receptor neurons that also can be gated by the cyclic nucleotides cAMP and cGMP. Here we report the effect of divalent cations on the activity of the olfactory channel at the single channel level. The channel was highly permeable to monovalent ions when divalents were absent. When Ca^{2+} or Mg^{2+} were added to the extracellular solution, a strong voltage dependent block of the open channel was induced. Channel block was accompanied by a decrease in single channel amplitude and an increase in open channel noise. Ca^{2+} was more effective than Mg^{2+}. From dose-response curves we obtained K_D=10 μM for the Ca^{2+} block, and K_D=220 μM for the Mg^{2+} block (at -60 mV). Thus at the normal resting potential and under physiological ionic conditions single channel events would no longer be resolvable. The channel block was strongly voltage dependent. For Mg^{2+} the block steadily increased with hyperpolarization. In contrast, channel block by Ca^{2+} was partially relieved with strong hyperpolarization, suggesting that Ca^{2+} both blocked and permeated the channel. Estimations of the rate constants for channel block and unblock were obtained from evaluating the single channel kinetics at different blocker concentrations. A different and novel effect of Ca^{2+} was seen when Ca^{2+} was applied to the intracellular face of the membrane under conditions preventing Ca^{2+} entry into the pore. In this case the channel's closed state was stabilized but there was no effect on the single channel conductance or mean open time. The IC_{50} value of Ca^{2+} for this effect was 0.9 μM. Whole cell recordings of the odor activated current indicated that the intracellular mechanism of Ca^{2+} could mediate short term adaptation of the response to sustained odor exposure.

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Evidence for an IP_3-gated Channel Protein in Isolated Rat Olfactory Cilia. D. RESTREPO[1], J.H. TEETER[1], E. HONDA[1], A.G. BOYLE[2], J.F. MARECEK[3], G.D. PRESTWICH[3] and D.L. KALINOSKI[1]. ([1]Monell Chemical Senses Center, Philadelphia, PA 19104, [2]Veterans Affairs Medical Center, Philadelphia, PA 19104 and [3]State University of New York, Department of Chemistry, Stony Brook, NY 11794)

Stimulation of rat olfactory cilia (ROC) with odorants leads to a rapid transient elevation in the levels of either cAMP or IP_3 (Breer and Boekhoff Chem. Senses 16:19-29, 1991). To study the mechanism of action of IP_3 in this preparation we have characterized the binding of [^{3}H]-IP_3 to isolated ROC. Micromolar concentrations of unlabeled IP_3 displaced [^{3}H]-IP_3 binding in a dose-dependent manner (K_d 3.9$\pm$0.65 μM). Binding was stereospecific and dependent on the number of phosphates in the inositol ring. A ciliary protein of apparent M_r 120 kDa was labeled specifically upon exposure of cilia membranes to u.v. light in the presence of the ^{125}I-labeled IP_3 analogue 1-O-[N-(4-azidosalicyloxy)-3-aminopropyl-1-phospho]-myo-inositol-4,5-bisphosphate ([^{125}I]-ASA-IP_3). The labeling of the protein displayed the same stereospecificity as the binding experiments. ROC membranes incorporated into a phospholipid bilayer at the tip of a patch pipette displayed an increase in conductance upon exposure to micromolar 1,4,5-IP_3 in 45% of the trials (n=88). The IP_3-gated conductance is relatively nonspecific for cations (P_{Ba}=8.4, P_K=1, P_{Ca}=0.8, P_{Na}=0.8) and is different from the cAMP-gated conductance. The conductance displayed stereospecificity consistent with the binding experiments. The results suggest that the site of action for odorant-stimulated elevations in IP_3 concentration in rat olfactory cilia is at a ciliary IP_3-gated cation channel.

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Taurocholic Acid Signal Transduction in Olfactory Rosettes from Atlantic Salmon.
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Taurocholic acid (TChA) and other bile acids are potent olfactory stimuli with threshold values at least an order of magnitude lower than amino acids in salmonids (Doving et al., 1980). In Atlantic salmon, amino acid signal transduction involves G protein dependent activation of phospholipase C (PLC) leading to phosphatidylinositol 4,5-bisphosphate (PIP2) breakdown. A plasma membrane-rich (PMR) fraction was prepared from olfactory rosettes of Atlantic salmon (Salmo salar) and used to investigate TChA binding and signal transduction. Initial binding studies indicated there was substantial specific binding of TChA when the PMR fraction was incubated with 3H-TChA (100 or 300 nM). While this may indicate the existence of a typical receptor-ligand interaction, the accompanying high levels of nonspecific binding may compromise further characterization. Initial signal transduction studies have focused on PLC activity. TChA stimulated PIP2 hydrolysis in a dose dependent manner, half maximal at 50-100 nM TChA. This effect of TChA required GTPγS and was eliminated upon preincubation with GDPβS, results consistent with G protein dependence. G protein dependent PLC activation by TChA was maximal below 10nM free Ca^{2+}. As the free Ca^{2+} was increased above 10nM, G protein dependent activation of PLC by TChA was accompanied by direct activation of PLC by Ca^{2+}, a possible mechanism for signal amplification. Along with our studies with amino acids, these results with TChA implicate Ca^{2+}-sensitive, G protein regulated, activation of PLC and PIP2 breakdown as critical components of signal transduction for two distinct classes of odors in Atlantic salmon.

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OLF-1: An Olfactory Specific DNA Binding Protein

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To study mechanisms of olfactory neuron-specific gene expression, we have focused on the olfactory marker protein (OMP) gene of the rat. OMP is an abundant, phylogenetically conserved, gene product expressed almost exclusively in mature olfactory receptor neurons. The OMP-gene lacks introns and canonical CAAT and TATA box motifs. The highly restricted pattern of OMP expression suggests that it may be a model of olfactory neuron-specific genes regulated by novel transcription factors. Our strategy utilizes both *in vivo* and *in vitro* approaches to identify cis-acting regulatory elements and their respective trans-acting proteins to understand the neuron-restricted and developmentally-regulated pattern of OMP expression. In transgenic mice 0.8kb or more of 5' upstream OMP sequence gives appropriate, cell-specific reporter gene expression. However, smaller fragments seem incapable of directing olfactory neuron-specific expression. Characterization of this upstream region using gel mobility shift assays and DNaseI footprinting have identified several putative regulatory motifs. Two of these exhibit high sequence homology and interact with nuclear protein enriched extracts from olfactory mucosa but not from liver or cerebellum. We have named the protein that binds to these two sites OLF-1. OLF-1 activity in olfactory mucosa is developmentally regulated and responds to olfactory nerve lesion. Synthetic oligonucleotides corresponding to both of these sites and to variants of them have been employed to characterize the specificity of the OLF-1/DNA interaction and to obtain partially purified OLF-1 by affinity chromatography. Nuclear protein enriched extracts from rat olfactory mucosa also contain activities that bind to consensus oligonucleotides for Oct-1, NF_KB, NF-1, and AP-3. Although a putative SP-1 site in the OMP gene is located just 3' of the proximal OLF-1 site SP-1 could not be demonstrated in nuclear extracts of olfactory mucosa. Cumulatively, these data demonstrate the presence of several known transcription factors in olfactory mucosa and present compelling evidence for an olfactory specific DNA binding protein, and putative transcription factor, OLF-1.

New Olfactory Genes Isolated by Enhancer Trapping in *Drosophila*

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As a means of isolating novel olfactory genes that cannot be identified through homology screens or other methods, we have screened 6,500 lines of *Drosophila*, each line containing an insertion of a transposable element carrying a B-galactosidase gene with a weak promoter but no enhancer. If the element inserts near an olfactory gene, the enhancer of the gene drives synthesis of B-galactosidase in the olfactory system, which can be recognized by a staining procedure. We have characterized 10 lines which show staining in both larval and adult olfactory organs, but relatively little staining elsewhere. Different subsets of the adult antenna are defined by different lines: some of the staining patterns resemble the distribution pattern of different classes of olfactory sensilla; others consist of small subsets of cells. Some lines show staining of other chemosensory organs as well. One line shows a sexual dimorphism in staining pattern, with antennae staining much more intensely in males than in females. Another line undergoes a change in expression pattern in the antenna at the time males begin to show normal sexual behavior towards females. Two of the insertions map near the site of an olfactory cDNA clone previously identified by subtractive hybridization, apparently within a cluster of three or more olfactory genes. Genomic sequences flanking four of the insertions have been cloned, and antennal cDNA clones flanking three of them have been isolated. The functions of three of the genes are being investigated by mutational analysis.

Molecular Genetics of Olfaction: *pentagon* is a gene required for response to a specific odorant.

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A behavioral and molecular genetic approach has been initiated which is designed to investigate three central problems concerning the olfactory system: the mechanisms of reception and transduction, the rules underlying the processing of chemosensory information and coding, and the principles by which genes determine the assembly and maintenance of this neuronal system.

Making use of *Drosophila's* sensitivity and stereotyped behaviors to a wide variety of odorant compounds, a number of Ethyl methane sulfonate induced mutants were isolated using a T maze behavioral paradigm. One of these mutants, *pentagon*, shows a remarkable specificity in its olfactory defect, being defective to the chemical odorant benzaldehyde, but normal to several other chemical odorants in two different behavioral assays. Preliminary electrophysiologic studies confirm this specificity and suggest that the defect may be at the level of the antennae. The specificity of the*pentagon*olfactory defect suggests that the mutant defines a molecule required in the reception, transduction or processing of a specific subset of chemical information in the olfactory system and has been the impetus for us to clone the *pentagon* locus.

A genetic analysis maps *pentagon* to between 8A1-2 and 8A4-5 on the X chromosome and we are in the process of cloning the *pentagon* locus. The cloning and characterization of the *pentagon* locus should provide information about the role *pentagon* plays in the olfactory system, furnish insights into the functional organization of the olfactory system, and provide molecular markers for examining the development of the olfactory system.

Paired single-unit recordings reveal synaptic interactions between local and output interneurons in the olfactory glomeruli of an insect.

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The antennal lobe of the large sphinx moth Manduca sexta has been a useful model for understanding how odors, including odorous mixtures, are processed in olfactory neuropil. We have begun to explore the patterns of connectivity among the various neural elements that participate in these circuits. Three physiological types of spiking local interneurons (LNs) were characterized. In response to stimulation of the antennal nerve, LNs exhibited: (1) a short-latency excitatory response; (2) a delayed excitatory response; or (3) a delayed inhibitory response. The structures of 38 LNs were revealed by dye injection following physiological recording. It was found that a given LN's physiological properties were not strongly correlated with its morphological characteristics. For the first time, interactions between LNs and projection neurons (PNs) were examined by paired intracellular recordings. Direct evidence that postsynaptic inhibition in a PN is mediated by a presynaptic LN was revealed by spike-triggered averaging. No reciprocal interactions were observed. Activation of a LN by injected depolarizing current led to a hyperpolarization and suppression of spikes in the PN in 20% of the 30 pairs examined. Moreover, suppression of LN firing led to a membrane depolarization and release of spike activity in quiescent PNs indicating that LNs may exert a background level of inhibitory influence over PNs. The LN-mediated suppression does not depend upon activation of firing in the PN, which suggests that it occurs through feedforward rather than feedback inhibitory pathways. Excitation in PNs may be mediated by a disinhibitory pathway involving two inhibitory LNs. Thus, spiking LNs are involved in a number of different local circuit interactions in the antennal lobe. The data suggest that the combined effects of several to many LNs in parallel are required to elicit the compound IPSPs frequently observed in PN responses to olfactory stimulation. A simple circuit model based on these results can explain all observed postsynaptic events in PNs without relying on either direct afferent input or feedback inhibition.

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Norepinephrine Inhibits Synaptic Transmission and Calcium Currents in Olfactory Bulb Neurons via a G Protein Coupled Mechanism. PAUL Q. TROMBLEY AND GORDON M. SHEPHERD (Section of Neurobiology, Yale Medical School, New Haven, CT 06501)

In the olfactory bulb the most pronounced effect of norepinephrine (NE) is a reduction in synaptic inhibition (Jahr and Nicoll, 1980). However, neither the site of action nor the mechanism underlying this effect is well defined. Recently, we have developed primary olfactory bulb cultures and have used whole cell recording techniques to examine synaptic interactions between identified mitral/granule cell pairs. In particular, we have sought to examine the potential neuromodulatory mechanisms of NE at the level of the single synapse. EPSPs recorded in granule cells, evoked by intracellular stimulation of a monosynaptically coupled mitral cell, were reversibly reduced by ~ 50% during flow pipe application of 30 μM NE. This effect was mimicked by clonidine but not by isoproterenol, suggesting that it was mediated through activation of alpha adrenergic receptors. NE did not, however, inhibit postsynaptic membrane currents in granule cells evoked by exogenous application of glutamate, indicating a presynaptic site of action. To define further the mechanism underlying the effects of NE on synaptic transmission we examined the effects of NE on whole cell calcium currents. Flow pipe application of 30 μM NE reversibly reduced calcium currents in mitral cells by ~ 30%. This inhibition was also mimicked by clonidine. The effect of NE on calcium currents was irreversible in the presence of internal GTPγS and blocked by pretreatment with pertussis toxin, suggesting the involvement of a G protein. Pretreatment with pertussis toxin also prevented NE mediated inhibition of EPSPs. These results indicate that presynaptic inhibition of calcium currents may mediate the effects of NE on synaptic transmission via a reduction in transmitter release. Evidence from several laboratories suggests that plasticity subserving olfactory learning may occur, in part, at the putative glutamatergic-GABAergic dendrodendritic reciprocal synapses between mitral cells and granule cells. The expression of olfactory learning is critically influenced by interactions between glutamate, GABA, and norepinephrine (NE), transmitters that have been implicated in synaptic modulation and plasticity in other regions of the mammalian brain including the hippocampus and the visual cortex. Our results suggest a mechanism in which arousal, mediated through NE, could influence olfactory learning by modulating synaptic plasticity of local circuits in the olfactory bulb.

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Analyses of Synaptic and Afferent Fiber Organization in Piriform Cortex of PCD Mice Following Mitral Cell Loss

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The murine mutant Purkinje Cell Degeneration (PCD) loses all mitral cells (MCs) in the olfactory bulb (OB) over a period of about 30 days beginning at 4 mon. postnatal. Tufted cells and denervated granule cells subsequently show evidence of reorganization of local synaptic circuits in the OB. Because a primary input of piriform cortex (PC) is derived from MCs, our current goal was to assess synaptic organization in the PC after loss of MC axons. In adult PCDs and controls the distribution of HRP labeled afferent fibers from the OB was studied in PC with light microscopy. In control mice there was a prominent rostral to caudal distribution of labeled fibers along the PC with a well demarcated sublaminar distribution limited to Layer Ia. In PCD mice there was a significant reduction in the rostral to caudal extent of labeled OB afferent axons. Rostrally, the sublaminar distribution appeared relatively normal through Layer Ia. However, the density of labeled axons was significantly reduced. Centrifugal axons in the lateral olfactory tract (LOT) did not appear affected since contributing nuclei were retrogradely labeled in both controls and PCD mice. Synaptological analyses of PC in controls showed small dark synaptic terminals, largely in Layer Ib, that correspond to those previously attributed to cortico-cortico association axons. LOT axon terminals were predominately in Layer Ia and appeared larger and less flocculent than the association axons. In PCD mice there was a decrease in the frequency of large pale synaptic terminals together with an increase in frequency and cross sectional diameter of dark synaptic terminals within Layer Ia. There was also evidence of an increase in multiple synaptic contacts per synaptic terminal in PCD mice. The increase in the number of dark terminals throughout Layer Ia after MC loss suggests that the cortico-cortico association axons may respond to the loss of MCs afferents by sprouting more robustly than do tufted cell axons. An increase in the diameter of dark terminals and an increase in the number of postsynaptic contacts made by individual fibers also suggests compensation for the loss of MCs afferents.

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MONOAMINERGIC INNERVATION OF RAT PIRIFORM CORTEX

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Piriform cortex (PC), one of the main targets of the olfactory bulb plays a key role in olfactory information processing. PC receives several modulatory transmitter inputs including the monoamines norepinephrine (NE), dopamine (DA) and serotonin (5-HT). The organization of these inputs to PC has not been characterized, however. Here, we directly compare the distributions and relative densities of NE, DA and 5-HT fibers in PC and other cortical areas using antibodies against dopamine-B-hydroxylase (NE), tyrosine hydroxylase (DA) and 5-HT.

All three monoamines robustly innervate the entire rostrocaudal extent of PC. The density of each of these monoamines in PC is generally greater than their corresponding innervation of hippocampus and neocortex. As in other cortical regions, 5-HT provides the densest innervation of PC, followed by NE, then DA (i.e., 5-HT>NE>DA). Monoaminergic innervation of PC also exhibited laminar specificity.

Similar to the NE innervation of neocortex, layers I and III of PC contain a moderate plexus of NE fibers; layer II is sparsely innervated. The density and laminar distribution of NE fibers is relatively uniform along the rostrocaudal axis of PC. In contrast to NE, the DA innervation of PC exhibited a marked rostrocaudal gradient. Rostrally, DA fibers are relatively sparse and confined primarily to layer III. The density of innervation systematically increases at more caudal levels and the DA fibers progressively invade more superficial layers of PC. At the caudal limits of PC, a relatively dense plexus of DA fibers extends from the deep part of layer I through layer III. The 5-HT innervation of PC is very heavy in layer I and the superficial half of layer III. The density of 5-HT fibers progressively decreases in the deeper parts of layer III. Layer II, by contrast, is sparsely innervated. 5-HT fibers in all layers are more densely packed, tortuous and have significantly more varicosities than NE or DA fibers. As for NE, there is little variation in laminar distribution or density of 5-HT fibers at different rostrocaudal levels of PC.

These findings demonstrate that PC receives robust NE, DA and 5-HT innervation. Like PC, the olfactory bulb receives dense and laminar specific extrinsic NE and 5-HT inputs; by contrast, DA innervation of the bulb appears to be derived exclusively from intrinsic DA neurons. In light of the potent modulatory actions of NE, DA, and 5-HT in hippocampal and neocortical circuits, these transmitters may play important roles in olfactory information processing. NE and 5-HT may coordinately regulate olfactory circuits whereas DA differentially modulates neural processing in PC and MOB.

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Enhanced Survival and Sprouting of Cultured Mouse Olfactory Bulb Neurons Following Treatment with NGF and FDU

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The effects of nerve growth factor (NGF) and fluorodeoxyuridine (FDU) on mouse (Balb/C x ICR) olfactory bulb cells were examined by light and scanning electron microscopy each week for four weeks. The number of neurons, the number of background cells, the size of the soma, the number of neurites, and the length of the neurites were recorded. Control cultures had minimal essential medium (MEM, Hyclone) only, whereas experimental cultures had MEM plus NGF (10 ng/ml, 2.5S-NGF, GIBCO). At day five after seeding, experimental cultures were exposed to FDU (6.4 μg/ml, Sigma) for 24 hours. If the number of background cells increased in later weeks, additional FDU treatments were subsequently given. Three cultures from each treatment were fixed and stained each week for light microscopy; another set of cultures were examined using standard scanning electron microscopy techniques. After the first week in culture, there were no differences in the measured parameters between the control and experimental groups. Olfactory neurons were small: 10 to 15 μm in diameter. There was a decrease in the number of neurons in the experimental group in the second week, probably due to effects of FDU. By the end of the fourth week there were statistically significant differences in the number of neurons, number of background cells, the number of neurites, and the length of the neurites. Generally in control cultures, neurons either did not survive or were not visible on the surface after four weeks, however, neurons thrived in experimental cultures. Neurons in experimental cultures have remained viable for up to three months. Thus a combination treatment of FDU and NGF aids in survival of cultured mouse olfactory bulb cells, increases the number of visible neurites and increases the distance over which neurites are visible.

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Analysis of Catecholamine Concentrations in the Salamander Olfactory Bulb. D.S. KNIGHT, S.S. FOSTER and K.A. HAMILTON (Department of Cellular Biology and Anatomy, Louisiana State University Medical Center, Shreveport, LA 71130).

The salamander olfactory bulb contains a large population of cells that are immunoreactive for tyrosine hydroxylase (TH) and dopamine (DA) (K.A. Hamilton and S.S. Foster, *Neurosci. Abstr.* 17:637, 1991). The cells do not exhibit immunoreactivity for aromatic L-amino acid decarboxylase, dopamine ß-hydroxylase (DBH) or norepinephrine (NE). However, scattered processes that appear to be centrifugal fibers are immunoreactive for TH, DA, DBH and/or NE.

In the present study, catecholamine concentrations in the salamander olfactory bulb were measured by HPLC with electrochemical detection, to complement a previous neurochemical analysis (K.A. Hamilton, R.M. Kream and J.S. Kauer, *Chem. Sens.* 12:663, 1987) and the immunocytochemical studies. The results indicate that the salamander olfactory bulb contains 2-3 times more DA than NE. The mean DA concentration was 317 ± 38 (S.E.) pg/mg wet weight (n=15) and the mean NE concentration was 126 ± 9 pg/mg. Low concentrations of the DA precursor L-dihydroxyphenylalanine (L-DOPA), the DA metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) and epinephrine (EPI) were also detected in some of the bulbs (n=7-14). The L-DOPA and DOPAC concentrations were estimated to be approximately 14% of the DA mean. The EPI concentration was estimated to be approximately 24% of the DA mean.

In other lower vertebrates, it has been suggested that L-DOPA may be released by TH-immunoreactive granule layer cells in the olfactory bulb, rather than DA (W.J.A.J. Smeets and H.W.M. Steinbusch, *J. Chem. Neuroanat.* 3:25-43, 1990; A. Gonzalez and W.J.A.J. Smeets, *Develop. Biol.* 303:457-477, 1991). The results of our HPLC analysis are consistent with our immunocytochemical results, which suggest that DA may be the catecholamine neurotransmitter of TH-IR cells in the salamander olfactory bulb.

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GABA-Immunoreactive Centrifugal Axon Terminals in the Frog Olfactory Bulb. I. KRATSKIN (Smell and Taste Center, Dept. Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA; Sechenov Institute, St. Petersburg, Russia); J.P. RIO (INSERM U-106, Hopital de la Salpetriere, Paris, France)

The pallial areas of the frog brain are involved in reciprocal connections with the main olfactory bulb (MOB). In a previous study, GABA-immunoreactive (GABA-IR) cell bodies were identified among bulbopetal pallial neurons by means of a double labelling technique which combined retrograde axonal tracing with light microscopic GABA immunohistochemistry. In the current study, we used anterograde axonal tracing combined with electron microscopic GABA immunocytochemistry to localize axon terminals of GABA-IR bulbopetal neurons in the frog MOB. After unilateral iontophoretic application of HRP solution in the lateral or medial pallium, transverse vibratome sections of the ipsilateral MOB were incubated with tetramethylbenzidine, stabilized in diaminobenzidine-colbalt solution and embedded in Araldite. The thin sections were processed using the immunogold technique. Double-labelled axon terminals contained pleiomorphic synaptic vesicles, whereas terminals single-labelled with HRP were filled with rounded vesicles. Anterogradely labelled axon terminals, including GABA-IR terminals, were concentrated in the granule cell layer where numerous GABA-IR neuronal perikarya and dendritic profiles have been observed. Some labelled axon terminals established synaptic contacts with a nearby granule cell dendrite or perikaryon. This study suggests that granule cells in the MOB of amphibians, like those in mammals, receive centrifugal inhibitory input and confirms that bulbopetal neurons within the same morphological formation of the frog brain may belong to several neuronal populations differing in their neurotransmitter specificities.

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Primary olfactory projections beyond the olfactory bulb in mammals.
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Immunohistochemical investigations with anti-substance P and anti-somatostatin sera have shown the presence of extensive extrabulbar olfactory projections in gymnotiform fish (Szabo et al., 1991) and three-spined stickleback (Honkanen and Ekström, 1990). In both animal species, primary olfactory fibers, collected in two fascicles, penetrate in several telencephalic and diencephalic regions. The present report shows that, in mammals too, primary olfactory fibers can extend beyond the olfactory bulb. Under deep anesthesia, adult rats were perfused with a mixture of aldehydes in sodium cacodylate buffer. The brains were removed from the skull and Vibratome cut sections, 50µm thick, were immunohistochemically stained for OMP by the peroxidase-antiperoxidase method. OMP-positivity was present in the glomerular layer of the olfactory bulb where the bulk of the olfactory axons terminates. In addition, however, numerous OMP-positive fibers, caudally directed, were observed past the olfactory bulb along the lateral olfactory tract. For their content of OMP, these fibers could be recognized as originating from primary olfactory neurons. Individually or collected in very small bundles, with abrupt change of direction, they penetrated the olfactory peduncle parenkyma and intermingled with neurons of the anterior olfactory nuclei without forming glomerular structures. The relationship that they establish with the local neurons is under investigation.

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Functional Activity Maps in the Olfactory CNS of the Lobster. E. ORONA[1], K.M. GUTHRIE[2], C.M. GALL[2], J.M. FADOOL[1], R.M. GREENBERG[1], and B.W. ACHE[1]. ([1]Whitney Laboratory, and Depts. Zoology & Neuroscience, Univ. Florida, St. Augustine, FL 32086; [2]Depts. Anatomy & Neurobiology, Univ. California, Irvine 92717)

Maps of functional activity (i.e., 2-deoxyglucose, cytochrome oxidase, c-*fos* induction) have been generated in the olfactory systems of primarily vertebrates to identify the neuronal ensembles activated by specific odors. Here we report on ongoing exploratory studies designed to generate such activity maps in the spiny lobster, *Panulirus argus*, encouraged by the finding that expression of the proto-oncogenes c-*fos* and c-*jun* can be induced by odors in the rat olfactory bulb (Guthrie et al, 1991, Neurosci. Abst. #56.14). c-*fos* and c-*jun* are members of the "immediate-early" gene family, which function as inducible transcription factors in stimulus-response coupling. Extracts of lobster brain tissue were subjected to SDS-polyacrylamide gel electrophoresis. Western blotting, employing "pan-fos" antisera ("panoramic": nuclear and cytoplasmic forms) raised against Fos and Fos-related antigens [Cambridge Research Biochemicals], detected these protein products in the lobster brain. Cryostat brain sections were processed for *in situ* hybridization with a c-*fos* ^{35}S-cRNA rat probe. High basal levels of c-*fos* mRNA were expressed in the somata of olfactory neurons innervating the olfactory lobe, but not in all somata of the brain. Experiments are in progress to determine if Fos and Jun expression can be modulated by odors and, if so, whether the pattern of expression is odor specific.

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PIONEERING OLFACTORY AXONS AND THE INDUCTION OF THE OLFACTORY BULB

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This study focuses on the initial events in the formation of the olfactory system. GAP43 is a well characterized marker for developing and regenerating axons. This molecule appears to be transiently expressed in all olfactory axons during development and a subpopulation of the olfactory receptor neurons in adults. In development, GAP43 is downregulated at about the same time that OMP is expressed. CDA 1 is an antigen that is specifically present in neuronal growth cones. Using a monoclonal antibody (mAb) that detects GAP43 independently of its phosphorylation state, and a mAb to CDA 1, we have investigated the possible role of olfactory axons in the induction of the olfactory bulb.

At E13 the olfactory epithelium has just formed from olfactory placode; some GAP43 positive olfactory axons are present at this age. A few axons enter the ventromedial part of the telencephalic vesicle and penetrate directly to the ependymal zone of the telencephalon. At E14, no obvious "olfactory bulge" is seen in the telencephalon, but large numbers of GAP43-IR olfactory axons penetrated beyond the olfactory nerve layer and the intermediate zone to reach the ventricular mitotic zone in this restricted area of the telencephalon. These deeply penetrating olfactory axons end as large, complexly shaped growth cones. Consistent with this observation, CDA 1-IR is also seen in the ventricular zone of the telencephalon at this age. By E15, the "olfactory bulb" is visible as a tiny evagination of the ventromedial part of the telencephalon. At this stage, most GAP43 labeled olfactory axons terminate in the intermediate zone of this bulge. By E16, olfactory axons have fasciculated into multiple nerve bundles that are strongly reactive for GAP43-IR. In the bulb, GAP43-IR axons are abundant in the intermediate zone, but very few are present in the ventricular zone. CDA 1-staining was in excellent agreement with the GAP43 observations: most of the CDA 1-IR was observed in the intermediate zone of the bulb with only occasional growth cone staining in the ventricular zone.

These results demonstrate that the olfactory axons reach the ventricular mitotic zone of the telencephalon prior to the induction of the olfactory bulb. We hypothesize, therefore, that olfactory axons play a role in the induction of the olfactory bulb. We speculate that pioneering olfactory axons at E13-14 have the ability to modulate the rate of neuronal proliferation in this restricted zone of the telencephalon to regulate the development of the olfactory bulb. To begin to test this hypothesis, we are examining BrdU incorporation into telencephalic neuroblasts at E12-15 to determine if there is increased proliferation in the region where pioneering olfactory axons penetrate to the mitotic zone.

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Patch clamp recordings of spiking and nonspiking interneurons from rabbit olfactory bulb slices.

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Physiological and morphological properties of visually identified peri- and juxtaglomerular cells were characterized by using a thin slice preparation in combination with the patch clamp technique without any enzyme treatment. During current clamp recording periglomerular neurons were characterized by their lack of action potentials upon depolarization. Consistent with these data Na^+ currents could never be elicited in voltage clamp experiments. Two types of outward currents were distinguished: an inactivating and a non inactivating K^+-current. The transient current was activable only from holding potentials more negativ than -80 mV and inhibited by DAP. About 70% of these cells responded to GABA (K_M 50 μM) and muscimol, one type showed a fast desensitizing (< 4s) and another type a slow desensitizing response behaviour (> 20 s). From single channel data a conductance of 24 pS of the GABA activated Cl^- current could be determined. These cells were activated also by the excitatory transmitter ACh, glutamate and norepinephrine showing a fast desensitizing response (<1 s) followed by an afterhyperpolarisation.
Juxtaglomerular cells always responded with only a single, TTX blockable action potential to maintained current injection. Two types of membrane currents could be discriminated: a fast inactivating Na^+ current and a sustained outward current that shared some properties with a delayed rectifier K^+ current. This type of cells was never found to be GABA sensitive.

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Responses of Single Olfactory Bulb Neurons in the Channel Catfish to Binary Mixtures of Amino Acids and to Their Individual Components.

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Responses of 65 olfactory bulb neurons in the channel catfish, *Ictalurus punctatus*, were recorded simultaneously with the EOG (to estimate the onset of the olfactory bulb response). For forming mixtures, acidic, basic and neutral amino acids were adjusted in concentration to provide approximately equal EOG response magnitudes. Two ml stimulus volumes (pH 8.0-8.5; flow rate, 7 ml/min) were injected into charcoal-filtered tap water that continuously bathed the olfactory organ. The olfactory bulb responses were initially classified as either excitatory (E) or suppressive (S) based on the initial distinguishable change from prestimulus activity (Meredith, 1986). Single neurons responded to different amino acids with E and/or S type responses, which were highly reproducible over time and over 3-4 log step increases in stimulus concentrations. No correlation was indicated between response classification and amino acid class. For 137 tests in which the responses to the components of a binary mixture were classified as both E or both S, 95% of the responses to the respective binary mixtures were similarly classified. To compare in more detail the similarity in temporal patterns among the responses to binary mixtures and their components, a nonparametric statistical analysis (Spearman Correlation) was performed on the number of action potentials occurring within successive 1 second time bins over 10 seconds of prestimulus and 20 seconds of response time. The results indicated that (a) in 95 tests when both components evoked similar response patterns, 57% of the response patterns to the binary mixtures were similar to those to the components; (b) for 202 tests in which the response patterns to the components were different, 49% of the response patterns to the mixtures were similar to the response pattern of one of the components. Studies are continuing to classify degrees of E and S type responses.

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Electrophysiological Characterization of Oscillatory Interneurons in the Crayfish Olfactory Pathway.

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The crayfish olfactory pathway originates as primary receptor neurons that supply antennular aesthetascs, with axons terminating in paired olfactory lobes in the brain; output of each olfactory lobe is via dendritic arborizations of about 10^5 deutocerebral globuli cells on each side, axons of which project out the olfactory-globular tracts (OGT) to the lateral protocerebrum in the eyecups. Terminals of the deutocerebral projection neurons are enlarged endings that form microglomeruli in the hemi-ellipsoid neuropile of the lateral protocerebrum; there they make excitatory synapses with a class of interneurons which are spontaneously active and oscillatory, generating regular bursts of action potentials. Different neurons exhibit burst frequencies ranging from one per second to one every thirty seconds. Interburst intervals are affected by injection of current through the recording electrode. Depolarizing currents increase interburst frequency, while currents that are hyperpolarizing reduce burst frequency or arrest bursting altogether. Electrical stimulation of the OGT interacts with burst activity in complex ways. Maximal stimuli, however, generate large, prolonged synaptic potentials in the hemi-ellipsoid interneurons; although not physiological these experiments suggest the existence of extensive convergence of OGT axon terminals onto individual hemi-ellipsoid neurons. We suspect that natural stimulation of the intact animal with behaviorally-relevant odorants must influence the ongoing activity of hemi-ellipsoid interneurons. Output of the hemi-ellipsoid neurons is upon neural elements within the terminal medulla of the lateral protocerebrum. This nexus may be the site of convergence of olfactory afference with both visual and mechanosensory input previously reported by other workers. Supported by The Whitehall Foundation.

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Laminar Contributions to Inhibition during Olfactory Bulb Response to Odor: Computer Simulation MICHAEL MEREDITH (Dept. Biological Science, Florida State University, Tallahassee, FL 32306 USA)

Inhibition of output cells in the olfactory bulb is primarily produced by two classes of cells in two different laminae. The periglomerular (PG) cells of the superficial layers make reciprocal dendrodendritic synapses in glomeruli with output (mitral and tufted) cells, which appear to be inhibitory towards the output cells. Their axons also appear to be inhibitory to output cells of neighboring glomeruli. In the deep layers, granule cells make reciprocal dendrodendritic synapses with output cell secondary dendrites, providing recurrent and lateral inhibition, segregated to some extent between different types of output cell. Previous work with a computer model based on olfactory bulb anatomy and physiology (AChemS 1991) demonstrated that complex spatial and temporal patterns of activity could be generated in response to relatively simply patterned olfactory input. Here the contribution of superficial and deep inhibitory circuits is examined in more detail. Lateral inhibition between glomeruli via PG cell axons is a potent contributor to spatial patterns of inhibition. *Feedforward* inhibition due to direct activation of PG cells by olfactory nerve fibers is especially important. Complex spatial patterns become more pronounced and inhibition increases faster, with increasing intensity of input, when the proportion of the PG cell input coming directly from olfactory axons (PROP) is high. At lower values of PROP, PG excitation is mainly from output cells and is, thus, self limiting. Mutual inhibition between PG cells, via their axons, restricts PG influence but does not affect the qualitative patterns generated. Similarly, intraglomerular inhibition between output cells of the *same* type (via PG dendrites) attenuates output with little effect on spatial patterns. Intraglomerular inhibition *between* output cell types within one glomerulus, however, can produce different patterns of activation in the different laminae of output cells. Granule cells have feedback dendro-dendritic connections to output cells and thus have less potent afferent inhibitory action (although potentially more important in efferent inhibition). To the extent that they are divided into populations connecting selectively with one output cell type, granule cells also contribute little to the generation of different activity patterns in different output cell laminae. Output cell collaterals, however, could generate interlaminar differences in output if they cross-connect (especially if asymmetrically) between output cells of one type and granule cells selectivly connected with another output cell type. The specificity of these connections is as yet unknown but the potential for complex pattern generation is considerable.

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Morphology and Electrophysiology of rat olfactory bulb mitral and tufted cells PATRICK I. EECH and JOHN W. SCOTT. (Dept. Anatomy/Cell Biology, Emory University, Atlanta, GA 30322)

The mitral and tufted cells exist in several forms with basal dendrites in different layers. Anatomical data suggest that the dendrodendritic interactions of these different types with the inhibitory granule cells are stratified so that the mitral cells probably do not share the same granule cell population as the most superficial tufted cells. This relationship suggested that these superficial cells might not be strongly inhibited by stimulation of the posterior piriform cortex, which would activate only longer axons of mitral cells. We tested the responses of biocytin-labeled mitral/tufted cells to stimulation of multiple sites on the olfactory nerve, on the anterior part of the lateral olfactory tract (LOT), and on the posterior piriform cortex (pPC). In most cells the recordings were done with acetate-filled electrodes, but in a few cells of each type we used intracellular injection of Cl^- ions to show that IPSPs in all types of mitral/tufted cells were chloride-based. Our results with 18 marked mitral cells show that they have their largest IPSPs in response to antidromic stimulation, while 12 marked superficial tufted cells exhibited their largest IPSPs in response to orthodromic stimulation. Mitral cells IPSP responses to olfactory nerve, LOT and pPC stimulation were similar in size, but superficial tufted cells IPSP responses to LOT and olfactory nerve stimulation were twice the size of the IPSPs produced by pPC stimuli. These physiological differences appear to confirm the morphological indication that there are separate inhibitory circuits acting on some mitral and tufted cells.

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On the Use of InformationTheory to Characterize the Network Dynamics of Cultured Olfactory Bulb Activity

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Olfactory bulb cultures form neural networks which burst. Bursts and the spikes that form them are discrete events. Sequences of measurements derived from these events can be demonstrated to be stationary. Thus, with a sufficient collection period, these sequences can be modeled as finite Markov chains. Shannon's fundamental inequalities can be exploited to construct a map from a data set which meets a well defined set of assumptions to a concise statistic, the Average Stored Information Index (ASI). This number represents a first order approximation to the true dynamics of the network activity as reflected in a unit. The magnitude of the ASI is reflected in the scatter of its corresponding phase space reconstruction (PSR). Experiments using bicuculline to disinhibit cultures cause PSRs to contract, indicating very predictable behavior. The ASI for this type of activity is relatively low. Spontaneous activity generates a PSR that is more scattered, and the magnitude of the ASI index is greater. This indicates a greater rate of information creation, and consequently a decrease in predictability. The ASI may be used to classify unit activity by generating a profile of the unit's response to pharmacological agents, as reflected in the magnitude of the index. Further, the comparison of the ASI index for "microscale" measurements (on the spike level) to "macroscale" measurements (on the burst level) can indicate information flow. For most units, during unpredictable regimes, information flows from micro to macro scale, (this is analogous to turbulent fluid flow), while the reverse is true for predictable regimes (which is analogous to laminar fluid flow). The information theoretic profiles for individual units simultaneously recorded from different locations are combined to generate a spatial and temporal description of the network dynamics.

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Participation of GABAergic and Glutamanergic Synaptic Pathways During Electrically-Evoked Responses in Salamander Olfactory Bulb *in vitro*. DAVID P. WELLIS and JOHN S. KAUER (Neuroscience Program, Tufts-New England Medical Center, Boston, MA 02111)

Odor-evoked patterns of neural activity in the olfactory bulb (OB) reflect properties of the activation of the olfactory epithelium and of the intrinsic organization of the OB. To characterize the synaptic interaction in the OB, we have applied whole cell patch and optical recording techniques simultaneously to an *in vitro* tiger salamander hemibrain preparation during electrical stimulation of the olfactory nerve and tract (ON/OT). Previously we reported whole cell recordings of spontaneous and ON-driven GABAergic synaptic currents in identified mitral/tufted cells (Wellis and Kauer, *Neurosci. Abstr.* 1991). These recordings indicate that the majority of synaptic current evoked by ON/OT stimulation consists of distinct $GABA_A$-mediated events. In order to observe the spatial patterns of activity in which such single cell responses are embedded, we stained the same preparations used for patching with the voltage-sensitive dye RH414 (kindly provided by A. Grinvald) and optically recorded responses under the same stimulus and pharmacologic conditions. While bicuculline (10-50 μM) consistently suppresses the GABA-mediated synaptic currents in single cells, optical signals are either enhanced or attenuated in the presence of bicuculline. During attenuated responses, the spatial distribution of activity within the OB is more restricted and the duration is shorter than in control records; during enhanced responses, activity spreads into previously inactive regions and is longer lasting. We are currently investigating the effects of pH, O_2 and CO_2 in our bath solutions. The evoked GABAergic currents and optical signals are substantially reduced in amplitude by bath perfusion of the non-NMDA antagonist CNQX (5 or 10 μM) and are attenuated in amplitude and spatial distribution by the NMDA antagonist AP5 (1-10 μM). Lowering bath Mg^{+2}, to facilitate NMDA-mediated synaptic transmission, enhances both the ON/OT-evoked currents and optical signals in both amplitude as well as space. These and other results suggest that glutamanergic synaptic pathways drive GABAergic neurons and that both GABA and glutamate play roles in shaping the spatial and temporal patterns of activity observed in the OB following electrical stimulation.

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Odor-induced glycogen metabolism in the olfactory bulb of the fetal rat.

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Neonatal rats can detect and respond to odors in the perinatal period: olfaction is a primary sensory modality mediating behaviors, such as feeding and learning. Near-term rat fetuses exhibit distinctive behavioral responses when different chemosensory stimuli, such as lemon odor extract or milk are delivered into the mouth. Olfaction may play a role in mediating these behavioral responses of fetuses. Recent evidence indicates that high levels of glycogen are found in olfactory bulb of the neonatal rat and that glycogen metabolism is modulated by sensory input. The purpose of this study was to extend these findings to the prenatal period by measuring (a) levels of glycogen stored in the olfactory bulbs of near-term rat fetuses (E20), and (b) changes in glycogen levels in bulbs after presentation of a chemosensory stimulus into the mouth of the fetus. Pregnant rats were prepared using techniques that permit direct observation and manipulation of rodent fetuses. Each fetal subject was exteriorized from the uterus into a saline bath (37.5° C). Olfactory bulbs were collected following decapitation into isopentane (-70° C) to rapidly freeze tissue samples for glycogen assay. Samples were collected immediately (untreated control) or at various times after intreoral infusion of a chemosensory stimulus (lemon extract or milk) to provide information about the time course of changes in glycogen levels in the olfactory bulbs. An initial experiment established that baseline levels of glycogen in untreated subjects did not differ from glycogen levels in subjects in cannula-only or saline infusion control groups. In a second experiment lemon or milk infusion resulted in a rapid breakdown of glycogen in the bulb within 5 s after the onset of infusion, which was still evident at 15-s. By 30-s glycogen levels exceeded baseline, and levels were returning to near baseline by 60-s. The high baseline level and the rapid, stimulus-induced turnover imply a role for glycogen in olfactory bulb processing of odor stimuli. Furthermore, these data suggest that chemosensory stimuli are capable of activating the olfactory bulb in fetuses.

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Effect of unilateral naris occlusion on the number of juxtaglomerular neurons in young rabbits.

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Unilateral naris occlusion of newborn rodents results in sensory deprivation and impairments in developing olfactory bulb (OB) (see Gudden, 1870; Meisami, 1976; Brunjes and Frazier, 1986). Neuronal constituents of the OB are in different developmental stages in these early postnatal (P) days, so, the deprivation may have different impact on various neurons. Since the glomerular circuit is very immature yet during the 2nd-3rd postnatal weeks and may be one of the principal targets of impairments, we focused our present studies on this layer. One nare of postnatal rabbits was surgically closed on the day of birth. On P30 OBs were removed and the juxtaglomerular cells (JGCs) were counted on haematoxylin-eosin stained sections of paraffin embedded tissue with the aid of camera lucida. The three main results were as follows: 1. It was no apparent difference between the number of JGCs of bulbs on the open and closed side of normal animals. 2.The number of JGCs was reduced significantly on the occluded side. 3. There was a significant increase in the number of JGCs when the control side of experimental bulbs was compared with the same side of unoperated rabbits.

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Changes in Growth Factor mRNA Expression in the Rat Olfactory Bulb with Unilateral Naris Occlusion

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A number of studies have demonstrated the expression of putative neurotransmitter substances by olfactory bulb neurons is influenced by sensory input. Recently, neurotrophic factor synthesis has been detected in many of these same neuronal populations. We have reported the localization of mRNA encoding nerve growth factor (NGF) in periglomerular and tufted cells and mRNA encoding brain-derived neurotrophic factor (BDNF) in granule and periglomerular cells. In addition, mRNA for insulin-like growth factor type 1 (IGF-1) is localized within periglomerular, tufted and mitral cells of the main olfactory bulb, and in output neurons of the accessory bulb. In the present study, in situ hybridization was used to determine if sensory deprivation, by unilateral naris occlusion, influences the expression of these neurotrophic factors. Rat pups were subjected to unilateral naris cauterization at postnatal day (PN) 2 and were sacrificed at PN21. Levels of ^{35}S-cRNA hybridization were determined using both film and emulsion autoradiography. In olfactory bulbs ipsilateral to naris closure hybridization to BDNF mRNA was reduced in both the glomerular and granule cell layers compared to the contralateral bulb. In addition, hybridization was reduced in the cellular layers of the ipsilateral anterior olfactory nucleus. In contrast, hybridization to IGF-1 mRNA appeared equivalent in both deprived and normal olfactory bulbs. Studies are in progress to evaluate the effects of olfactory deprivation on the expression of NGF mRNA in the olfatory bulb. These results indicate that expression of BDNF mRNA is influeneced by sensory input; this is consistent with other studies done in this laboratory demonstrating that expression of this neurotrophin is regulated by physiological activity in other brain regions. In contrast, the expression of IGF-1 mRNA by target neurons of olfactory nerve afferents appears to be independent of sensory input.

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Developmental Changes in c-*fos* mRNA Expression in the Rat Main Olfactory Bulb

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We have previously reported that exposure of young rats (PN21-23) to odors results in dense hybridization of c-*fos* cRNA over neurons in spatially discrete regions of the glomerular layer. The pattern of labeling often defines the boundaries of individual glomeruli. Tufted and granule cells in the underlying external plexiform and granule cell layers also exhibit dense hybridization. Labeling in the granule cell field extends well beyond the width of an activated glomerulus, but still retains well-defined boundaries. A few mitral cells also appear labeled. We have proposed that this translaminar, columnar pattern of cell labeling seen with odor stimulation defines a basic functional anatomical unit of odor processing in the olfactory bulb. Because many of the anatomical elements of the bulb develop postnatally, we examined the maturation of this response pattern in postnatal rats using in situ hybridization of c-*fos* cRNA. Hybridization was examined over a range of postnatal ages in both normal rat pups and pups unilaterally deprived by naris occlusion at postnatal day (PN) 2. Animals were sacrificed (1) after 30 min exposure to clean air followed by 30 min exposure to odor or (2) after exposure to clean air for 60 min. At PN4, odor stimulation increased c-*fos* mRNA expression throughout the developing mitral cell layer, with a small amount of cell labeling apparent in the granule cell layer. The labeling pattern did not appear spatially restricted, and cell in the glomerular layer was lacking. Air-controls exhibited lower levels of labeling in the same cell populations. Unilateral naris occlusion at PN2 dramatically reduced c-*fos* expression by mitral and granule cells in deprived bulbs at PN4, particularly along the medial aspect of the bulb. By PN8, odor-stimulated expression of c-*fos* by mitral cells had diminished, whereas robust labeling was evident in the granule cell layer. Scattered clusters of cells in the glomerular layer were also labeled and the spatial response pattern was emerging. Air-exposure and naris occlusion reduced c-*fos* expression substantially. By PN14, mitral cell labeling with odor exposure had diminished further, granule and glomerular layer cell labeling was robust, and the spatial nature of the odor response was clearly evident. Air-controls exhibited far less cell labeling and neonatal naris occlusion significantly reduced c-*fos* mRNA levels in the deprived olfactory bulb at both PN14 and PN21. These data demonstrate that c-*fos* expression is odor responsive as early as PN4, but the spatial topography of the odor response does not emerge until the end of the first postnatal week.

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Characteristics of the Reinnervated Olfactory Bulb Following Recovery From Olfactory Nerve Transection. NANCY L. KOSTER and RICHARD M. COSTANZO (Department of Physiology, Virginia Commonwealth University, Medical College of Virginia, Richmond, VA 23298-0551)

Olfactory receptor cells have the remarkable capacity to replace themselves when injured and reestablish axon connections with the olfactory bulb. To study the reconnection process and the morphological characteristics of the reinnervated olfactory bulb, we examined adult hamsters at various recovery times following a nerve transection procedure. Complete denervation of the left olfactory bulb was achieved by transection of olfactory nerve fibers emerging from the left nasal cavity at the point where they enter the cranium (cribriform plate). This results in a retrograde degeneration of mature olfactory receptor cells in the left nasal cavity. During the recovery period, new receptor cells grow axons that reestablish connection with the olfactory bulb. Nerve fibers from the right nasal cavity that project to the right olfactory bulb were left intact. One day prior to the end of recovery periods (1-90 days), horseradish peroxidase (HRP) solution was introduced into both the right and left nasal cavities to label control and newly reconnected axon projections. HRP labeling on the transected side determined the extent to which replacement axons reestablish contact with the left olfactory bulb. Labeling of uncut axon projections to the right bulb served as a control. The amount of reinnervation increased with recovery time. The morphology of the HRP-labeled areas on the recovered side differed from the typical glomerular formations observed on the control side. On the recovered side, HRP projections often terminated as dense clusters of HRP-labeled areas. These HRP-labeled areas were usually small and irregular in their arrangement. Axons reinnervated most of the glomerular layer and occasionally were found penetrating into the inner layers of the bulb. In some animals, we inserted teflon barriers to block the nerve's access to selected bulbar regions. Blockers were effective in preventing direct axon connection to the bulb; labeled axons occasionally were seen extending around the edge of the blocker. At recovery times of 30, 60, and 90 days, we observed substantial reconnection of axon projections on the transected side. This suggests that there may exist sufficient recovery to support restored sensory function.

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Developmental Expression of OMP and N-CAM in the Nasal Chemosensory Systems of the Postnatal Brazilian Short-tailed Opossum, *M. domestica*.

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Metatherian mammals give birth to embryo-like young, equivalent to about a 12-day-old rat embryo. We used one representative of this group, *M. domestica*, in our investigation of the development of their olfactory and vomeronasal systems. Immunocytochemical techniques were used to localize olfactory marker protein (OMP, a gift of Dr. F. Margolis), and neural cell adhesion molecule (N-CAM, a gift of Drs. G. Edelman and K. Crossin). On the day of birth, OMP-positive neurons were clearly present in the olfactory epithelium (OE) with well-stained cell bodies, dendrites and dendritic knobs lining the nasal cavity, and axons reaching and surrounding the anterior portion of the presumptive main olfactory bulb (MOB). N-CAM was also present at this time in the olfactory pathway: it appeared as a granular reaction product lining the nasal cavity and surrounding the nuclei of epithelial cells. Olfactory cell axons (main olfactory nerve, MON) and their terminations in the MOB were well-stained with this antibody. By the end of the first week of life, there was an increase in the number of OMP-positive cells in the OE, but the appearance of the epithelium stained with N-CAM did not change at this or at any other age looked at in this study. At 7 days of age, an occasional OMP-positive axon penetrated deeply into the MOB, almost to the level of the ventricles; these "aberrant" fibers did not express N-CAM, however. At 10 days of age, the MON was OMP- and N-CAM-immunoreactive and the terminations of these axons had started to segregate into olfactory nerve and glomerular layers, although no glomeruli could be distinguished. By 21 days of age, glomeruli had formed in the MOB and were well stained by both antibodies. By the end of the first month of life, antibodies to OMP stained the MOB glomeruli very well, while the AOB appeared lighter-stained, with glomeruli that were just discernable. N-CAM immunoreactivity showed a similar pattern of staining, although it did not distinguish between the MOB and AOB patterns of staining as clearly as OMP did. By the time of weaning (around 8-9 weeks of life), as in the adult, an interesting pattern of immunostaining was evident in the AOB with the two antibodies. Whereas OMP differentiated between the anterior and posterior portions of the AOB, staining the anterior part darker than the posterior AOB, N-CAM immunoreactivity in this region was homogeneous. These results suggest that these (and other) proteins may play different roles in the two chemosensory systems.

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The Role of Serotonin in Early Olfactory Learning. C.L. KIRSTEIN, S. RANGEL and M. LEON. (Dept. of Psychobiology, University of California, Irvine, CA 92717-4550).

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. This odor preference is associated with physiological, anatomical and neurochemical changes in the olfactory bulb. We have begun examining which neurotransmitter systems which may be involved in these changes. Using *in vivo* microdialysis in awake 3 and 7 day-old rat pups, we have shown that olfactory preference training increases extracellular dopamine (DA) concentrations 400% in the olfactory bulbs of 3 day-olds. Increases seen in the olfactory bulbs of 7 day-olds are significant but not as large or as long lasting suggesting an attenuation in dopaminergic effects in these neurobehavioral responses at time points later in the sensitive period for this type of learning. However, norepinephrine (NE) increases in response to stroking only at P7 and a conditioned NE release can be elicited by the odor in trained 7 day-olds. Recently, we have focused on serotonin (5-HT) in this paradigm. Specifically, rat pups were trained in the olfactory paradigm from postnatal day 1-18 (P1-18). On P19, pups were given either the serotonergic antagonist methysergide, prior to odor preference testing or 2-deoxyglucose prior to odor exposure. As seen previously, pups trained with the odor spent significantly more time over the odor than control pups. Methysergide significantly increased the amount of time pups in both groups spent over the odor. This suggests a general inhibitory role of 5HT in olfactory preference training such that administration of a 5HT antagonist increases the amount of time pups spent over the odor. The results of this blockade on 2-DG uptake in the regions of the glomerular layer specific to the odor will be discussed.

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Developmental Course Of Early Olfactory Learning. CYNTHIA C. WOO AND MICHAEL LEON (Univ. of California, Irvine).

Young rats will develop both a preference and an enhanced neural response to an odor following training with concurrent odor and tactile stimulation on postnatal days (PND) 1-18. Correlated with these neurobehavioral changes is an increase in the number of juxtaglomerular cells underlying the focal regions of high ^{14}C-2-deoxyglucose (2-DG) uptake on PND 19. In the current study, we examined these changes developmentally. Pups were trained with peppermint odor or clean air with tactile stimulation on PND 1-2, PND 1-8, PND 1-11, or PND 1-14, and were tested on the following day. At each test age, trained pups showed enhanced 2-DG uptake, compared to control pups, in response to the trained odor, . At each test age, trained pups also spent more time over the odor when tested with a two-odor choice test. 2-DG uptake peaked in both groups at PND 12, as did total respirations, mean respiration rate, and number of high frequency respirations. There were not, however, any differences in respiration between experimental and control pups on any test day. Juxtaglomerular cell counts underlying the 2-DG uptake foci of trained and control pups revealed no differences in cell number or glomerular layer width on PND 12. It appears, therefore, that changes in respiration may contribute to the developmental peak in baseline 2-DG uptake, but the enhanced neural response at each age is not due to differential respiration. Since neither differential respiration nor increased juxtaglomerular cell number is required for the enhanced uptake of 2-DG, it may be that changes in centrifugal input or metabolic changes induced by training underlie the enhanced focal 2-DG uptake.

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Cats Can Discriminate Odors Without the Prefrontal Cortex. YOICHI OGAWA and FUMIAKI MOTOKIZAWA(Department of Physiology, Nara Medical College, Japan)

In order to clarify the role of the PFC in olfaction, the effects of lesions of the PFC or mediodorsal thalamic nucleus(MD) on odor detection, odor discrimination or visual discrimination were studied in cats. Sham lesions were made in the coronal gyrus(COR). The animals were preoperatively trained in an enclosed T maze to detect an odor, to discriminate between two odors, or to discriminate between white and black colors until they reached a criterion of at least 9 correct choices in 10 trials for 3 consecutive days. Cats with lesions of the COR had excellent retention of the preoperative learned task. Whereas cats with lesions of the PFC as well as the MD had no retention of the task for the first few days of the postoperative training. They could however reattain the criterion after fewer than or the same as the number of days required for the preoperative learning. Visual discrimination learning was also impaired by lesions of the PFC and MD, though this impairment was more temporary than that in olfactory detection or discrimination learning. These results suggest that the PFC is neither involved in detecting nor in discriminating odors.

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Binding sites of two lectins in the vomeronasal system of rats. SHIGERU TAKAMI, PASQUALE P. C. GRAZIADEI (Department of Biological Science, Florida State University, Tallahassee, FL 32306) & MASUMI ICHIKAWA* (Tokyo Metropolitan Institute for Neurosciences, Fuchu-City, Tokyo 183, Japan).

Ichikawa et al. have found that two lectins, *Bandeiraea simplicifolia* lectin I (BSL-I) and *Vicia villosa* aggulutinin (VVA), stain the vomeronasal nerve and glomerular layers (VNL and GL) of the accessory bulb (AOB), but not the olfactory nerve layer and the GL of the main olfactory bulb (MOB) of rats (Neuroscience Res., 1992, in press). The present project examines the possible co- and differential localization of the binding sites of BSL-I and VVA in the vomeronasal organ (VNO) and the AOB of Sprague-Dawley rats. Deeply anesthetized rats were transcardially perfused with Ringer's solution followed by 4% paraformaldehyde containing 0.1 M phosphate buffer. Removed brains were postfixed in the same fixative overnight. By using biotinylated BSL-I, FITC-VVA, and texas red-labeled avidin D or rhodamine-labeled avidin D, a double labeled fluorescence microscopic method was carried out; 40 µm thick sagittal sections of the AOB and MOB, and 20 µm thick transverse sections of the VNO were processed. The BSL-I stains very homogeneously the whole VNL and GL of the AOB. The VVA, however, stains very lightly the anterior one-third, but more intensely the rest of the VNL and GL of the AOB; many VN axons in the rostral part of the GL do not have VVA binding sites. The binding sites of VVA are visualized as a gathering of granular products which vary from 0.2 to 0.5 µm in diameter. Even in the posterior part of the GL, VVA negative VN axons can be seen. The above staining pattern is first found in 2 week old rats and constantly present in the adult rats of both sexes. In the VNO, the luminal surface of its sensory epithelium is strongly stained by BSL-I but not by VVA. These lectins stain many VN sensory cells and the VN nerve bundles in the submucosal layer. The present results indicate that in the rat AOB the majority of VN axons are BSL-I positive; and some of them are also VVA positive, and the percentage of co-localization of these two lectins is especially low in the rostral part of the GL. Further studies are needed to clarify if a clear topographical distribution of VVA positive VN receptor cells is present in the VNO.

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Removal of the Vomeronasal Organ Impairs Reproduction in Male Prairie Voles.

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& JOHN J. LEPRI (Department of Biology, The University of North Carolina at Greensboro)

Rodents use social odors to coordinate social and reproductive behaviors. Previous work has identified the vomeronasal organ (VNO) in the nasal cavity as the chemosensory mediator of many behavioral and endocrine social responses, e.g., in short-duration tests on sexually-inexperienced house mice and Syrian hamsters, the removal of the males' VNO reduces copulatory behavior and eliminates female-odor induced surges in testosterone secretion. We investigated the role of the VNO in the reproductive and aggressive behaviors of male prairie voles, *Microtus ochrogaster*. We used a surgical approach through the oral cavity to remove the entire VNO of adult males (VNX). Other males were exposed to sham surgery as a control (SHAM). Only 2 of 9 VNX males sired offspring after having been paired with females for 2 months. However, 9 of 12 SHAM males sired offspring in that same interval. Our data support observations of VNX-induced deficits in sexual responses during short-term tests. In behavioral tests, subject males in their home cages were briefly presented with an anesthetized "intruder" male at 3 different times during the experiment. These "aggression" tests occurred once before each male was paired with a female, again after being paired, and finally after the pair produced pups. The females and offspring were removed during the second and third tests. The levels of aggression in all males dramatically increased after they sired offspring. Since only 2 VNX males sired offspring, our data are inconclusive regarding the VNO's role in this response. We conclude that the chemosensory stimulation of the vomeronasal system is an important component of heterosexual responses of virgin male prairie voles.

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c-Fos expression in Vomeronasal pathways during mating behavior in male golden hamsters. GWEN FERNANDEZ and MICHAEL MEREDITH. (Program in Neuroscience., Florida State University., Tallahassee, FL.).

Vomeronasal chemosensory input is important for mating behavior responses in male hamsters. The vomeronasal system has projections via the accessory olfactory bulb to the medial (MN) and posterior-medial cortical nuclei (PMCN) of the amygdala, the medial preoptic area, (MPOA) and bed nucleus of the stria terminalis, (BNST) central structures important in reproductive behavior. Ablation of these nuclei abolish mating behavior in male hamsters. In this study c-fos immunoreactivity was used as a marker of neural activity to identify specific populations of neurons activated during mating behavior. Sexually inexperienced male hamsters, either intact or with their vomeronasal organs removed (VNX) were exposed to a naturally cycling behaviorally receptive female for 45 mins and perfused 45 mins later. Control animals were put into a clean box with fresh bedding and perfused 90 mins later. All animals were perfused with 4% paraformaldehyde and 50u vibratome sections were processed for immunocytochemistry using a polyclonal c-fos antibody. (Cambridge Research). Preliminary results show a clear difference in c-fos expression in males exposed to females compared to controls. Densely stained nuclei were evident in MN, BNST and MPOA of stimulated animals. VNX males exposed to females had fewer densely stained c-fos positive cells in MN & accessory olfactory bulb than was the case for controls. Ongoing studies include double labelling for fos and LHRH to explore the participation of LHRH in facilitation of mating behavior by vomeronasal sensory input.

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HRP Transport from Epithelium to Olfactory Bulb in Rats Treated with 3-Methylindole.

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DR. BURTON M. SLOTNICK (The American University).

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A number of chemicals have specific or preferential direct or indirect toxic effects on olfactory epithelium (OE) resulting in pathological changes including inflammation and/or degeneration of olfactory receptor cells and other cellular elements (e.g. Gaskell, Envir. Health Perspec. 1990). Recently Peele et. al. (Toxic. Appl. Pharm., 1991) reported that 400 mg/kg but not 100 mg/kg 3-methylindole (3MI) produced severe degeneration in most but not all areas of OE and severe deficits in acquisition of an olfactory detection task. We assessed anterograde transport of horseradish peroxidase in olfactory receptor axons of rats treated with 250 mg/kg or 350 mg/kg 3MI. Five days after treatment there was little evidence of anterograde transport in bulbar glomeruli of HRP applied to the olfactory epithelium. In rats that survived 10 or 20 days there was dense anterograde HRP transport to glomeruli but only in parts of the lateral and ventral medial aspects of the bulb. Reaction product on the lateral surface had a more ventral position anteriorly and extended further up on the dorsal wall in the posterior third of the bulb. Little reaction product was observed in neighboring glomeruli and virtually none in the band of glomeruli extending from the most dorsolateral to the mid-medial part of the bulb. A dose of 150 mg/kg of 3MI also resulted in a restricted, although possibly more extensive, area of HRP transport to glomeruli. These preliminary results suggest that 3MI may have selective effects on olfactory epithelium and might serve as a useful tool in studies of regional chemical differences in the epithelium and of olfactory coding.

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3-Methylindole Impairs Olfactory Function while Leaving Nasal Trigeminal Chemoreceptors Intact. W. L. Silver (Wake Forest U.). S. Khajenasir (U.N.C.- Chapel Hill). C. Wirsig-Wiechmann (Wake Forest U.) T. E. Finger (U. Colorado School of Medicine)

Nasal chemoreception involves both olfactory and trigeminal receptors. However, behavioral study of trigeminal chemoreceptors has been complicated by an inability to eliminate easily olfactory receptors while leaving trigeminal receptors intact. In the present study we attempted, using 3-methylindole (3-MI) injection, to eliminate olfactory function in rats without affecting trigeminal receptors. 3-MI is reported to produce functional olfactory deficits in rats by causing a severe degeneration of the olfactory sensory epithelium (Peele et al., 1991, *Tox Appl Pharm.* 107:191). In the present study, rats injected with 3-MI (400 mg/kg) took significantly longer than controls to find a buried cookie in a behavioral assay up to 15 days after the injection at which time they were sacrificed for histological examination. The "cookie test" is a simple test for olfactory function in rodents in which the latency to find a buried cookie is measured. All rats, however, reacted to acetic acid soaked cotton pellets by moving away. In addition, electrophysiological recordings from the ethmoid branch of the trigeminal nerve of control and 3-MI-treated animals exposed to amyl acetate, cyclohexanone, and propionic acid revealed identical thresholds and suprathreshold responses, demonstrating that 3-MI does not affect trigeminal chemoreceptor function. Anatomically, olfactory marker protein immunoreactivity in the epithelia and bulbs of the experimental animals was robust when examined 16 days after 3-MI treatment. The distribution and degree of innervation of the bulbs appeared normal. In addition, at that time light microscopy of the olfactory epithelium of 3-MI-treated rats revealed a normal-appearing cell body layer. However, the dendritic layer appeared to have been greatly reduced which may explain the impaired olfactory function. The results of these experiments suggest that 3-MI affects olfactory receptors in the nasal cavity while leaving trigeminal chemoreceptors intact. Thus we may now have a tool for examining behaviorally the sensitivity and specificity of nasal trigeminal receptors to volatile chemical stimuli.

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The Effect of 3-Methylindole on Measures of Olfactory Function. LLOYD HASTINGS, DOUGLAS G. KOHLRIESER, JAMES E. EVANS and MARIAN MILLER (Dept. of Environmental Health, University of Cincinnati, Cincinnati, OH).

While exposure to many compounds (*e.g.* zinc sulfate, methyl bromide, acrylic acid, etc.) often results in extensive damage to the olfactory epithelium, recovery of function is usually swift and complete. However, recovery of structural integrity usually occurs at a much slower rate. A recent report in the literature (Peele, *et al, Toxicol. Appl. Toxicol.*, 107:191-202,1991) suggests that exposure to 3-methylindole (3-MI), a potent olfactotoxin, results in complete and permanent anosmia, even when exposure is i.p. To verify this observation and to provide a more rigorous examination of olfactory function, we evaluated rats on two different measures of olfactory function–detection thresholds and odor discrimination behavior–after 3-MI exposure. Rats were trained in operant test chambers with odor cues provided by a precision flow dilution olfactometer. A go-no-go discrimination paradigm was used for both the threshold detection task and the discrimination task. The detection threshold task involved exposure to varying concentrations of ethyl acetate; the discrimination task consisted of exposure to either ethyl or methyl acetate. Once the rats were displaying stable performance, they were exposed to 3-MI, 400 mg/kg bodyweight, i.p. After repeated testing for nearly one month, the rats displayed no evidence of recovery of olfactory function. Histological examination of the nasal cavity revealed that the epithelium covering the turbinates and septum in the nose after 3-methylindole was identifiable as olfactory epithelium only because of its location. There were a few areas where the epithelium was several cells thick;infrequently were there cells whose nuclei were small and replete with heterochromatin resembling bipolar cells, but neither sustentacular cell nuclei nor bipolar cells were arranged in typical architecture. The primary covering of lamina propria where olfactory epithelium should have been was about half as thick as controls, and was comprised by a cuboidal type cell which did not possess cilia or mucous granules (5 μm sections, H&E). Respiratory epithelium contained ciliated and mucous cells, and appeared to be a thickness which was consistent with control animals.

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The Effect of Longlasting Formaldehyde Gas Exposure on the Olfactory Epithelium and on the Olfactory Discrimination Ability in the Ferret

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In the carnivorous ferret (*Mustela putorius f. furo* L.) olfaction is of major importance, especially for prey recognition. Since the behavior of ferrets is well documented and due to the fact that the postnatal development of the olfactory epithelium is known this animal was selected to investigate possible noxius effects of formaldehyde gas on neuronal structures. Ferrets exposed to formaldehyde gas (0.25 ppm and 0.5 ppm respectively; formaldehyde gas was generated by thermal depolymerization of paraformaldehyde at room temperature) for 3 - 12 months showed distinct deficits in olfactory discrimination tasks (discrimination between methyl acetate and clean air as well as discrimination between methyl acetate and ethyl acetate). The olfactory epithelium was studied by examining toluidine blue stained semithin tissue sections (1 μm thick). Already after 3 months of continuous exposure quantitative changes in the neuronal structures of the olfactory epithelium can be detected: The percentage of olfactory receptor cells is reduced while the percentage of basal cells is increased. In addition the appearance of the sensory cells seems altered.

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Intranasal Administration of Dopaminergic Agents: Transport to the Central Nervous System.

C.C. STAHLBAUM (University of Pennsylvania), A. GIOVANNI (University of Pittsburgh), R.E. HEIKKILA (UMDNJ-Robert Wood Johnson Medical School), R.L. DOTY (University of Pennsylvania)

These experiments characterize the pattern of uptake into the central nervous system (CNS) of dopaminergic agents when administered intranasally to rats. Rats administered the dopaminergic proneurotoxin n-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and its neurotoxic metabolite n-methyl-4-phenyl-pyridinium (MPP+) intranasally were subsequently found to have MPP+ in their brains. The pattern of uptake of these two neurotoxins differed. MPP+ was found only in the ipsilateral olfactory bulb subsequent to its unilateral intranasal administration, whereas MPP+ was found in both the ipsilateral and contralateral olfactory bulb, frontal cortices and caudate nuclei subsequent to unilateral intranasal MPTP administration. MPP+ concentrations were highest in the caudate nucleus after intranasal MPTP administration.

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Odorant Specificity in Olfactory Identification Deficits in Aging and Alzheimer's Disease

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Objective: Olfactory identification deficits have repeatedly been demonstrated in all phases of Alzheimer's disease (AD). We have investigated the possibility that the ability to identify some specific odorants may be relatively preserved in the AD process despite a global decline in identification ability. If identification of some odorants is preserved, it may be instructive to characterize the nature of those odorants. Methods: Odor identification was tested by using the University of Pennsylvania Smell Identification Test. Each odorant had previously been subjectively rated on a 10 point scale by a control group with intact olfactory abilities. The ratings encompassed five features: familiarity; intensity; irritation; pleasantness/unpleasantness; warmth/coolness. Two hundred and six subjects underwent olfactory testing. Control subjects were classified as young (YC), ages 15-59, (n=47); old controls (OC), ages 60-90 (n=97). Cognitively impaired subjects were classified as mildly impaired (Global Deterioration Scale 3) (n=16), mild AD (GDS 4) (n=22) and moderate AD (GDS 5) (n=24). In our analysis, we clustered groups of odorants that each subject group identified most frequently ("best") and least frequently ("worst"). We analyzed differences between clusters on the basis of the five features mentioned above. We also attempted to determine odorants that most clearly distinguished AD from OC. Results: The clusters of "best" odorants were significantly "cooler" than the "worst" odorants in YC ($p<.04$), OC ($p<.006$) and showed a trend in this direction in GDS 3 ($p<.08$). In contrast, AD patients (GDS 4 and 5) showed no difference in features, including warmth/coolness, between their "best" and "worst" odorants. Early AD (GDS 4) can be distinguished from OC based upon performance on four items (licorice, turpentine, paint thinner and lemon) with a sensitivity of 91% and a specificity of 83%. Conclusions: All groups studied except AD can identify "cool" odorants better. The AD process may involve some mechanism that compromises this ability. Certain odorants can be used to distinguish AD from normal aging and may be useful as early diagnostic markers. Future research might focus on the molecular differences of these odorants.

Taste Discrimination Testing II: Modification of Thurstonian Modeling. SUSAN TEDJA, RYUICHI NONAKA, MICHAEL O'MAHONY (Dept. Food Science & Technology, Univ. California, Davis) DANIEL ENNIS (Philip Morris Co., Richmond, VA)

"Triangle and 3-AFC discrimination tests for measuring taste sensitivity vary only in their instructions. For the latter test, the attributes of the stimuli are specified; for the former they are not. Both tests have the same chance levels and are generally analysed statistically in the same way using binomial statistics. Yet, for a given sensitivity to NaCl, a subject will perform better using 3-AFC tests. Clearly, the tests are not equivalent. Thurstonian modeling provides a suitable explanation why, for a given d', the search procedure set up by the subject renders superior performance for the 3-AFC. Ennis and Frijters have modeled these tests and provided tables of d' for their analysis. Yet, the tables are theoretical and require experimental confirmation. Subjects performed triangle and 3-AFC tests, discriminating low concentration NaCl from water, while also providing samples of whole saliva before and after tasting each stimulus. These were analyzed by atomic absorption spectrometer to indicate their NaCl content; the concentration change on tasting a stimulus gave a measure of its physical signal strength. Performance on the 3-AFC was superior to that on the triangle test, confirming earlier reports. For a given subject and a given NaCl concentration, the d' obtained from the tables of Ennis or Frijters for a 3-AFC test corresponded to the d' obtained for the less powerful triangle test. Thus, the tables were internally consistent. Yet, the data for the physical signal strengths suggest that the assumption of two independent distributions used to describe NaCl and water tastes for Thurstonian modeling is incorrect because of interactions between the stimuli when they are tasted in sequence. Instead of two frequency distributions, one for 'NaCl' and one for 'water', sequencing effects caused there to be four distinct distributions; one for NaCl tasted after water, another for NaCl tasted after NaCl, one for water tasted after water and another for water tasted after NaCl. Thus, classical Thurstonian modeling would seem inappropriate for taste. A new four distribution model, marrying the Thurstonian and Sequential Sensitivity Analysis approaches would seem a more desirable starting point for modeling.

Taste Discrimination Testing III: Higher Salivary NaCl levels reduce discrimination between NaCl and water by altering physical stimulus strengths. JEANNINE DELWICHE, MICHAEL O'MAHONY (Dept. Food Science & Technology, Univ. California, Davis)

According to current theory, the taste system has an automatic tare mechanism. The 'taste zero' for a given stimulus (the concentration perceived as tasteless) is generally set at the concentration in the medium surrounding the receptors. This has been confirmed using various artificial manipulations like stimulus flow delivery techniques. Simple adaptation theory would further predict that a secreted saliva higher in NaCl concentration would raise the NaCl 'taste zero' and render the judge less sensitive to NaCl taste. Evidence for this in situ effect is either questionable or indirect. Yet, simple adaptation theory would seem to be an oversimplification, because taste sensitivity tests require subjects to discriminate between low concentration NaCl and water. A higher 'taste zero' would certainly render NaCl stimuli weaker tasting but it would also make water stimuli taste stronger and thus not necessarily reduce discriminatory performance. To test the consequences of higher concentrations of secreted salivary NaCl, subjects performed 'A-Not A' discrimination tests with added sureness judgements to circumvent response bias. Between tests, subjects chewed a tasteless gum for 10 seconds to stimulate salivary flow and raise its NaCl concentration. In a second condition subjects did not chew and so maintained a lower secreted salivary NaCl concentration. Salivary NaCl concentrations were measured before and after tasting stimuli and analysed by atomic absorption spectrometer for NaCl content; the concentration changes indicated the physical signal strengths of the stimuli. The data confirmed that with higher secreted salivary NaCl concentrations, the subject was worse at discriminating NaCl from water. Yet this did not appear to be a simple adaptation effect; it appeared to be caused by changes in the physical signal strength of the stimuli. Interestingly, comparison of the variances of the physical signal strengths and the perceived signal strengths obtained from ROC curves, indicated that afferent input added more 'noise' for water stimuli than for NaCl stimuli.

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Perceptual Integration in Heterogeneous Taste Percepts.
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J.E.R. FRIJTERS (Dept. of Food Science, Wageningen Agricultural University, The Netherlands)

Tasting a mixture of dissimilar tasting substances usually results in a heterogeneous percept. Such a percept can be hypothesized to be composed of the specific taste sensations (sweetness, sourness, bitterness, and saltiness) elicited by the mixture compounds. Two models have been postulated that predict the total taste intensity of a mixture on the basis of the total taste intensity of the unmixed components: the Vector Summation model (Berglund et al., 1973) and the Dominant component model (McBride, 1989). Two other models describe total taste intensity as the result of integration of the specific taste sensations within the percept: the Dominant Component model as described by Ganzevles & Kroeze (1987) and the Sum of Sensations model by De Graaf & Frijters (1989). The predictions made by these four models are evaluated empirically in experimental data on sucrose/NaCl, sucrose/citric acid, and quinineHCl/NaCl mixtures. The predictions of the two Dominant Component models are inferior to those made by the two other models. The Vector Summation model performs well as a predictive model, but contains weaknesses which make it unfit for use as a psychological integration model. The Sum of Sensations model is the only model that provides good predictions and that can account for all the phenomena observed.

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PROP Supertasters and the Perception of Sweetness and Bitterness. L.M. BARTOSHUK, K. FAST, T.A. KARRER, S. MARINO (Yale University School of Medicine), R.A. PRICE, and D.R. REED (University of Pennsylvania).

It has been proposed that individuals who have high thresholds (at or above .0002 M) for PROP (6-n-propylthiouracil) are nontasters and carry two recessive genes for "taste blindness." Those who have lower thresholds (at or below .0001 M) are tasters and carry one recessive and one dominant gene or two dominant genes. We report both threshold and suprathreshold evidence that there may be three phenotypical groups rather than two. The distribution of PROP thresholds (N>700) is fit better by hypothesizing three underlying distributions than by two. We suggest that those three distributions are produced by nontasters (two "recessive" genes), medium tasters (one "recessive" and one "dominant" gene), and supertasters (two "dominant" genes). Suprathreshold taste scaling of PROP also supports the existence of three groups. We compared suprathreshold scales for NaCl (.01-1.0 M) and PROP (.000032-.0032 M). When these scales are superimposed, nontaster PROP functions fall considerably below NaCl functions, as expected. Taster PROP functions show variability. Some fall considerably above the NaCl functions while others overlap them. The taster population presumably contains 1/3 homozygous tasters and 2/3 heterozygous tasters. We classified the 1/3 of our tasters with the highest PROP functions as supertasters of PROP. We then used magnitude matching (with a sound control) to compare responses of nontasters, medium tasters and supertasters to a variety of sweet and bitter stimuli. For certain bitter and sweet stimuli, the supertasters perceived much greater taste intensities than both medium tasters and nontasters. We suggest that supertasters account for most of the suprathreshold taster-nontaster differences and that these individuals may be homozygous "dominants."

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Irritation as a Component of Saltiness and Sourness: Effects of Capsaicin Desensitization. MAGDALENA M. GILMORE and BARRY G. GREEN (Monell Chemical Senses Center).*

It has previously been shown that at moderate and high concentrations, the perception of saltiness is composed of sensations of both taste and irritation. In the first experiment, we examined whether the same is true for sourness. In separate sessions, NaCl (.4, .71, 1.26, 2.25, and 4M) and citric acid (.0126, .02, .04, .07, and .126M) were presented via filter paper disks to the tip of the tongue. Subjects rated the intensity of taste and irritation after 5 and 25 sec. The perceived intensity of taste remained approximately constant over time, whereas the perceived intensity of irritation grew, particularly at the highest concentration. In the second experiment, we investigated the effect of capsaicin desensitization on the taste and irritation components for both saltiness and sourness. Subjects rated taste and irritation before and after exposure to either capsaicin or zingerone. Zingerone, a non-desensitizing irritant, was included to control for possible context effects. For citric acid, the taste decreased only at the highest concentration; for NaCl, there was a tendency for taste to decrease across all concentrations. The perceived irritation of both stimuli decreased, suggesting that the perception of saltiness and sourness is at least partly mediated by capsaicin-sensitive fibers. However, similar though somewhat smaller effects were obtained with zingerone, which suggests that the observed reductions in both taste and irritation may have been due in part to perceptual (context) effects rather than to desensitization. We are testing this possibility.

*This study was supported in part by NIH grant DC00249.

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Interactions among salivary flow rate, sample composition and perception of sweetness, sourness and fruitiness
S. BONNANS and A.C. NOBLE (University of California, Davis, CA 95616)

Nineteen subjects (S) rated sweetness, sourness, and fruitiness by time-intensity methodology (TI) in 18 orange flavored samples varying in sweetener (Aspartame [APM] and sucrose [Suc]) and in levels of sweetener and citric acid. In a separate test, while the Ss rated sourness for a subset of 6 of the 18 samples (all at one acid level), saliva was collected for 2 min from one parotid gland. When Ss were grouped on the basis of accumulated flow, no significant differences in TI parameters for any of the attributes were found among the low, medium and high-flow groups in contrast to previous studies in which higher-flow Ss rated sourness intensity higher than low-flow Ss. Across all Ss, as sweetener levels were raised, sweetness and fruitiness increased, but the reduction in sourness was not significant. Although equisweet at maximum intensity at each sweetener level, APM samples elicited longer persistence of sweetness and fruitiness than Suc samples. Despite this, the salivary response to the two sweeteners did not differ. Even with an insignificant difference in TI parameters for sourness across the 6 samples, greater salivary flow was elicited by samples containing lower sweetener levels for all Ss.

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The Influence of Stimulus Context and Instructional Set on Odor-induced Enhancement of Taste. ROBERT A. FRANK & NICOLETTE VAN DER KLAAUW (Univ. of Cincinnati)

We have shown that changes in the instructional set given to subjects can greatly influence the ability of strawberry odor to enhance the sweetness ratings of sucrose solutions (Frank, Wessel & Shaffer, 1990, Chem. Senses, 15:576). The ability of instructional set and stimulus context to modify odor-induced alterations of sweetness were studied in the present experiment. A factorial design was employed using three different instructional conditions and two different sets of stimuli. In one instructional condition, only the sweetness of the stimuli were rated. In a second, the sweetness, sourness and fruitiness of the stimuli were assessed. In the third condition, the stimuli were rated for total intensity, which was then broken down into sweetness, saltiness, sourness, bitterness, fruitiness and other. [All ratings were performed using a 21-point category scale.] One stimulus set consisted of aqueous solutions of sucrose (0.25 M), citric acid (2.5 mM), artificial strawberry flavor (1.0%), artificial lemon flavor (0.4%), artificial almond flavor (0.6%) and distilled water. The other set of stimuli included all those listed above (except almond) plus sodium chloride (0.14 M), quinine sulfate (0.15 mM), wintergreen flavor (0.2%) and artificial chocolate flavor (0.5%). Consistent with our previous findings, odor-induced enhancement of sweetness declined as the number of response alternatives increased. In fact, sweetness was suppressed by the odors in the condition with the most alternatives. Stimulus context had no influence on odor-induced enhancement of sweetness. It appears that changing the response alternatives available to a subject can have a dramatic impact on the magnitude & direction of taste-odor interactions.

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Unexpected Congruence in Odor Quality and Intensity Ratings. HARRY LAWLESS (Cornell University).

Two groups of subjects judged the odor character of citrus, woody, mixed and ambiguous aroma materials. One group (N = 60) profiled the odors by rating intensity on citrus and woody category scales (weak-strong). The second group (N = 30) rated the degree to which each stimulus was a good (vs. a poor) example of a citrus or a woody odor. The procedures gave nearly identical profiles. Correlations between intensity ratings and goodness-as-example ratings were .87 for woody scales and .90 for citrus scales. Three subgroups in each main group differed in the number of citrus and woody examples they were given to smell in a warmup session, but this manipulation had no measurable effect on either group's data. Interpretations include 1) intensity and similarity judgments are inextricably linked, 2) subjects substitute one type of judgment for another, 3) some other type of decision underlies both tasks (e.g., applicability of term, distinctiveness of odor), 4) human subjects don't read instructions. In a later session, each group was also asked to evaluate whether one, two, or more than two odors were present in each stimulus jar. As expected, mixtures were judged less singular than more prototypical odors Ambiguous odors such as dihydromyrcenol were also low in singularity, even though they were single compounds. Paradoxically, when odors which were judged low in singularity (alone) were mixed, they were not judged to be two or more odors (i.e. less singular) more often than mixtures of highly singular odors. In other words, mixtures of singular odors were recognized more readily as two-odor mixtures. This result suggests a principle of olfactory mixtures, a conservation of confusion of mixed-ness (confused alone, confused together).

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Switch and Bait: Probing the Basis for Errors of Odor Identification WILLIAM S. CAIN and BONNIE POTTS (J. B. Pierce Laboratory & Yale Univ., New Haven, CT 06519).

When people attempt to identify everyday odors, they often make errors. These commonly seem subjectively to be failures to retrieve appropriate labels. How then should we interpret the incorrect labels subjects give? If subjects call peaches by the name pears, should we assume that they knew the item not to be peaches and simply assigned another convenient, but retrievable, label? Or should we assume that they actually perceived pears? We probed the matter in a recognition memory task in which we asked subjects first to inspect and label 40 odors, and then, two days later, to recognize which among 20 items (10 "old" and 10 "new") had occurred previously. Subjects recognized the previous occurrence of items they had identified correctly better than those they had not (performance of 93% vs 74% re chance level of 50%) and those they labeled consistently at inspection and recognition better than those they had not (89% vs 72%). The outcome of that experiment confirmed a role of semantic processing in recognition memory and set up a key manipulation in the recognition paradigm. In a manipulation we call switch and bait, we interspersed five items that corresponded to incorrect labels subjects had given at inspection. In so far as bait simulated the original items and in so far as the labels subjects had given at inspection corresponded to what they had actually perceived, then we expected the subjects to fall for the bait and to identify it correctly. The subjects commonly fell for the bait (60% vs 32% of other new items) and showed much better ability to identify it correctly than to identify items they had failed to identify correctly at inspection (70% vs 22%). The results revealed a) that errors of odor identification are commonly perceptual, rather than memorial, and therefore uncommonly represent failures of retrieval, and b) that the labels subjects give reflect how they have encoded the odors. Hence, when subjects call peaches by the name pears, they have actually "perceived" pears.

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The Role Of Temporal Coding In The Perception Of Odour Mixtures DAVID G LAING[1], ANDREW EDDY[2], GEOFFREY W FRANCIS[2], and LINDA STEPHENS[3]
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In a series of recent experiments, Laing and Francis (Physiol. Behav. 46, 809, 1989) and Laing and Livermore (in press) reported that humans have great difficulty in identifying more than 3 or 4 constituents in complex odor mixtures. A possible explanation of these results is that discrimination and identification of the constituents of mixtures may be dependent and limited by the temporal separation of input from each mixture constituent. The smaller the temporal separation between two constituents, the greater the difficulty in identification. In addition, perception of "slow" odorants may be disadvantaged since these odours may be suppressed through competitive inhibition at the periphery and lateral inhibition centrally. To test the temporal hypothesis an olfactometer capable of delivering odors separated by intervas of $\leq$10ms was constructed. By varying the intervals between odorants in binary mixtures as they entered the nose (by between 0 and 400 ms), the following results were obtained: (i) With each of 4 odor pairs the suppressed component was the slower odorant. Latencies ranged between 628 ms for the mixture carvone-benzaldehyde (slower) to 93 ms for carvone-limonene (slower). (ii) Latency was dependent on odorant type and odorant concentration. (iii) Increasing the concentration of an odorant decreased the latency of perception and could reverse which of two odors was the suppressor. The data, however, is extremely noisy suggesting that as in vision Saarinen and Jules, PNAS 88, 1812, 1991) humans have considerable difficulty in consciously determining the temporal order of perception of the constituents of mixtures even though this appears to be an integral part of the mechanism by which mixture constituents are encoded.

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Does descriptive analysis of vanilla samples by two independently trained panels provide similar results?
HILDEGARDE HEYMANN (University of Missouri)

In descriptive analysis (DA) panelists create, through training, a consensus language to describe the perceived differences among the samples. If DA gives reliable, objective descriptions of samples, it would be expected that data obtained from independently trained panels be consistent. The objective was to train two DA panels independently using the same set of vanilla samples. Vanillin and four vanilla samples each at 3-fold, 10-fold and 20-fold concentration were evaluated. Panel 1 used 14 terms to describe the vanillas and panel 2 used 16 terms. Analysis of variance indicated that 11 and 13 of these terms, respectively, significantly discriminated among the samples. Principal component analyses (PCAs) for the two panels were visually similar. PCAs from both panels separated vanillin, Bourbon, Bourbon Processed Bali, Indonesian and Indonesian Non-smoky vanillas in this sequence across the first PC (accounting for 45% and 47% of the variance, respectively). Procrustes analysis of the data spaces gave a 0.80 fit value with a least squares loss of 0.061. In both the PCAs and the Procrustes analysis there were considerable overlap for similar descriptive terms. Thus from these results it appears that DA indeed gives reliable consistent results across independently trained panels.

Vanilla samples were donated by Beck Flavors, St Louis, MO.

Perception of Fat Content: Effect of Fat Type and Processing Parameters. D.J. MELA, K.R. LANGLEY, A. MARTIN (Dept. of Consumer Sciences, AFRC Institute of Food Research, Reading UK)

Little is known about the fundamental aspects of the sensory perception and gauging of fats in foods. In an initial experiment, oil-in-water (O/W) emulsions were prepared with 0, 5, 10,...,40, 45, and 50% sunflower oil (SUN) and homogenized at pressures of 100 and 300 Bar. These were rated for "fat content" on a 9-point category scale. There were significant effects of of fat concentration and processing pressure. Higher pressures, associated with a decreased fat particle size/number ratio, generated an enhanced perception of fat content. In a second experiment, O/W emulsions were prepared with 0, 12, 24, 36, and 48% fat at 100 and 300 Bar, from 2 oils differing in fat saturation: SUN (predominantly unsaturated) and Hycoa 5 (HY5, a highly saturated commercial cocoa butter substitute). Preliminary ANOVA results indicate significant main and interactive effects of fat type and concentration. Increased processing pressure did not significantly affect judgements of fat content in this experiment. HY5 emulsions had a substantially greater measured viscosity and were judged higher in fat content than those prepared from SUN at all fat levels, particularly at higher levels. However, analyses indicate a significant independent contribution of fat concentration (in addition to viscosity) in determining perceived fat content. Current experiments are focussed on the role of melting, particle size and number, and other factors which may be implicated in "fat perception."

Effects of Sucrose and Aspartame on Hunger, Taste Responsiveness and Energy Intake in Humans. ADAM DREWNOWSKI (University of Michigan School of Public Health), JEANINE LOUIS-SYLVESTRE, CHRISTINE MASSIEN (University of Paris VI), JACQUES FRICKER, DIDIER CHAPELOT and MARIAN APFELBAUM (INSERM U286, Faculte de Medecine X. Bichat, Paris).

Twelve non-restrained normal-weight subjects (6 men and 6 women) consumed 4 breakfast preloads consisting of creamy white cheese ("fromage blanc") sweetened with sucrose or aspartame. Hunger ratings, taste preference profiles, and energy intake measures were obtained for up to 10 hours following preload consumption. The study was conducted using a within-subjects design with experimental sessions spaced a week apart. Two high-calorie (700 kcal) and two low-calorie (300 kcal) preloads were employed. High calorie preloads were sweetened with either sucrose or aspartame, while low-calorie preloads were sweetened with aspartame or not sweetened at all. Three buffet-style meals: lunch, snack, and dinner always composed of the same 21 foods were served in the laboratory during the test days. All foods were weighed by the experimenters to determine the amounts consumed. Following preload consumption, the subjects also rated their preferences for a range of sugar/fat sensory stimuli and rated their hunger and desire to eat. Hunger ratings were influenced only by the caloric value of breakfast preloads, and not by the presence or the nature of the sweetener. Sensory preference profiles for sweet taste decreased relative to baseline following preload consumption. Analysis of energy intakes showed evidence of incomplete compensation: subjects who consumed low-calorie preloads at breakfast time also had lowest total caloric intakes by the end of the day. These data do not support the hypothesis that the use of intense sweeteners promotes hunger or increases energy intake in normal-weight subjects.

Effect of Meal Sensory Properties on Post-prandial Hunger and Taste Reactivity in Human Subjects. ZS WARWICK and SS SCHIFFMAN (Duke University, Durham, NC)

Taste and smell sensations initiate physiological responses which prepare the body for the influx of nutrients. Altering or eliminating the oral stimulation contingent with feeding affects nutrient processing. Food sensory properties are also a potent determinant of behavioral responses such as meal size and subsequent satiety. The sensory properties and palatability of a food are closely linked to its macronutrient composition, particularly fat content. For this reason, orosensory and nutrient effects are often unavoidably confounded in feeding research. The purpose of the present study was to evaluate the independent and interactive effects of orosensory and nutrient factors on postprandial satiety and taste processing. Four isocaloric meals representing two levels of palatability (high, moderate) crossed with two nutrient formulations (high-fat, high-carbohydrate) were consumed by normal-weight volunteers on four separate days. Ratings of hunger, fullness and the pleasantness of chemosensory stimuli were obtained before the meal and at intervals following the meal. Meal palatability affected subsequent interoceptive sensations: hunger decreased and fullness increased to a greater extent following highly palatable meals when compared to moderately palatable meals. Alliesthesia was noted with regard to a set of taste stimuli: the average pleasantness ratings decreased immediately following the meal, and gradually increased in pleasantness during the subsequent four hours.

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Juice and Cookies: Is There a Mutual Compensation of Sweetness?
H. TUORILA[1], L. HYVÖNEN[1] & L. VAINIO[2] (University of Helsinki, [1]Department of Food Chemistry and Technology and [2]Department of Nutrition, Viikki, SF-00710 Helsinki, Finland)

The compensation of sweetness between two snack items, red currant juice and filled cookies, was investigated in two experiments. Samples of red currant juice contained either 5 or 10% sucrose, and the cookies were filled with either non-sweet or sweet vanilla flavored filling. In Study 1, all four combinations of juice and cookies were presented to 62 subjects, who were asked to rate the pleasantness of both items and that of the combinations. Using another set of samples, the subjects subsequently rated the intensity of sweetness of each item. In spite of the considerable differences in sweetness intensity, the two sweetness levels in juice and cookies were rated as equally pleasant. Furthermore, there were no differences in pleasantness ratings of combinations. Subjects of Study 2 (N=41) rated, after ad libitum consumption, the same four combinations in four separate sessions. Ratings were similar to those of the Study 1, and the mean consumption rates did not vary with sweetness. Based on these mean values, it seems that the perceived sweetness was not critical for the acceptance of juice and cookies, nor did it affect the appreciation of their combinations. Besides responses to samples of juice and cookies, subjects were also asked to report their frequency of consumption of 12 specified sweet foods and their preferences for them; further analyses will show if subgroups, differing in their use and preferences for sweet foods, will also differ in their responses to samples of juice and cookies.

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Ultrastructure of the Nasal Epithelial Surface in Land-Phase Tiger Salamanders (*Ambystoma tigrinum*). HEATHER L. EISTHEN* and DOLORES M. SCHROEDER†*, *Program in Neural Science and †Medical Sciences Program, Indiana University, Bloomington.

Despite the widespread use of tiger salamanders (*Ambystoma tigrinum*) as a model animal for studies of the electrophysiological properties of the olfactory system, the ultrastructure of the nasal epithelia has not been described using transmission electron microscopy (TEM). We have therefore used TEM to investigate the ultrastructure of receptor dendrites and their supporting cells in the olfactory and vomeronasal epithelia in metamorphosed (land-phase) tiger salamanders. The surface of the olfactory epithelium contains dendrites that terminate in either cilia or microvilli. In different regions of olfactory epithelium one of the two predominates, although a few regions appear to contain exclusively ciliated dendrites. The sustentacular cells of the olfactory epithelium terminate in microvilli. We find no substantial ultrastructural differences between the flat sheet of olfactory epithelium located in the medial nasal cavity and the ridged, "larval" epithelium found in the ventrolateral portion of the cavity. In contrast, the vomeronasal epithelium contains microvillar dendrites as well as two types of supporting cells, both of which surround and contact the dendrites. One type of supporting cell resembles the sustentacular cells of the olfactory epithelium, with microvilli and secretory granules; however, the other type lacks secretory granules, terminates in a thick cluster of cilia, and contains densely-packed mitochondria. This latter type may play a role in moving mucus across the epithelium. In both the olfactory and vomeronasal epithelia, the sustentacular cell contains a bundle of filaments that span the cell below the tight junctions. As a sustentacular cell wraps around a dendrite, this bundle of filaments appears to constrict the dendrite. These filaments resemble the band desmosomes that are involved in microvillar motility in skin and intestinal epithelial cells. The ultrastructure of the olfactory and vomeronasal epithelia of terrestrial tiger salamanders does not differ from that of aquatic salamander species that we have examined.

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Structural and Enzymatic Processes Facilitating Stimulus Access and Clearance in a Chemosensory System.
RICHARD A. GLEESON, HENRY G. TRAPIDO-ROSENTHAL, LORRAINE M. MCDOWELL, HENRY C. ALDRICH, and WILLIAM E. S. CARR (The Whitney Laboratory and Department of Microbiology and Cell Science, University of Florida)

A dense tuft of from 1000 to 2000 olfactory sensilla (aesthetascs) is located on each antennule of the spiny lobster, *Panulirus argus*. Each hair-like aesthetasc has a thin cuticle which is permeable to odor molecules in seawater and contains the highly-branched dendritic extensions of over 300 receptor cells. We propose that the unique arrangement and orientation of these sensilla within the tuft promotes efficient exchange of boundary layer water during a flick of the antennule (="sniff"). The hair-like morphology of aesthetascs, together with their orientation during a flick, minimizes the distance odor molecules must diffuse to and from receptors. Within the aesthetasc, three types of ectoenzymes, which dephosphorylate nucleotide odor molecules, have been localized to the transitional zone (i.e., that region where the sensory dendrites develop cilia and branch extensively to form the outer dendritic segments). Odor diffusion within the transitional zone is limited by a high density of cellular processes, thus the presence of degradative enzymes may represent an important means of clearing odor stimuli from this region. We propose that these structural and enzymatic processes, by facilitating stimulus access and clearance, can contribute significantly to optimizing the temporal resolution and sensitivity of chemoreceptors.

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Heterogeneity of Membrane Particles in Rapidly-Frozen, Freeze-Fractured Rat Olfactory Cilia Replicated with Tantalum/Tungsten
BERT PH. M. MENCO (O. T. Hogan Hall, Northwestern University, Evanston, IL 60208, U. S. A.)

Unfixed rat olfactory epithelial samples were rapidly frozen with a Gentleman Jim bounce-free liquid nitrogen/copper-block impact-freezer and then fractured at -150°C. Subsequently, they were reproducibly replicated with tantalum/tungsten (Ta/W) at angles of 20° and/or 45° in 4 to 6 seconds. The replicas were reinforced with carbon obliquely evaporated from above. During both evaporations the sample stage rotated at 300 to 400 rpm. The Ta/W replicas were about 0.2 nm, i.e., 10% to 20% of the average mass thickness of platinum/carbon (Pt/C) replicas. The size of the smallest Ta/W grains is about 0.4 nm, whereas that of the smallest Pt/C grains is about 1.5 nm. Ta/W replicas differ in several aspects from those obtained with Pt/C evaporation; more subtle distinctions can be discerned among membrane-associated particles and many of these display a fair amount of substructure. The latter may be due to different protein subunits and/or α-helices. Many particles seem to have pores, at least some of which are most likely genuine; particles with pores and without pores occur within the same membrane leaflet and, when present, pores may seem partially plugged. The pores may represent transmembranous ion channels of, e.g., voltage-sensitive sodium channels, or adenylyl cyclase. Other particles are likely to be receptors or part of the IP_3 transduction cascade. Particles of ciliary necklaces never had pores and could clearly be distinguished from other membrane particles (Menco et al., *J. Electron Microsc. Techn.*, 8: 441-442 (1988)). The same was true for the special rod-shaped particles of supporting cell apices. Eventually we would like to relate each type of particle to their prospective function.

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The Chemosensory System of Channel Catfish, *Ictalurus punctatus*, Following Immersion Exposure to *Edwardsiella ictaluri*.

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The channel catfish, *Ictalurus punctatus* is the most important commercially cultured catfish species in the United States. Production of channel catfish has increased significantly in recent years however, along with expanded production their has also been an increase in disease. The bacteria *Edwardsiella ictaluri*, the causative agent of enteric septicemia (ESC) is the most important pathogen to affect channel catfish. The pathogenesis of *E. ictaluri* is unclear, however the olfactory system is thought to be the natural site of entry. In a preliminary study we examined fingerling catfish olfactory organ following immersion exposure to *E. ictaluri*. Acute form of ESC was produced experimentally by placing channel catfish fingerlings (N=45) in water containing 1 x 10^6 (ml/l) *E. ictaluri*. Following one hour exposure catfish were returned to home aquaria. Animals were euthanized at day 1,5,7,10 and 30. Heads were removed and placed in fixative. Nasal sacs and olfactory bulbs were dissected and placed in fresh fixative overnight, then processed for light or electron microscopy (SEM,TEM). Early signs of infection were observed (day 1) in olfactory sac neuroepithelium, with some animals showing inflammation response in the lamina propria. Supporting cells showed increased signs of secretory activity as compared to control. Olfactory neuroepithelium during acute infection showed an irregular patchy distribution of sensory cilia and by day 5, some animals showed considerable ciliary loss. At later stages (day 30) of infection, olfactory neuroepithelium thickness was decreased (15-25 μm) and patchy areas of necrosis were observed. Inflammatory fluid was present within the olfactory organ and extended along olfactory axons into the olfactory bulb. These preliminary results indicate the olfactory organ is affected by *E. ictaluri* during early stages of infection and is the probable pathway by which bacteria infect the CNS. We are currently investigating if the olfactory neuroepithelium and olfactory bulb have the capacity for morphological and functional recovery. At the present time aquaculture lacks the basic information about both infectious and noninfectious causes of disease. Our results provide anatomical and pathological data to better understand the disease process of the major catfish bacterial pathogen, *Edwardsiella ictaluri*.

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The Olfactory Mucosa from Prolarval to Spawning Stages in the Sea Lamprey *Petromyzon marinus*

JAMIE VANDENBOSSCHE and BARBARA ZIELINSKI, (Dept. of Biological Sciences, Univ.. of Windsor, Windsor, ON Canada N9B 3P4)

We have investigated the development of the sea lamprey olfactory mucosa by light microscopy of 1μm epoxy sections and by transmission electron-microscopy. Sea lamprey metamorphose from larvae that are benthic filter feeders to voracious parasites of large fish. To determine changes in structure of the olfactory mucosa during the life cycle, we examined prolarvae (shortly after hatching, 15mm length), larvae (40-80 mm length), transformed and spawning stages. Morphologically mature ciliated olfactory receptor cells (ORC) were present in all stages. In prolarvae and larvae, the olfactory mucosa was located on the posterior and lateral surfaces of the nasal cavity. The olfactory receptor cell density in larvae was remarkably consistent at 26 cells per 100 μm length of olfactory epithelial surface. The ORC had an olfactory knob diameter of 1.5 μm and sustentacular cell (SC) apical width was up to 4 μm. At the transformer and spawner stages, the density of ORC varied from 5 to 9 olfactory knobs per 100 μm length of mucociliary surface within individual samples. The ORC knob diameter increased in size from larval stages by a factor of 1.8 to 2.7 μm. The widest SC also increased from larval stages by a factor of 1.8 to 7μm. At all stages, both the ORC and SC were ciliated, with the SC containing prominent secretory vesicles. The high ORC density in larval stages indicates that the larvae are able to use olfaction. The increase in diameter of both ORC and SC by a factor of 1.8 following metamorphosis suggests that large cell size in the adult olfactory epithelium is regulated by metabolic or hormonal changes associated with metamorphosis.

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Ultrastructural Localization of Odorant (AMP) Binding Sites on Olfactory Receptor Cells of the Spiny Lobster. DAVID BLAUSTEIN, MICHELE BURGESS, ROBERT SIMMONS, AND CHARLES DERBY (Georgia State University)

Transmission electron microscopy was used to examine binding of a labeled odorant molecule to dendrites of olfactory receptor cells. The olfactory cells under study innervate the aesthetasc sensilla in the lateral filament of the antennules of the spiny lobster *Panulirus argus*. The labeled odorant was adenosine 5'-monophosphate (AMP) conjugated to a marker. AMP was selected since it is known from biochemical, electrophysiological, and behavioral studies to be a major stimulatory odorant for the spiny lobster. The ability of the labeled AMP to bind to and activate AMP receptor sites was confirmed in extracellular electrophysiological experiments: olfactory receptor cells that are excited by AMP are also excited by equimolar labeled AMP. The labeled AMP was visualized using monoclonal murine primary antibody to the marker and a donkey anti-mouse secondary antibody conjugated to colloidal gold. Results reveal AMP-specific binding sites on the outer membranes of cilia of the outer dendritic segments of these cells. We are currently performing additional TEM and biochemical studies to examine the distribution of these binding sites and to determine if they are AMP receptor molecules involved in chemosensory transduction.

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Naris Closure and Olfactory Bulbectomy Induce P-glycoprotein-like Immunoreactivity in Mouse Olfactory Epithelium. JEFF HENEGAR, ERIC WALTERS and JOEL A. MARUNIAK (Division of Biological Sciences, University of Missouri-Columbia, Columbia, MO 65211).

Overproduction of P-glycoprotein, the product of the multidrug resistance gene, confers resistance to cytotoxic drugs in many malignancies. The selection for resistance to one cytotoxic drug renders cells capable of acquiring resistance to other drugs which are unrelated in structure or function. P-gp is thought to confer the mdr phenotype in cells by decreasing the net accumulation of intracellular drug. We have extended our previous study [Walters and Maruniak, Chem. Senses 16 (5): 596] of P-glycoprotein (P-gp)-like immunoreactivity in adult mice and have investigated the short term-effects of unilateral naris closure and surgical olfactory bulbectomy. Bouin's fixed, paraffin-embedded tissue from animals that had undergone naris closure for periods ranging from 2 weeks to 5 months revealed induced P-gp-like immunoreactivity in supporting cells on the open side olfactory regions only. In animals that had experienced regrowth of receptor cell populations after closure (4 months) there was no immunoreactivity of our antibody. No immunoreactivity was found in any other cell types. In animals that had experienced unilateral olfactory bulbectomy, we saw the same immunoreactive patterns on the ipsilateral side at 1, 2, and 3 weeks post-op. In all cases, antibodies revealed the presence of distinct apical and intracellular compartments that were heavily stained. These data suggest that trauma associated with receptor neuron death and/or regeneration may influence gene expression in associated non-neuronal cells in the olfactory epithelium.

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Patterns of Immunohistochemical Staining for Proliferating Cell Nuclear Antigen (PCNA) follow Staining Patterns Established with the BrdU Method and Provide an Endogenous Marker for Cell Proliferation Studies of the Adult Mouse Olfactory Epithelium. DAVID S. REASNER and ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545 USA)

Proliferating Cell Nuclear Antigen (PCNA) is a 36kd nuclear protein associated with sites of DNA replication. PCNA is elevated during late G1 and S phase of the cell cycle and can thus be employed as an endogenous marker for cell proliferation studies. Unlike certain other cell cycle markers, PCNA can be labeled in paraffin sections and the PCNA antigen site can be recognized following a variety of common histological treatments (e.g., decalcification). A commercially available mouse monoclonal antibody (19A2; Ogata *et al., Exp. Cell Res.* 168:475-486, 1987) was used to compare the staining pattern of PCNA positive nuclei in the adult mouse olfactory epithelium with the distribution of S phase nuclei labeled by the BrdU method. The number of PCNA positive nuclei observed was larger than the number of BrdU labeled nuclei, but appeared to have the same spatial distribution in the nasal cavity. The number of BrdU labeled nuclei is equivalent to 65% of the number of PCNA positive nuclei in the same rostral-caudal position of the nasal cavity, as measured in nearby sections. The larger number of PCNA positive nuclei reflects the population of cells in the cell cycle, expressing PCNA, but not yet actively synthesizing DNA, presumably late G1 phase cells. The nuclear pattern of PCNA staining closely followed BrdU staining patterns in which light to medium nucleoplasmic staining surrounds intensely labeled spots thought to represent replication domains. Attempts to increase the S phase specificity of PCNA staining using methanol fixation, which has been successful in other cell types, lead to a loss of PCNA positive nuclei. Fixation with Formol Alcohol (ETOH, acetic acid, and formaldehyde), standard paraffin processing, and an HCl denaturing step before immunohistochemistry lead to clear and consistent PCNA labeling in adult mouse olfactory epithelium. No cytoplasmic PCNA staining was observed in the olfactory epithelium although the squamous epithelium of the ventral nasal cavity and certain other non-olfactory tissues did show cytoplasmic staining patterns. Antibodies to PCNA and other endogenous cell cycle markers are important for an assessment of proliferative activity in retrospective studies and as a measure of cell cycle activity outside the S phase. Furthermore, as a non-intrusive endogenous marker, PCNA expression can be used to evaluate the proliferative compartment following BrdU treatment in those situations where an assessment of BrdU toxicity is of concern.

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Immunohistochemical localization of GTP-binding proteins and neuronal antigens in the catfish olfactory epithelium
FE C. ABOGADIE and RICHARD C. BRUCH (Dept. of Neurobiology & Physiology, Northwestern Univ., Evanston, Il)

In catfish, Ictalurus punctatus, there are at least two olfactory signal transduction pathways, the adenylate cyclase system and the phospholipase C system. Both pathways are believed to be mediated by GTP-binding proteins. We are investigating which G-proteins are expressed in the catfish OE using antibodies to mammalian G-proteins: AS$_1$ (anti-G_{i1}/G_{i2}), affinity-purified antiserum to G_{o1}, and RM$_1$ (anti-G_o/G_{o1}). Western blotting of catfish cilia showed bands which are immunoreactive to AS$_1$ and RM$_1$, but none that is immunoreactive to the G_{o1}-specific antibody. Subsequent immunohistochemistry however showed that all 3 antibodies exhibit specific staining on the surface of the catfish olfactory epithelium. The heterogeneous nature of the cell population in the olfactory epithelium necessitates discrimination of the neurons from the non-neuronal cells. Towards this end we have investigated several neuronal markers that may be useful in evaluating the results of denervation studies. Immunohistochemical studies showed that anti-NSE (neuron-specific enolase) and anti-b-tubulin (type III), but not OMP, are useful neuronal markers in the catfish olfactory epithelium.

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Receptor Neuron Losses Result in Decreased Cytochrome P-450 Immunoreactivity in Associated Non-neuronal Cells of Mouse Olfactory Mucosa. KAY BUCHHEIT, ERIC WALTERS, AND JOEL A. MARUNIAK (Division of Biological Sciences, University of Missouri, Columbia, MO 65211).

Our laboratory previously reported that unilateral naris closure for extended durations caused time-dependent changes that were characteristic of the open-side epithelium, while the closed side appeared to be unaffected. The most deleterious effects occurred in the rostral regions of the epithelium where there were dramatic losses of olfactory receptor neurons at 3 months, regrowth at 4 months, and losses at 5, 6, 7, and 8 months of closure (Maruniak et al., 1989). We extended our study to investigate the effects of naris closure on the expression patterns of cytochrome P-450 nasal detoxification enzymes. Using antibodies against rabbit nasal cytochromes P-450NMa and P-450NMb (Ding and Coon, 1988), we performed immunohistochemical analysis on olfactory tissue of animals that had losses of olfactory receptor neurons (3 and 5 months); results showed decreased immunoreactivity of enzyme in supporting cells and Bowman's glands on the open-side, with the closed side seemingly unaffected. To our surprise, naris closure animals that had undergone replacement of receptor cells (4 months) showed increased immunoreactivity on the open-side cavity, comparable to or even higher than the closed-side. In all cases, the data seemed to indicate that expression of P-450 in the olfactory mucosa was linked to receptor cell numbers. To test this, surgical olfactory bulbectomy (which ensures the precipitous death of mature receptor neurons) was performed and immunoblots of the corresponding tissue revealed reduced P-450 immunoreactivity as compared to controls. These data strongly suggest that receptor cell populations may contribute significantly to the regulation of gene products in non-neuronal cells of the vertebrate olfactory system.

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Effects of the Interferon-Inducing Agent, Poly(I)·Poly(C) on Cytochrome P-450 in the Mouse Olfactory Epithelium. ERIC WALTERS and JOEL A. MARUNIAK (Division of Biological Sciences, University of Missouri, Columbia, MO 65211).

There is a wealth of information regarding the suppression of hepatic P-450s by agents that induce interferon production. With the recent characterization of olfactory-specific cytochrome P-450s, we initiated experiments to determine whether the interferon inducing agent, poly(I)·poly(C) caused suppressed P-450 immunoreactivity in olfactory tissue. Mice were given intraperitoneal injections of poly(I)·poly(C) at 10 mg/kg while control animals received sterile saline. Both groups of animals were sacrificed after 24 hours. Olfactory tissue was perfused with ice-cold PBS, excised and homogenates prepared for Western blotting. Blots were probed with anti-cytochrome P-450NMb antibodies. Our preliminary studies revealed that there was no significant loss of P-450 immunoreactivity in any of the animals (n=4) that received poly(I)·(C) injections. These preliminary studies suggest that interferon-inducing agents may not influence suppression of olfactory-specific P-450 expression as in similar hepatic studies. We are currently obtaining more definitive data regarding the effects of interferon-inducing agents on olfactory P-450 expression.

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Localization of Fucose Residues in Glycoconjugates of Human Olfactory and Respiratory Mucosae using Lectinoprobes. K.M EASTON[1], M.L. GETCHELL[2,3], G.A. BELL[1], and T.V. GETCHELL[2,3,4]. [1], CSIRO Div. of Food Processing, North Ryde, NSW 2113 Australia; [2], Div. of Otolaryngology, Dept. of Surgery; [3], Sanders-Brown Center on Aging; [4], Dept. of Physiology and Biophysics, University of Kentucky College of Medicine, Lexington, KY 40536.

Human nasal mucosa was stained with 2 lectins, Lotus tetragonolobus agglutinin (LTA) and Ulex europaeus-I agglutinin (UEA-I), to identify and localize fucose residues in glycoconjugates. LTA and UEA-I bind differently to fucose residues depending on the saccharide linkage. The biotinylated lectins were visualized using ABC techniques in tissue obtained from 15 individuals at autopsy (age range from 10 weeks to 85 years). Olfactory mucosa (OM) was identified by staining with an antibody to olfactory marker protein. In OM, LTA stained olfactory receptor neurons within the epithelium, about 1/4 of which were stained more intensely than others, as well as axonal bundles in the lamina propria. A patchy distribution of positive staining was observed in the mucociliary complex (MC) overlying the epithelium. A small subset of Bowman's glands were weakly stained. In respiratory mucosa (RM), serous and mucous subepithelial glands were intensely stained, with corresponding staining in the overlying MC. A different pattern of staining was obtained with UEA-I. In OM, olfactory receptor neurons were all stained with about the same intensity; UEA-I also stained basal cells. Patchy staining of the MC was also observed. In RM, serous and mucous glands and overlying MC in most individuals were stained. In addition, goblet cells and basal cells in the epithelium were positive. The endothelium of all blood vessels in both mucosae was also stained. Preabsorption of each lectin with fucose abolished staining. Lectin staining patterns suggest that human olfactory receptor neurons contain two types of fucosylated glycoconjugates, one of which is also present in basal cells, that there are subpopulations of human olfactory receptor neurons, and that fucosylated glycoconjugates are associated with secretions predominantly from RM.

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Expression of Class I MHC-associated and Virus-specific Antigens by Bowman's Glands in Infected Olfactory Mucosa. M.L. GETCHELL[1,2], G. SHIH[3], and T.V. GETCHELL[1,2,3]; [1], Div. of Otolaryngology, Dept. of Surgery; [2], Sanders-Brown Center on Aging; [3], Dept. of Physiology and Biophysics; University of Kentucky College of Medicine, Lexington, KY 40536.

Immunoreactivity for β_2-microglobulin (β_2-m), a peptide associated with class I MHC on cell surfaces, and for IgA and IgG was localized in the olfactory mucosa (OM) of virus-antibody-free (VAF) and conventional rats. Conventional rats had positive serum antibody titers for sialodacryoadenitis virus (SDAV+), a coronavirus that infects nasal epithelial cells and serous glands in the head and neck. Sections of OM from 6-week-old rats (2 VAF, 2 SDAV+) were stained with FITC-labeled antibodies for β_2-m, IgA, and IgG. In the OM of VAF rats, a few lymphocytes in the lamina propria were immunoreactive for β_2-m. Immunoreactivity for IgA was observed along acinar cell membranes in Bowman's glands (BG) and in infrequent B lymphocytes near BG. IgG stained the connective tissue stroma and a few B lymphocytes in the lamina propria. In SDAV+ rats, patches of infected nasal mucosa, characterized by the presence of large numbers of IgG-immunoreactive intraepithelial B lymphocytes, were interspersed with non-infected mucosa, which had lymphocyte-free epithelium. In infected OM, BG acinar and duct cell membranes were immunoreactive for β_2-m as were numerous lymphocytes in both the epithelium and lamina propria. Immunoreactivity for IgG and IgA was observed in numerous B lymphocytes and, for IgA, in acinar cell membranes as well. In addition, BG acinar cells displayed numerous punctate foci of immunoreactivity. These were never observed with IgA or IgG staining in BG of VAF rats or in BG of SDAV+ rats stained for IgD or IgE; they most likely represent binding of virus-specific antibodies. These results suggest that the effects of SDAV infection on BG include 1) enhanced expression of class I MHC-associated molecules, involved in antigen presentation, 2) expression of viral antigens, and 3) binding of virus-specific antibodies, marking BG cells as targets for cytotoxic T cells, complement and/or macrophages. Supported by NSF BNS-88-21074 (MLG) and NIH DC-00159 (TVG).

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Human olfaction in an atmosphere of helium or nitrogen: Individual and odorant variation in the need for oxygen. ALEXANDER M. FEIGIN[1], EDWARD P. ZINKEVICH[2] and CHARLES J. WYSOCKI[1] ([1]Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA and [2]A. N. Severtsov Institute of Evolutionary Animal Morphology and Ecology, 33 Leninsky Prospect, Moscow, V71, Russia).

Recently (Feigin et al., 1991), we demonstrated that replacing air in the nasal cavity with helium decreases olfactory sensitivity for some odorants, but has little effect on the perception of other odorants. In a series of additional experiments, we further increased the number of odorants that have been tested and obtained single-presentation detection thresholds from five subjects to six additional odorants (geraniol, citralva, menthone, lilial, methylbenzylamine, and ethyl vanillin) in delivery streams of air and helium. Subjects exhibited unique patterns of change in sensitivity to odorants in helium. In two subjects, the thresholds for a single odorant, geraniol, shifted; for one subject, thresholds for lilial, menthone and methylbenzylamine were altered; for another subject, lilial, citralva and menthone thresholds were affected; and for one subject, thresholds for all six odorants shifted. We also explored an alternative to helium and presented odorants in an oxygen-free nitrogen stream. In this experiment, five additional subjects and three odorants were studied; amyl acetate, lilial, and phenol. As was true for odorants in helium, substituting air with nitrogen affected sensitivities in individual- and odorant-specific fashions. In an atmosphere of nitrogen three subjects had higher olfactory thresholds for phenol; the thresholds to amyl acetate shifted in two subjects. Interestingly, one subject was more sensitive to phenol in nitrogen then in air. These results suggest that the presence of oxygen in breathing air appears to be involved in olfaction; its elimination through the use of either helium or nitrogen affected thresholds. Further, they demonstrate the expression of individual differences in the apparent utilization of intra-nasal oxygen in olfaction.

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Electrical Responses Of Endogenous Receptors In *Xenopus* Oocytes To Amino Acids.

MASAYA ETOH AND KIYONORI YOSHII (Kyushu Institute Of Technology, Iizuka, 060 Japan).

We investigated electrical responses of the endogenous receptors to various amino acids under voltage clamp conditions. 1) Oocytes responded to 10 mM Ser (1.7 ± 1.8 nA, n=34) and Leu (3.2 ± 4.4 n=33) dissolved in a saline salt solution (pH 7.4 ± 0.1) at -50 mV though a third of oocytes did not. Since addition of sucrose slightly changed the voltage clamp currents, it was indicated that changes in osmotic pressure by adding amino acids had little effect on the responses. 2)Although 10 mM Arg, His, and Glu also elicited responses, which were contaminated by responses to NaOH or HCl added to adjust pH of the solutions. 3) The responses to Leu steeply rose and reached steady states in 15 sec whereas those to Ser slowly reached the steady states in 2 min. 3) The responses to Leu and Ser decreased on depolarization. The voltage clamp currents did not reverse even at + 50 mV. 4) The responses to both amino acids appeared in solutions where Na was replaced with Tris, choline, or K. 5) Ouabain did not change the responses to the amino acids.

Further Characterization of the Chemoattractant Binding site(s) in Vomeronasal (VN) Organ of Garter Snakes

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Studies of function and structure of the VN system in garter snakes have established that some species-typical behaviors, such as prey detection, prey odor trailing and several social behaviors, are mediated through the VN system and not the olfactory system. Earthworms are a favorite prey of garter snakes and detection of chemoattractants from earthworms requires a functional VN system. Three snake-attractive proteins, a 20KDa protein, a sulfhydryl-contianing protein and a low molecular weight (LMW) protein, have been isolated from earthworm preparations. The 20KDa snake-attractive protein has been shown to bind to the VN sensory epithelium in a saturable and reversible manner. We have further characterized the binding site(s) and provide evidence that specific receptor(s) to these chemoattractants exist in the VN organ.The 20KDa chemoattractive protein was labeled with tritium by reductive methylation. A saturable and reversible binding site was confirmed with Kd=0.45 uM and Bmax=720 pmols/mg protein. This binding can be competitively inhibited by the LMW chemoattractant, but is not affected by unrelated proteins, such as cytochrome C and trypsin inhibitor. Furthermore, we have found that this binding is calcium dependent, and can be abolished by EGTA. EGTA added to the 20 KDa chemoattractant decreased attractivity to garter snakes, and this inhibitory action could be largely restored by addition of calcium. The binding is tissue specific. Binding of the 20KDa chemoattractant to other tissues including olfactory epithelium, Harderian gland, tongue, brain and heart is less than 20% of the binding to VN tissue. Removing the accessory olfactory bulbs, which causes degeneration of primary sensory neurons in VN epithelium, resulted in loss of about 60% of the VN tissue binding.These data indicate that specific binding site(s) in the VN sensory epithelium are most probably receptors and mediate garter snake response to earthworm chemoattractants.

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Diversity and Localization of Putative Olfactory Receptors

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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 novel components of a second messenger cascade expressed exclusively in olfactory sensory neurons. The identification of these components in olfactory cilia has led to extensive efforts to isolate G-protein coupled olfactory receptors based on the similarities in structure and sequence shared by members of this family. Recent work by Buck and Axel has identified a large multigene family expressed exclusively in olfactory epithelium. Our laboratory has extended the apparent size of the gene family and have demonstrated that the messenger RNA that encodes the putative receptor protein is largely confined to the sensory neurons. The generation of antibodies capable of recognizing most members of this receptor family confirms the expression of this family in the neurons and localizes the protein to the dendrites and cilia. We have recently generated antibodies that should recognize only a subset of the members of the receptor family. These will provide considerable insight into the patterns of receptor expression throughout the epithelium. The expression of these putative olfactory receptors in a mammalian cell line may allow one to examine specificity in ligand binding and G-protein interaction. In addition, the genomic organization of the genes which comprise this large family is currently being examined. The combined results of these studies will provide some insight into the regulation of expression of these receptors and the mechanism of odorant coding.

Specific anosmia to isovaleric acid is a peripheral deficit of genetic origin in the C57BL/6J mouse. HAI-WEI WANG, CHARLES J. WYSOCKI and GEOFFREY H. GOLD (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104).

Specific anosmias are thought to be peripheral deficits, caused by abnormalities in olfactory receptor proteins. We tested this hypothesis using the male C57BL/6J mouse, which, in behavioral experiments, exhibits *ca.* 10-fold lower sensitivity to isovaleric acid than do males of several other strains of mice, including AKR/J (Wysocki et al. Behav. Genetics 7, 171 (1977)). Peripheral sensitivity was measured by recording the EOG, *in vitro*. Olfactory stimulation was accomplished by using a pneumatically actuated valve to change the gas bathing the olfactory epithelium from clean air to air which was in equilibrium with aqueous solutions of K-isovalerate or isoamyl acetate (stimulus duration 1 sec). Isoamyl acetate was used as a reference odorant, because the behavioral thresholds for this odorant do not differ significantly between the C57BL/6J and AKR/J strains. The aqueous concentrations of K-isovalerate and isoamyl acetate were 10^{-2}-10^{-5} M and 10^{-2}-10^{-4} x saturation, respectively, at pH 4.8 (buffered with 10^{-3} M citric acid). Experiments were performed on 10 C57BL/6J and 15 AKR/J mice and responses to all of the stimulus concentrations stated above were typically measured at 2 locations in each of 4 turbinates on the left side of each animal. The data were subjected to a repeated measures analysis of variance, with responses to isoamyl acetate serving as a covariate (in a separate analysis of variance, the responses to isoamyl acetate were not significantly different between the two strains). The peak EOG amplitudes in response to isovaleric acid were smaller in the C57BL/6J mouse than in the AKR strain at all concentrations ($p=0.06$; no regional differences were significant). The electrophysiological observations obtained from these two strains are consistent with the behavioral measurements of olfactory sensitivity cited above and provide evidence that the specific anosmia to isovaleric acid, exhibited by male C57BL/6J mice, results from a genetic deficit within the nasal cavity, suggesting a defect in the olfactory receptor cells.

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Odorant Modification by Olfactory Epithelial Glutathione S-Transferase.

NISSIM BEN-ARIE and DORON LANCET (Weizmann Institute of Science, Rehovot, Israel).

The olfactory epithelium is exposed to a variety of airborne chemicals, including odorants and environmental pollutants. Recently, we have described two novel olfactory xenobiotic-metabolizing enzymes: cytochrome P-450olf1 and olfactory UDP-glucuronosyl transferase (UGT_{olf}), which could underlie the detoxification and clearance of such compound, as well as the rapid termination of odorant signals. We have now extended our research to another biotransformation enzyme, olfactory glutathione S-transferase (GST). GSTs are a family of cytosolic enzymes, which are divided into several classes (Ya, Yb, Yc and Yp). The olfactory epithelial cytosol shows a relatively high GST activity toward the common substrate, 1-chloro-2,4-dinitrobenzene: 54%±4 of the activity in liver, and 2-7 times higher than in other tissues tested, including respiratory epithelium. Thus, olfactory epithelium is the most GST-rich extrahepatic tissue in the rat. SDS-gel electrophoresis and high performance liquid chromatography of the affinity-purified enzymes showed that the olfactory GSTs are mainly composed of the Yb class, while other tissues usually show a mixture of several subclasses. N-terminal protein sequencing and Polymerase Chain Reaction (PCR)-directed cDNA cloning identified the olfactory enzymes as identical to the liver Yb_1 and Yb_2 isoenzymes. The olfactory GSTs catalyzed the conjugation of several odorants (e.g. cinnamaldehyde, carvone and citral) to the tripeptide glutathione. Olfactory epithelial GST may thus complement the activity of UGT_{olf}: while the latter acts only on alcohols, fatty acids and thiols, the newly identified GST activity could biotransform and eliminate many unsaturated aldehydes, ketones and hydrocarbons.

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Cellular Localization and Molecular Cloning of Phospholipase C Isoenzymes in Rat Olfactory Epithelium. RICHARD C. BRUCH and FE C. ABOGADIE (Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208).

Biochemical evidence supports the hypothesis that the phosphoinositide signaling pathway is involved in vertebrate olfaction. However, the molecular nature of the proteins mediating this pathway in olfactory neurons has not been established. We have therefore investigated phospholipase C (PLC) isoenzyme expression in rat olfactory epithelium (OE) using monoclonal antibodies and molecular cloning techniques. Immunoblots probed with monoclonal antibodies to PLC beta, gamma and delta showed that both PLC gamma and delta, but not PLC beta, are expressed in rat OE. Immunohistochemical studies showed that PLC delta immunoreactivity was localized in the apical dendritic regions and cilia of the receptor cells. A cDNA library was prepared from poly (A)+ RNA isolated from rat OE. The average insert size of the library was 1.3 ± 0.8 kbp. A mixture of nine PLC oligonucleotides was used to screen the cDNA library. After two rounds of screening, two clones with inserts of 5.2 and 4.8 kbp were isolated. Further characterization and expression studies of these clones will be presented.

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Phosphoinositide-specific Phospholipase C: Activation by Odors and Feedback Inhibition by Protein Kinase C. LEE-JU CHENG, YING HAR LO, AND DENNIS E. RHOADS (Univ. of Rhode Island).

In Atlantic salmon, signal transduction for at least two classes of odors, L-amino acids and bile acids, involves G protein dependent activation of phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis. The specificity for PIP2 was partially tested using phosphatidylcholine (PC). Odorants had no effect on PC metabolism and there was no evidence of either phospholipase C or D activity toward PC in membrane preparations where PIP2 metabolism was observed. Thus, PIP2 breakdown was assumed to be catalyzed by a phosphoinositide-specific phospholipase C (PLC) and, through generation of diacylglycerol and elevation of internal calcium, should lead to activation of Protein Kinase C (PKC) in a signal transduction cascade. Using isozyme specific monoclonal antibodies and immunoblotting of proteins separated by polyacrylamide gel electrophoresis, two PLC isozymes, PLC- γ1 (strong reactivity) and PLC- β1 (weak reactivity), were identified in both cytosolic and plasma membrane rich fractions from salmonid olfactory rosettes. PKC was also identified in these cytosolic and plasma membrane rich fractions. Preliminary experiments were designed to test the effect of PKC on PLC activity. When the plasma membrane rich fraction was pretreated with ATP and phorbol ester to promote PKC activity, subsequent activation of PIP2 hydrolysis by amino acids was significantly decreased. We interpret this result as evidence of a negative feedback mechanism whereby protein kinase activity, as a later component of the olfactory signal transduction cascade, limits further activation of PLC by amino acids. This study identifies several key enzymes that may take part in an olfactory signal transduction cascade.

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G Protein Dependent Stimulation of Ca^{2+} Regulated Olfactory Phospholipase C (PLC) by Amino Acids. YING HAR LO, TERENCE M. BRADLEY AND DENNIS E. RHOADS (Univ. of Rhode Island).

L-amino acids are potent olfactory stimuli for Atlantic salmon. A plasma membrane fraction, previously shown to be rich in amino acid binding sites, was prepared from olfactory rosettes of Atlantic salmon (Salmo salar) and utilized to investigate the role of phosphatidylinositol 4,5-bisphosphate (PIP_2) hydrolysis in olfactory transduction. A cocktail of L-amino acids (Ser, Glu, Lys and Gly) stimulated PIP_2 hydrolysis by PLC in a dose dependent manner with half maximal stimulation when each amino acid was 0.7 uM. Interestingly, the effects of individual components of the cocktail were not additive. Stimulation of PIP_2 hydrolysis by amino acids required GTPγS, which alone had no effect on PLC activity . Unlike GTPγS, AlF_4^- alone stimulated PIP_2 hydrolysis about two fold. Preincubation with 1mM GDPβS eliminated the effect of amino acids and AlF_4^- on PIP_2 hydrolysis, suggesting the involvement of G protein regulation. The lack of stimulation of GTPγS alone suggested that there was negligible exchange of GTPγS for GDP in the absence of odorant. The effect of the amino acid cocktail was maximal when free Ca^{2+} was below 10 nM . At 100 nM free Ca^{2+} or above , no effect of amino acids on PIP_2 hydrolysis was found. However, between 100 nM and 100 uM, Ca^{2+} directly stimulated PLC activity in a dose dependent manner. This stimulation by Ca^{2+} appeared to be G protein independent because it did not require GTPγS and was not inhibited by GDPβS. Direct activation of PLC by elevated Ca^{2+} may contribute to amplification in olfactory signal transduction.

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IP_3-induced Depolarization in Isolated Rat Olfactory Neurons. Y. OKADA, J.H. TEETER and D. RESTREPO (Monell Chemical Senses Center, Philadelphia, PA 19104)

Dialysis of inositol-1,4,5-trisphosphate (IP_3) from a whole cell patch pipette into the cytoplasm produces a large transient depolarization in isolated catfish olfactory neurons (Restrepo *et al.* Science 249: 1166-1168, 1990). In stopped-flow experiments, some odorants stimulate IP_3 formation in rat olfactory cilia (Breer and Boekhoff Chem. Senses 16: 19-29, 1991). In the present experiments, we studied the effect of IP_3 on the membrane properties of freshly isolated rat olfactory neurons. When the membrane was ruptured to attain the whole-cell configuration with K^+ internal solution, the resting membrane potential varied from -38 to -60 mV (-49 ± 6 mV, mean ± SD, n=10), the input resistance ranged from 0.5 to 5 GΩ (2.4 ± 1.4 GΩ, n=8), and the membrane capacitance averaged 3.3 ± 0.9 pF (n=8). Under current clamp, the internal dialysis of 1,4,5-IP_3 (10 μM) produced a large sustained depolarization (37 ± 17 mV, n=5) from an initial membrane potential of -24 ± 9 mV. Responses to IP_3 were observed in 70% of trials (n=10). The sustained response produced by IP_3 was not inhibited by external ruthenium red (5-20 μM), but was completely blocked by internal ruthenium red (10 μM). Under voltage clamp, internal IP_3 (10 μM) induced an inward current whose magnitude varied from 10 to 50 pA at a holding potential of -60 mV. When IP_3 was released from its caged precursor by ultraviolet irradiation, olfactory neurons displayed a transient depolarization. Internal 2,4,5-IP_3 (50 nM) also induced a depolarizing response in rat olfactory neurons. These responses may be mediated by IP_3-gated channels similar to those in catfish olfactory neurons.

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Rapid Kinetic Measurements of Second Messenger Formation in Isolated Olfactory Cilia from the Channel Catfish (I. Punctatus). DIEGO RESTREPO[1], INGRID BOEKHOFF[2], TAKENORI MIYAMOTO[1], JOHN H. TEETER[1] and HEINZ BREER[2] ([1]Monell Chemical Senses Center, Philadelphia, PA and [2]University of Stuttgart-Hohenheim, Stuttgart, F.R.G.).

The effect of stimulation of olfactory cilia with odorant amino acids on the formation of cAMP, cGMP and IP_3 was studied in the subsecond time scale using a stopped flow technique (Breer et al, Nature 345:65, 1990). L-alanine and L-cysteine (100μM) elicited a transient elevation in IP_3 levels that peaked at 25 msec. In contrast, even at high concentration a mixture of odorant amino acids (1 mM L-ala, L-cys, L-nle, L-glu, L-pro and L-arg) did not elicit a change in cAMP levels in this time scale and caused only relatively slow and minor increases in cGMP. Odorant amino acid-stimulation of IP_3 formation was GTP-dependent and was inhibited by GDPβS suggesting that the response was G-protein mediated. To determine the effect of the increase in IP_3 concentration on membrane conductance we studied second messenger-modulated conductances in isolated catfish olfactory neurons using the whole cell mode of the patch clamp technique. We found that these neurons possess both cAMP and IP_3-modulated conductances that differed on the basis of pharmacology and voltage dependence. These experiments support a hypothesis according to which odorant amino acids exert their effect through an odorant-stimulated increase in IP_3 formation which causes opening of a ciliary IP_3-gated channel (Huque and Bruch, BBRC 137:36, 1986, Restrepo et al, Science 249:1166, 1990, Kalinoski et al, Biochemical J. 281:449, 1992), and indicate that cAMP may play a role in olfactory transduction in catfish for non-amino acid stimuli.

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An $InsP_3$ (Inositol-1,4,5-trisphosphate) Receptor is Localized to the Ciliary Surface Membrane in Olfactory Sensory Neurons and may Mediate Odorant-Induced Signal Transduction.

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Recent evidence suggests that in the olfactory sensory neuron the phosphoinositide (PI) second messenger system plays a role in odorant-induced signal transduction in addition to the adenylyl cyclase (AC) cascade. Using primary cultures of rat olfactory receptor neurons (ORNs) we have assayed components of both systems in intact cells. The five odorants previously shown to augment AC [Ronnett et al., *PNAS*, 1991, 88:2266-2269] were found to also enhance PI turnover. Maximal production of inositol phosphates (IP) occurred within 1 second of odorant exposure and this response was found to desensitize upon repeated exposure to odorant. $InsP_3$ and odorants also augmented calcium^{45} flux in isolated cilia, suggesting a physiological role for IP production in olfaction. To further clarify the role played by $InsP_3$ in the transduction process, we studied the immunohistochemical localization of the $InsP_3$ receptor and compared it to components of the cAMP cascade, G_{olf} and type III adenylyl cyclase. $InsP_3$ receptor was highly enriched in the olfactory cilia, the primary site of chemotransduction. All neuronal forms of the $InsP_3$ receptor previously described have been found to be associated with the endoplasmic reticulum. However, by immunogold EM we localized $InsP_3$ receptor to the surface membrane of cilia, both the proximal and distal parts. Our results are consistent with the previous report of a channel in catfish cilial membranes [Restrepo et al., *Science*, 1990, 249:1166-1168]. These findings raise the possibility that this $InsP_3$ receptor represents a surface membrane channel which opens in response to the binding of an odorant ligand to its receptor. The putative odorant receptors [Buck & Axel, *Cell*, 1991, 65:175-187] are members of a large family of 7-transmembrane receptors. We are now interested in examining interactions between the PI and AC signalling pathways, such as whether an individual odorant receptor is able to activate both second messenger cascades.

Single Channel and Immunochemical Evidence for Inositol 1,4,5-trisphosphate as a Second Messenger in Lobster Olfactory Neurons. FADOOL, D.A. and B.W. ACHE (Whitney Laboratory and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086).

It was previously demonstrated that inositol 1,4,5-trisphosphate (IP_3) activated a GTP- dependent inward current. We now report that IP_3 applied to the inside face of cell-free patches of membrane activated two types of channels in 39 of 47 patches. One channel had a slope conductance of 30.0 ± 1.6 pS (n=16) with Pr_{open} independent of voltage between -90mV and 60mV. In symmetrical solutions this channel reversed at 0 mV. The second channel had a slope conductance of 73.7 ± 5.7 pS (n=12), strongly inactivated at voltages greater than 0 mV, and reversed at 30 mV in symmetrical solutions. Both channels had "flickery" kinetics with mean open times best fit by a double exponential; t_1 = 0.45 ± 0.07 msec and t_2 = 5.47 ± 1.6 msec. Neither channel inactivated under continuous stimulation. The Pr_{open} increased in a dose-dependent manner between 2.4×10^{-7} and 2.4×10^{-4} M IP_3. Pr_{open} also increased with duration of IP_3 application (500 msec to 5 sec) where Pr_{open} saturated at 0.51 at -60mV. Pr_{open} was independent of up to 50mM ATP. Both channels were completely and reversibly blocked by 10 μM ruthenium red or 2.5 μM heparin applied to the internal face of cell-free patches. In one cell it was possible to calibrate an inside-out patch of membrane to IP_3 and then "cram" it into a second cell. Applying an excitatory odor mixture to the bath subsequently activated a channel with properties identical to the one in the control calibration. Immunocytochemical evidence suggests that these channels are expressed in lobster olfactory receptor cells *in situ*. Confocal microscopy of 2 μm thick frozen sections of the olfactory organ showed immunolabeling of the outer dendrites with an antibody directed to the 19 amino acid carboxyl terminal of a cDNA clone of the IP_3 receptor in rat cerebellum (generously supplied by Dr. Pietro DeCamilli). This IP_3 receptor antibody (αIP_3-R) recognized a band greater than 200kDa in purified membrane preparations of mouse brain, lobster olfactory dendrites, and 36h cultured olfactory neurons, but not in preparations of lobster brain. A second band ≈ 100 kDa was also uniquely labelled in the dendrites. In double-patch experiments of cultured cells voltage-clamped in the whole-cell configuration, the addition of αIP_3-R to the patch pipette selectively increased odor-evoked inward current an average of 427 ± 48% (n=5). The antibody failed to alter the odor-evoked outward current (n=9). Collectively these data support IP_3 as a second messenger mediating excitatory transduction in lobster olfactory neurons and suggest that IP_3 acts through direct activation of the channel without the requirement of phosphorylation.

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cAMP Mediates the Odor-evoked Inhibitory Conductance in Lobster Olfactory Receptor Cells. W.C. MICHEL, D.A. FADOOL and B.W. ACHE. (Whitney Lab. and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086)

Lobster olfactory receptor cells support both odor-evoked excitatory and inhibitory conductances. While inositol trisphosphate has been implicated as the second messenger mediating excitation (Fadool *et al.*, AChemS XIII, XIV) the transduction pathway underlying the inhibitory conductance is unknown. Here we present evidence implicating cAMP as the second messenger mediating odor-evoked inhibition. Forskolin stimulation of adenylyl cyclase (AC) elicited an inhibitory (outward) current in 68% of the cells tested (n=27). Phosphodiesterase (PDE) inhibition with 3-isobutyl-1-methylxanthine (IBMX) also elicited an inhibitory response in 79% of the cells tested (n=33). The magnitude of the forskolin and IBMX evoked currents were *ca.* 50% and 85% of the odor-evoked responses, respectively. A mixture of forskolin and IBMX elicited a current that was 178% of the odor-evoked current (n=6). The response to an inhibitory odorant was larger if co-presented with either forskolin or IBMX. Continuous superfusion with IBMX, however, resulted in a persistant outward current and elimination of the odor-evoked response. Membrane permeant analogs of cAMP and cGMP, 8-bromo-cAMP and 8-bromo-cGMP, also elicited outward currents. Introduction of GTP-γ-S and GDP-β-S through the patch pipette into cultured receptor cells increased and decreased, respectively, the magnitude of the odor-evoked inhibitory responses implicating a G-protein in the inhibitory transduction cascade. Preliminary single channel data obtained from cultured olfactory receptor cells suggest that cAMP directly activates a potassium conductance. This study provides direct evidence that cAMP is an olfactory second messenger in an invertebrate.

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Properties of Cyclic Nucleotide-gated Channels in Rat Olfactory Receptor Cells

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The properties of cyclic nucleotide-gated cation channels were studied in membrane patches excised from the apical knob of rat olfactory receptor neurons. Apical membrane patches usually contained high channel densities, with peak conductances of up to 10 nS, whereas those from the soma contained few or no channels. Channels were activated by both cAMP and cGMP, with activation constants at -50 mV of 4.0 µM for cAMP and 1.8 µM for cGMP. Hill coefficients of dose-response curves were 1.3 - 1.8, indicating cooperativity of ligand binding. The equilibrium selectivity sequence to monovalent alkali cations was: Na (1) > K (0.81) > Li (0.74) > Rb (0.60) > Cs (0.52), indicating a relatively high field strength site. The cAMP-induced current was inhibited by cytosolic acidification with an apparent $K_{1/2}$ at pH 5.1. Bath application of amiloride inhibited the current in 9 out of 11 inside-out patches. The block was voltage-dependent and at -50 mV required 1 mM for complete inhibition. The cAMP-induced currents in inside-out patches were also blocked in a voltage-dependent manner by bath application of Ca and Mg ions. At -50 mV, between 1 - 3 mM Ca or Mg was required for 50% inhibition. Both of these ions also appeared to be slightly permeant through the channels. These results demonstrate a high degree of functional similarity with the cyclic nucleotide-gated channels of the frog (Frings and Lindemann (1991) *J.Gen.Physiol.* 98:17a), but have marked differences with those from the salamander (Zufall *et al.* (1991) *Proc.R. Soc.Lond.B* 246:225).

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Protein Kinase C Sensitizes Olfactory Adenylate Cyclase

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The role of protein kinase C (PKC) in signal transduction was investigated in frog olfactory epithelium. Spike rate and response duration after stimulation with the odorant eugenol (1 µM), but not basal spike rate, were significantly increased by application of 0.5 µM phorbol-dibutyrate (PDBu), an activator of PKC. In intact epithelium, odorant-stimulated, but not basal accumulation of cAMP was enhanced by a factor of 5.5 (SEM=1.1, n=4) in the presence of 0.5 µM PDBu. When tissue was stimulated with forskolin (0.3-100 µM), PDBu (0.5 µM) potentiated the measured cAMP production (RIA) by a factor of 2.8 (SEM=0.3, n=22) at all forskolin concentrations tested. This potentiation was suppressed by inhibitors of PKC (0.1 µM staurosporin, 0.25 mg/ml polymyxin B, 1 µM Goe 16). At forskolin concentrations below 1 µM, PDBu had no effect on cAMP accumulation. The diterpene mezerein (0.5 µM), a PKC activator without phorbol structure, also induced potentiation of cAMP production in the presence of 10 µM forskolin (factor 2.2, SEM=0.4, n=4), without affecting basal production. The Mezerein effect was blocked by 1 µM Goe 16 (n=3). Increasing cell Ca by 2 µM ionomycin (1 mM Ca in the bath) potentiated forskolin-induced cAMP production by a factor of 2.6 (SEM=0.9, n=3), while ionomycin only slightly increased basal cAMP accumulation. When cell Ca was lowered by 1 mM BAPTA, cAMP production was blocked and no longer increased by forskolin. These results suggest that PKC enhances the sensitivity of olfactory adenylate cyclase, possibly, as in other systems, by phosphorylating its catalytic subunit.

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Modulation of Voltage-Gated Ionic Currents in Channel Catfish Olfactory Receptor Neurons

T. IVANOVA AND J. CAPRIO. (Louisiana State University)

Voltage-gated currents of olfactory receptor neurons (ORNs) in the channel catfish, *Ictalurus punctatus*, were investigated with the whole-cell patch-clamp method. ORNs were isolated from the olfactory mucosa mechanically without enzymatic treatment. Depolarizing pulses (20 mV increments) were applied to the soma membrane under voltage clamp from holding potentials of -80 mV, -100 mV and -120 mV. These depolarizing pulses elicited fast inward currents followed by delayed outward currents. The inward transient currents reversed near +50 mV as expected for sodium currents. Steady-state inactivation studies indicated that the mean voltage for half inactivation was ca. -80 mV, comparable to that for ORNs of the grass frog (Pun and Gesteland, 1991). With more negative holding potentials, both the activation of the inward currents and their maximum amplitudes shifted to more negative potentials while the activation of the outward currents was not affected. At holding potentials of -80 mV, inward currents began to activate at -60 mV to -50 mV, while at both -100 mV and -120 mV inward currents activated at -80 mV to -70 mV. Outward currents began to activate at -50 mV to -40 mV irrespective of the change in holding potentials. The application of the amino acid mixture (0.25 mM, L-Ala, L-Arg, L-Glu, L-nVal) during the depolarizing pulses at each of the three holding potentials resulted in a further shifting of the peak amplitudes of the inward currents to more negative potentials. This effect was never accompanied by a shift in the reversal potential and occurred despite the fact that amino acids did not directly activate inward or outward currents in these cells. The shift in the maximum current of an ORN at a particular holding potential in the presence of amino acids was similar to the shift that occurred at a -20 mV more negative holding potential without the presence of the stimuli. In some ORNs, the amino acid mixture affected the inactivation of the outward currents. Preliminary data suggest that individual amino acids alter the voltage-gated currents differentially.

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Reduction of Olfactory Neuron Adenylate Cyclase Activity by Bromocriptine In Vitro

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D2 dopamine receptors are present in relatively high concentrations in both the nerve layer and the glomerular layer of the olfactory bulb (Nickell et al., 1991); this suggests that these receptors are produced in olfactory sensory neurons and are localized on their terminals. In general D2 receptors in other neurons inhibit the enzyme adenylate cyclase. Because this enzyme is present in very high concentrations in olfactory neurons we investigated the effect of a D2 agonist, bromocriptine, on forskolin stimulated adenylate cyclase activity. This was done *in vitro* using olfactory membrane preparations from normal and unilaterally bulbectomized (OB-X) adult rats. In unoperated animals bromocriptine (5.0-20.0 µM) significantly inhibited forskolin-stimulated AC activity. To determine if this effect was specific for neurons in olfactory epithelium we made membrane preparations from OB-X animals 4 days after surgery. In these preparations we saw significantly lower AC levels and a decrease in response to bromocriptine on the bulbectomized side. Our data indicate that bromocriptine lowers AC activity in olfactory tissue and this decrease is probably specific for olfactory neurons.

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Molecular characterization of degradative enzymes associated with the olfactory organ of the spiny lobster.
HENRY G. TRAPIDO-ROSENTHAL, KARL F. HOFFMANN, MARGARET O. JAMES, JAMES C. NETHERTON, RICHARD A. GLEESON, and WILLIAM E.S. CARR. (Whitney Laboratory, University of Florida, St. Augustine, Florida).

Each olfactory sensillum of the spiny lobster, Panulirus argus, contains the dendrites of several hundred chemosensory cells, and processes of a number of auxiliary cells. Biochemical studies have shown that these sensilla contain extracellular enzymatic activities that dephos-phorylate the nucleotide odorants AMP, ADP, and ATP. Sensilla also have an enzymatic activity that metabolizes the cytochrome P-450 substrates progesterone and 7-hydroxycoumarin when these compounds are present in seawater. Messenger RNA (mRNA) isolated from the lobster's olfactory organ was used to construct a cDNA library in the vector λZAPII. The library was screened for cDNAs that code for ectophosphatases and cytochrome P-450s by means of the polymerase chain reaction (PCR). When PCR was performed using primers based on the conserved N-terminal amino acid sequence of 5'-ectonucleotidases from vertebrate sources, and the T7 promoter site of the vector, six cDNA sequences were amplified, ranging from 200 to 1500 base pairs. When PCR was performed using primers based on the N-terminal amino acid sequence of lobster hepatopancreas cytochrome P-450 and the T7 promoter sequence, a 600 base-pair product was amplified. Sequencing of this PCR product revealed a region showing 61% homology with cytochrome P450IIA from mouse, rat, and human sources.

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Cross-Adaptation of Spiking Responses of Individual Olfactory Receptor Cells of Spiny Lobsters Reveals Multiple Receptor Sites and Shared Excitatory Transduction Processes.
JACQUELINE FINE-LEVY, CHARLES DERBY, PETER DANIEL, AND M.-N. GIRARDOT (Georgia State University)

The intent of this study was to determine the relative independence of receptor sites and excitatory transduction processes for chemical stimuli in the peripheral olfactory system of the spiny lobster *Panulirus argus*. Single-unit spiking responses of 116 olfactory receptor cells in the lateral antennular filament were extracellularly recorded subsequent to presentation of a set of 7 odorant compounds (adenosine 5'-monophosphate, ammonium chloride, betaine, L-cysteine, L-glutamate, DL-succinate, and taurine). Individual receptor cells often had narrow excitatory response spectra, but the most excitatory compound was different from cell to cell. These results suggest that these compounds can exert most of their excitatory effects through relatively independent receptor site types. This conclusion is also supported by direct biochemical measurements of odorant-receptor binding (see Abstract by Olson et al.). To determine the relative independence of excitatory transduction processes for these stimuli, spiking responses of these olfactory receptor cells under conditions of self- and cross-adaptation were analyzed. The results demonstrate extensive cross-adaptation between pairs of the seven stimuli. When averaged across all cells and all cross-adaptation conditions, cross-adaptation resulted in an mean reduction of 86% of the unadapted response. However, there were differences in the degree and pattern of adaptation for different pairs of compounds and for different cell types. Additionally, in some cases, there were asymmetries between cross-adaptation efficacies for given pairs of compounds. Together, these results suggest that these seven compounds bind to relatively independent receptor site types, but that they share excitatory transduction processes at some point subsequent to odorant-receptor binding.

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Towards a coding strategy in the fish olfactory system
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Information processing in the olfactory system is still poorly understood. It is not known to what extent differences in odorant structure are coded already at the level of the olfactory epithelium. Alternatively, coding may occur by sorting out of projections at the level of the olfactory bulb. As a contribution towards elucidation of the odorant coding strategy, we are currently cloning zebrafish odorant receptor molecules to obtain probes for determining the spatiotemporal pattern of odorant receptor expression. Odorant signal transduction is mediated by G proteins, and G protein-coupled receptors share several highly conserved sequence motives. We choose oligonucleotides corresponding to regions from membrane-spanning domains three and seven to amplify genomic DNA via PCR. Amplified fragments were cloned and grouped by restriction enzyme digestion and T track sequencing. Fragments with differing patterns were sequenced. Sequence comparison with rat odorant receptor molecules [Buck and Axel, Cell 65, 175 ,1991] and other G protein-coupled receptors allowed the identification of eight different putative odorant receptor fragments. Amino acid homologies between any two members of this group range between 20 and 85% . Concurrently we analyse the degree of ordering in the anatomical projection pattern of olfactory receptor cells into the olfactory bulb with retrograde and anterograde tracing of the fluorescent dye DiI.

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The relation between the number of classes of primary olfactory neuron and the ability to distinguish the components of a mixture.
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Identification of odors is thought to depend upon the selectivity of classes of primary olfactory neurons (PONs). Neither the number of these classes nor their pattern of selectivity is known. If the response of each class of PON to an odor can be characterized by a single parameter, then the only information about the odor that is available to the brain is contained in these numbers. At sufficient odor concentrations, the noise due to transduction mechanisms may be negligible and a small number of PON classes would be sufficient to discriminate among a large number of odors. However, if the nose is presented with a mixture of two familiar odors, the response pattern of the PON classes will differ from both familiar patterns. The difference between the response pattern to the mixture and the response to the individual odors is essentially "noise." The ability of the olfactory system to recognize a previously presented odor in a mixture of odors is constrained by this "noise," which is inherent in the nature of olfactory transduction. It is probable that the ability to distinguish biologically significant odors in mixtures is a major objective of central olfactory processing. To investigate the capacity of plausible central processing mechanisms to recognize the components of mixtures, we simulated the responses of PONs to hypothetical odors. In this simulation, each of 100 classes of PON was randomly assigned an affinity for 100 odors. A non-linear model of the relation between odor concentration and PON activity determined the neural firing rate for any of the classes of PON for any concentration of the 100 "odors" and for mixtures of the "odors." The neural responses to each of the "odors," presented at a standard concentration, was calculated and stored as a matrix. This matrix simulated the memory of previously presented odors. We then simulated the presentation of a "test" odor by calculating the neural responses to one of the odors presented at a different concentration or mixed with a second odor. The behavioral task of odor recognition was simulated by determining whether a matching procedure (linear correlation) could select the correct match between the generated "test" response and the "remembered" response. If the number of classes of PON was relatively large (>80) linear correlation reliably distinguished both components of a mixture when one component was 1/2 the other. For smaller numbers of receptor classes, spurious correlations with unrelated odors made identification unreliable.

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A Model to Explore the Relationship Between Olfactory Receptor Cell Specificity and Mitral Cell Response. DAVID A. BERKOWICZ, *KENSAKU MORI AND GORDON M. SHEPHERD (Section of Neurobiology, Yale University School of Medicine, New Haven, CT 06510 and *Osaku Bioscience Institute, Osaka, Japan)

Olfactory receptor proteins are members of a large multigene family (Buck & Axel, 1991). This has raised many questions regarding odor molecule:olfactory neuron interactions. For instance, do olfactory receptor neurons express more than one type of receptor? Do odor receptors show different affinities for different odors? How do these variables affect mitral cell activity? Our approach to these problems has been to construct computational models of olfactory neurons, and connect them to a model mitral cell via multiple synapses. Each cell incorporates the known electrotonic, electrophysiological and morphological information. The model epithelium consists of a hundred olfactory neurons; each neuron consists of 5 compartments, containing an odor ligand gated conductance sited in the cilia (Firestein et al., 1990; Lowe & Gold, 1991) and voltage-gated Na^+ and K^+ conductances placed in the soma. The ligand-gated conductance displays a differential response to stimuli based upon the number of C-atoms in the fatty acid side chain (Mori et al., 1992). Each olfactory neuron is thus capable of responding independently and in a manner that reflects the affinity of its receptor for structurally related odor molecules. The mitral cell has compartments for synaptic inputs and outputs and for voltage-gated conductances. The temporal aspects of the afferent input to the glomerular dendrites can be precisely controlled, as can the time course of the synaptic current. The model allows parameter manipulation and variation through menu driven instructions and so permits for the rapid testing of hypotheses relating to the nature of olfactory neuron 'tuning', mitral cell receptive fields and glomerular specificity.

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A Computational Model of Adaptation and Disadaptation in Chemoreceptor Cells: Consequences for the Coding of Temporal and Intensity Patterns in Odor Signals. PAUL A. MOORE (Department of Pharmacology, University of Colorado Health Sciences Center, 4200 East Ninth Ave., Denver, CO 80262)

The environmental stimulus for olfactory receptor cells is a turbulent odor plume. Due to turbulent dispersion, odor signals arriving at chemoreceptor cells are spatially and temporally dynamic. Odor concentrations can fluctuate widely within discrete bursts and individual bursts are very intermittent and unpredictable. Chemoreceptor cells have the temporally dynamic properties of adaptation and disadaptation that serve to alter their responses to these various environmental odor patterns. A computational model, modified from a previously published model (Moore and Atema, 1988) was used to investigate the effect that both adaptation and disadaptation had on the spiking output of olfactory receptor cells under realistic environmental stimulus conditions. The response characteristics of model cells were based on electrophysiology work on the dose-response, adaptation, disadaptation, and flicker fusion properties of peripheral olfactory cells. The individual physiological properties of the cell (adaptation and disadaptation rate and the dose-response relationship) could be modified independently from each other, which allowed assessment of the role of each of these properties on the responses of the model cell. Complete adaptation and disadaptation time courses ranged from 100 ms (Fast cells) to 10 s (Slow cells). The stimuli for the cells were quantified odor plume recordings done under a variety of biologically relevant flow conditions. As expected, the Fast cells had different response characteristics than the Slow cells to identical temporal odor profiles. Responses of the cells depended upon their time constants and the frequency characteristics of the odor presentation. These results indicate that adaptation and disadaptation serve to set a window of intensity fluctuations that are the best stimulus for a particular cell. These properties function as an olfactory equivalent of a band-pass filter in electronics. This type of filtering has implications for the extraction of information from odor signals, such as the coding of temporal and intensity features.

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Time course of electrical responses and blood flow changes recorded at the human nasal respiratory mucosa induced by painful chemical stimulation

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Negative mucosal potentials (NMP) and blood flow changes can be recorded from the human nasal mucosa after stimulation with chemical irritants. In order to exclude the NMP being an epiphenomenon of induced blood flow changes both signals were measured in eight healthy volunteers under different stimulus conditions. During the first part of the experiment painful CO2-stimuli of different durations (500, 1000, 2000 ms; 52 % v/v) were presented. During the second part CO2-stimuli of different concentrations but constant duration were presented (45 %, 52 %, and 59 % v/v; 1000 ms). Subjects rated stimulus intensities using a visual analogue scale. NMPs were recorded with tube-electrodes from the surface of the respiratory epithelium. Measurements of changes in blood flow at the recording position of the NMP were made using a laser Doppler blood flow monitor (time constant 0.1 s). Whereas amplitudes and areas under the curve of the NMP significantly increased with increasing stimulus concentrations and were closely related to intensity estimates blood flow only marginally changed in relation to stimulus parameters under these experimental conditions. Onsets of the NMP (546-784 ms) shortened with rising stimulus concentrations, whereas latencies of the maximum NMP-amplitude only increased with increasing stimulus durations. In comparison to the early onsets of the NMP blood flow changes were recorded with significantly longer latencies (3270-4377 ms). Based on this result we conclude that the NMP does not represent an epiphenomenon of preceding or coinciding blood flow changes. The close relationship between intensity estimates and the NMP parameters characterize the NMP as a nociceptive response useful in quantifying trigeminal activitation by chemical stimuli.

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Nasal Pungency and Odor from Nonreactive Airborne Chemicals.

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A group of clinically diagnosed anosmics served in an experiment looking at nasal detection (pungency) thresholds for homologous ketones and selected secondary and tertiary alcohols and acetates. A group of age-, gender- and smoking-status-matched normosmics provided odor thresholds for the same substances. Both types of thresholds decreased with carbon chain length, as seen before for homologous alcohols (Physiol. Behav. 48(5) 719-725, 1990) and acetates (Pharmacol. Biochem. Behav. 39(4) 983-989, 1991). All the relatively nonreactive substances studied so far elicit nasal pungency - as perceived by anosmics - at a fairly constant percentage of vapor saturation (≈32%) irrespective of molecular size or chemical function. As before, the outcome for pungency implies an important role for a physical, rather than chemical, interaction with the nasal mucosa. It also shows that low volatility, high molecular weight compounds (octanol, octyl acetate, nonanone) not generally thought of as irritants do evoke pungency and act at lower concentrations than highly volatile members of the respective homologous series (methanol, methyl acetate, acetone). An emerging issue is the possibility that chemicals of the low-volatility type might elicit pungency at substantially lower concentrations in complex mixtures and over repeated exposures. This has importance both for the mechanism of nasal pungency and for the understanding and validation of symptoms from polluted indoor environments where no single substance accounts for the symptoms observed.

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Monorhinal stimulation as a method for differentiating between thresholds for irritation and odor. CHARLES J. WYSOCKI, BARRY G. GREEN and THOMAS P. MALIA (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104)

Preliminary experiments on human sensitivity to the irritation caused by intranasal carbon dioxide (CO_2) revealed possible odor confounds. This is consistent with reports of olfactory nerve responses to CO_2 (Ottoson, 1956; Coates & Ballum, 1990). In an effort to eliminate olfactory stimulation as a possible contributor to irritation measurements, it was decided that thresholds for localizing a monorhinal stimulus within the nasal cavity would be used to indicate irritation thresholds. When two air streams are each directed to separate nostrils, but one contains an odorant, localizing the stimulus is possible only when the odorant also stimulates intranasal trigeminal fibers (Kobal, Toller, & Hummel, 1988). In the present study, clean air or air with CO_2 was delivered to each nostril simultaneously. Subjects were asked to indicate the side receiving the CO_2 from the irritation that it produced. Although our methods differ from those used previously, the results revealed thresholds for irritation that are consistent with expectations, given the limited data available in the literature. These similarities include a trend towards lower thresholds for females. Research is continuing to determine possible differences between thresholds for simple detection of CO_2 (by mechanisms not yet understood) and thresholds for localizing the irritation produced by intranasal CO_2, as well as other odorants, in both anosmics and normals. It is anticipated that these procedures may be used to evaluate the relative thresholds for olfactory and trigeminal components of odorants, and may provide a new standard for the evaluation of subjects as anosmic.

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Capsaicin Cross-Desensitization with Zingerone: Evidence that Desensitization is Accelerated by the Removal of Capsaicin.
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A remarkable feature of capsaicin desensitization on the tongue is that it can develop within minutes, but only if stimulation is interrupted; if capsaicin continues to be applied, sensitization rather than desensitization tends to occur. The simplest explanation for the necessity of an interruption in stimulation is that desensitization cannot take place as long as the affected fibers remain active. An alternative explanation, which seemed paradoxical and therefore less likely, was that the continued presence of capsaicin itself delayed the onset of desensitization. To differentiate between these two hypotheses, zingerone, a chemical that stimulates but does not desensitize capsaicin-sensitive fibers, was used as a probe for desensitization. In the main experiment a series of zingerone stimuli was presented immediately after a series of capsaicin stimuli; the object was to see if desensitization would be delayed, as it is when capsaicin stimulation is continued, or would begin to develop almost immediately, as it does when capsaicin stimulation is stopped. Subjects therefore rated the perceived irritation produced by a zingerone test stimulus after 10 capsaicin (10 ppm) stimuli were followed by either no stimulation for 10 min (the interrupted condition) or by 10 equi-intense zingerone (1%) stimuli (the stimulated condition). All stimuli were applied to the tip of the tongue on filter paper disks. Desensitization occurred in both conditions: most importantly, the perceived intensity of the zingerone stimuli, initially even higher than the perceived intensity of the capsaicin stimuli, fell nearly to zero by the last exposure. The induction of desensitization during continuing stimulation with zingerone rules out the possibility that rapid desensitization cannot occur while capsaicin-sensitive fibers are being stimulated. Instead, the results imply that although capsaicin must be present to initiate the desensitization process, thereafter desensitization is accelerated if capsaicin is removed.

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Rapid interactions between proline-rich proteins (PRPs) and 5-caffeoylquinic acid (5-CQA): A spectroscopic study of oral astringency.
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MICHAEL N CLIFFORD (University of Surrey, U.K.)
GORDON G BIRCH (University of Reading, U.K.)

Astringency is a sensation of taste, generally recognized as a feeling of dryness, constriction and puckeriness, experienced as a diffuse stimulus in the mouth. It has been demonstrated that tannins (phenolic compounds, which elicit the sensation) have a considerable affinity for proline-rich proteins (PRPs) such as gelatin and those which occur in saliva. By analogy with vegetable tanning it has been assumed that sensory astringency is caused by the precipitation of these proteins in the mouth, with concomitant loss of their lubricating effect. However, this has not been demonstrated, nor has the involvement of a specific taste receptor definitely been excluded. The present two-part study investigates the rapid structural changes which follow the interaction of a phenolic compound known to be astringent, 5-CQA, with PRPs, in an attempt to ellucidate some of the mechanisms through which oral astringency may be mediated. The sources of PRPs used in the first and second part of the study were gelatin, and freeze-dried pooled human saliva, respectively. The analytical technique chosen was a sensitive means of monitoring the structural transitions expected to occur upon PRPs/5-CQA interactions. UV difference spectroscopy was used, under controlled pH and temperature conditions. The results to be reported are interpreted as indicators of the reactions that may take place in the mouth, within the time-course of the perception of astringency.

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Y-Chromosomal Influence on Chemosignal Production and Olfactory Discrimination Affecting Urine Marking in Mice.
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Previous research from our laboratory has suggested evidence for chemosignal-dependent and -independent effects of Y-chromosomal gene(s) on aggression in mice. For the two Y-chromosome-congenic strains studied, DBA1 responded more aggressively when the genotypes of the opponents and the urine daubed upon them were concordant. This response was independent of genotype. In contrast, DBA1.C57BL10-Y did not show this differential response toward members of its own genotype. In the present study these two strains were tested for their urine marking responses when presented with the odors of another DBA1 mouse or its urine, another DBA1.C57BL10-Y mouse or its urine, or nothing. It was found that the only significant difference occurred when DBA1.C57BL10-Y was presented with the odors of another DBA1.C57BL10-Y mouse. Considered *in toto*, these data suggest that the Y chromosome may affect chemosignal production and olfactory discrimination differently in terms of how they relate to two different reproductive strategies.

Individual discrimination in hamsters: sources of cues and role of vomeronasal system.

ROBERT E. JOHNSTON (Cornell University)

Most examinations of individual discrimination by scent have used either whole body odors or one particular source of scent, most often urine. In order to really understand this process and the underlying mechanisms, however, we need to know all of the sources of individual odors for a given species. Using an habituation technique we have investigated eleven scents as possible sources of individual identity information. Hamsters discriminated the differences between individuals on the basis of five of these scents - flank glands, ear glands, urine, feces and vaginal secretions; they did not make such discriminations using the scent of saliva, feet, chest fur, back fur, behind the ear, or flanks of flank glandectomized males. Thus individual signatures are located only in discrete locations on the body. Contrary to expectations grooming does not distribute such information to other locations. This discrete distribution pattern suggests evolutionary specialization of scent sources for this communication function. Preliminary experiments suggest that lesions of the vomeronasal system disrupt discrimination of individual differences, but only to one of the relevant scents, namely vaginal secretions. Thus individual discrimination appears to be mediated by both vomeronasal and olfactory systems; the system used depends on which scent is being investigated. Implications for the evolution of vomeronasal and olfactory system functions will be discussed.

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The Effects of a Conditioned Taste Aversion on Gustatory Evoked Activity in Rat NTS Remain After Behavioral Extinction

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Chang & Scott (1984) reported modification of gustatory evoked activity in the rat NTS as a consequence of a conditioned taste aversion (CTA). We investigated whether this modification would be lost with the complete behavioral extinction of the aversion. Rats were conditioned to avoid .0025 M sodium saccharin (CS) by pairing its taste three times with i.p. injections of 128mg/kg LiCl. The aversion was then extinguished over a period of weeks by daily presentations of the CS when the rats were 23-hour fluid deprived. A control group received physiological saline injections. After rats consumed the CS at pre-illness levels, responses of single units in the NTS were recorded to the CS plus an array of salts, acids, sugars and quinine. The stimulus space from the control group presented a typical distribution of chemicals, with the CS among the sugars. In the CTA-extinguished group, the CS shifted decisively away from the sugars toward salts and quinine. This change in location of the CS was similar to that reported in rats with intact CTAs. Therefore, the effect of an intense aversion on the taste system remains even after the rat has regained full acceptance of the CS. This vestige of the initial conditioning may account for the ease with which relearning occurs following extinction.

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The Role of Parabrachial Lesions in the Disruption of Conditioned Taste Aversions

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Three experiments investigated the process by which electrolytically-guided bilateral lesions of the pontine parabrachial nucleus (PBN) disrupt acquisition of a conditioned taste aversion (CTA) in rats. Experiment 1 demonstrated that rats with lesions of the PBN failed to learn to avoid either a gustatory or an olfactory cue that had been followed by lithium chloride (LiCl) poisoning (sham taste mean=2.0 ml, lesion taste mean=22.8 ml; sham odor mean=3.1 ml; lesion odor mean=15.4 ml). Experiment 2 provided evidence that the failure to learn a CTA probably was not due to an inability to process CS information because both sham (CS+ mean=23.4 ml; CS- mean=5.2 ml) and lesioned (CS+ mean=23.5 ml; CS- mean=9.8 ml) rats learned to prefer a non-nutritive orosensory stimulus (orange or grape Kool-Aid) when it was paired with another, nutritive one (sucrose). Experiment 3 indicated that the failure to learn either a CTA or an odor aversion was not due to an inability to respond to the effects of the US (LiCl). Specifically, all of the sham animals and more than half of the lesioned subjects learned to avoid the preferred side of a shuttle box after that side was paired with LiCl injections (sham mean=141.2/900 sec; lesion mean=154.8/900 sec). Taken together, these data suggest that the failure to learn a CTA following lesions of the PBN is not due to disruption of CS or US processing per se, but to a failure to form an association between these stimuli.

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Sodium Appetite after Chorda Tympani Nerve Transection in Wistar and Fischer 344 Rats.

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Acute sodium (Na) depletion in rats leads to dramatic increases in intake of hypertonic NaCl solutions, a behavior known as sodium appetite. The importance of signals conveyed by the chorda tympani nerve (CT) in the expression of sodium appetite is unclear. Although the CT is generally viewed as the major pathway for gustatory fibers selective for NaCl, bilateral transection of the CT does not alter need-free NaCl intake in most rat strains. The present study examined the effects of bilateral CT transection on the short and long term response to Na depletion in two rat strains, Wistar and Fischer 344 (F344). Wistar and F344 strains are contrasted with each other because Wistars normally display a NaCl preference in the absence of need while F344s avoid NaCl. We have recently reported that the F344 NaCl aversion is completely reversed by CT transection (Sollars, et al, 1990). In the present study animals (N = 6-8 per group) underwent bilateral transection of the CT (CTX) or sham operations (SHAM). Following recovery animals were injected with furosemide (10mg/kg) or saline and placed on a Na-free diet. Twenty-four hours later rats were given 0.3M NaCl and water; intake was measured for 24 hr. Wistar Strain: While SHAM animals displayed a strong Na appetite which appeared within the first 5 min of testing and lasted throughout the 24 hr test, CTX rats had a delayed and blunted Na appetite which emerged at 15 min and was evident at the 3 hr but not the 24 hr time point. F344 Strain: SHAM animals displayed a significant appetite, which emerged at 1 hr and persisted throughout the 24 hr test. At no time point did depleted CTX animals ingest significantly more NaCl than nondepleted controls. These results suggest that the chorda tympani nerve is important in the expression of a Na appetite in Wistar and F344 rats. Effects on F344 rats are particularly interesting because CTX surgery appears to have opposite effects on NaCl intake depending on whether they are Na replete or Na deplete.

The Contribution of Anterior Tongue and Nasoincisor Duct Taste Receptors in the Behavioral Responsiveness of the Rat to Sucrose. ALAN C. SPECTOR (Univ. of FL.), SUSAN P. TRAVERS (Ohio State Univ.). and RALPH NORGREN (Penn. State Univ.).

Based on electrophysiological studies in rat, the taste buds lining the naso-incisor ducts (NID) appear to be substantially more responsive to sucrose than the receptors of the anterior tongue (AT). The present study examined the relative contribution of these two gustatory receptor fields to the licking behavior elicited by suprathreshold concentrations of sucrose. Water-deprived Sprague-Dawley rats were trained in a specially designed gustometer to lick a drinking spout to obtain 10 sec trials of various sucrose concentrations (0.01 - 1.0 M). These stimuli were randomly delivered during three, 30 min sessions. The number of licks during the latter 8 sec of the trial was used as data. The rats were then given water ad-lib. Three days later the rats were retested in a non-water-deprived state for 3 sessions. Next they received 1 of the following surgical treatments: bilateral naso-incisor duct cautery (NIDX; n=7); bilateral chorda tympani nerve section (CTX; n=7); combined NIDX + CTX (n=6); non-surgical control (n=7). After recovery rats were water deprived and tested for one session as described above. During this session there were no significant differences in the number of licks to water among the groups. The rats were given ad-lib water and retested 3 days later in a non-water-deprived state for 3 sessions. Sigmoidal functions representing the concentration-response relationship in the non-water-deprived state were fit (least squares) to the data for each rat. The change in the area under the curves (before vs. after surgery) was used as the primary measure of altered behavior. Control and NIDX treatment did not significantly change responsivity to sucrose. In contrast, CTX significantly reduced the area under the curve by 17% and CTX+NIDX reduced the area by 33%. In light of electrophysiological experiments, the observation that CTX blunted sucrose responsivity, but NIDX did not, at first appears paradoxical. The same electrophysiological data, however, also may provide an explanation. Most gustatory neurons in the nucleus of the solitary tract (NST) do respond better to sucrose that is applied to the NID rather than the AT. Nevertheless, very few neurons respond only to stimuli applied to the NID, but about half respond only to stimuli applied to the AT. Thus, removing NID taste input alone may have little effect on overall neural activity in the NST, but removing AT input or both AT and NID input will reduce the activity of a far greater number of cells. These data also indicate that the taste receptors that remain following CTX+NIDX surgery play a substantial role in sucrose responsivity.

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Functional Recovery of the Gustatory System Following Peripheral Nerve Crush In The Hamster. M.A. BARRY, D.C. LARSON, and M.E. FRANK. Univ. of Connecticut Health Center, Farmington, CT 06032

Following peripheral nerve damage, gustatory nerves readily regenerate, and there is recovery of taste buds and responsivity of afferent fibers. However, little is known about recovery either at the level of the central nervous system or of behavior. Fluid deprived adult male golden hamsters (experimental animals) were given injections of apomorphine paired with a drink of 0.1 M NaCl, and developed a conditioned taste aversion (TA) specific for sodium salts (test stimuli: 0.1 M NaCl, 0.3 M KCl, 0.1 M sucrose, 0.003 M citric acid). Control animals were given water in place of NaCl, and did not show a TA. The chorda tympani nerves (CT) were then crushed bilaterally (distal to their ganglia) in half of the experimental and half of the control animals. The other animals received sham surgeries. When tested 1-3 weeks after surgery, the experimental animals with crushed CTs did not show a TA to any test stimulus, whereas the sham animals retained the NaCl specific TA. The control animals learned no TA, and showed no specific effect of the surgery itself on drinking behavior. After a survival period of 11 weeks, the experimental animals were again trained, and both the crushed CT and sham animals were able to learn the TA. In order to show that the TA was mediated by regenerated CT nerves (and not other intact gustatory nerves) in the crushed CT group, the CT nerves were cut bilaterally, whereas the sham experimental animals again received sham operations. Only the sham operated animals retained the TA. Thus, normal adult hamsters depend on information carried by the CT to form conditioned taste aversions to sodium salts, and that information can be supplied by regenerated CT nerves following nerve crush.

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Sucrose and Polycose Reduce the Salt Intake of Sodium Depleted Rats. SANDRA P. FRANKMANN, JOHN H. DOKKO, & 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. Last year we presented data which demonstrated that sucrose intake suppresses the depletion induced intake of salt and that the suppressive effect of the sucrose depends on oral rather than postingestive stimulation. The current experiment was designed to test the hypothesis that the suppression is common to preferred taste stimuli and not specific to sweet taste. Polycose (composed of starch derived polysaccharides) is treated by the rat as having a taste which is distinct from sucrose and is thought to interact with a separate population of receptors than sucrose. Male, Sprague Dawley rats (n = 10) were sodium depleted (10 mg furosemide and sodium deficient diet overnight) and the 1 h intake of 0.3M NaCl was measured following 15 min intake of 0.6M sucrose (7.8±0.9 ml), 2% polycose (12.6±2.3 ml) or nothing.

TABLE 1. CUMULATIVE 0.3M NaCl INTAKE, ml (Mean ± SEM)

Pre Test Solution	5 min	15 min	60 min
None	7.1 (±0.6)	13.2 (±1.5)	17.3 (±2.0)
Sucrose	4.2 (±0.8)*	7.8 (±1.0)*	10.9 (±1.4)*
Polycose	4.9 (±1.0)*	8.6 (±1.5)*	10.4 (±1.8)*

* = $p < .05$ vs None

Thus under conditions of sodium depletion both sweet and non-sweet carbohydrate solutions can contribute to the satiation of salt appetite that is normally produced by NaCl. The effect is not specific to sweet solutions. This supports the hypothesis that the satiation of salt appetite depends in part on oral stimulation by pleasant tasting stimuli. When in a state of sodium depletion, this pleasant stimulation can be produced by NaCl, sucrose and polycose. Future studies will extend this finding to other, non-carbohydrate solutions, such as corn oil emulsions, that are also preferred by the rat.

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Distribution of Interspike Intervals in the Responses of Pheromone Receptor Neurons of *Trichoplusia ni*. PAOLA F. BORRONI, ROBERT J. O'CONNELL, and ANGELA M. ZAPATA (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545).

Extracellular recordings from the HS sensilla trichodea on the antenna of male *T.ni* have revealed the activity of two neurons: the "A" neuron, which responds to the major component of the female pheromone blend,(Z7,12:AC) and the "B" neuron which responds to Z7,12:OH. The temporal characteristics of the responses of these receptor neurons to rectangular pheromone pulses were recently described (Borroni & O'Connell, in press): they both showed a fast-adapting phasic burst (~100 ms), followed by a slow-adapting tonic component. Stimuli of increasing concentration and duration effected stronger adaptation of the tonic portion of the responses. A second type of "B" neuron (LR "B") was also described, which lacked a phasic response component and showed virtually no adaptation with prolonged stimulation. Here, we continue our temporal analysis, focussing on the effects of adaptation on the distribution of interspike intervals (ISI) in the response of these receptor neuron types (ie. "A", HR "B", LR "B"). We measured the distribution of ISI during long periods (5 min) of spontaneous activity, and during responses to stimuli of increasing duration (1, 5, and 10s). Unstimulated neurons fire infrequently, with a clear preference for very short ISIs (3-9ms) in both "A" and "B" neurons: typically, bursts of 2-5 closely spaced impulses are interspersed with long periods of silence. Within spontaneous bursts, the ISI could be as short as 3-4ms, whereas the *interburst* interval varies from tens of milliseconds to tens of seconds. Both tungsten and tip recordings from a variety of other studies show that moth pheromone receptors tend to fire in bursts of action potentials. In *T. ni*, stimulation differentially changes the distribution of ISIs within bursts in the different neuron types: in "A" neurons, a significant concentration-dependent lengthening of the preferred ISI from 3-6 to 6-9ms is observed. In HR "B" neurons, the preferred ISI is significantly shortened by moderate stimulus doses, but it is lengthened with higher doses. In LR "B" neurons, the distribution of ISIs within bursts is not changed with any of the stimulus doses. In all of the neurons *interburst* intervals are gradually shortened with increasing stimulus doses. We hypothesize that the lengthening of the ISIs within bursts may be a further concentration-dependent effect of adaptation: as neurons are driven more strongly to produce more frequent bursts of action potentials, brief ISIs cannot be sustained as effectively as in unadapted receptors.

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Detection of Gender-Specific Stimuli by Olfactory Receptor Neurons on the Antenna of *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).

Many Lepidoptera possess specialized glands and structures that produce and release the chemical signals involved in courtship and mating. Both males and females of the ornate moth, *Utetheisa ornatrix* (Lepidoptera: Arctiidae) release complex odors that are detected by members of the opposite sex and serve to coordinate many aspects of their reproductive behavior. Previously, our studies of this communication system focused on the detection capabilities of specialized receptor neurons housed in morphologically distinct sensilla on the antennae of both sexes. *Utetheisa* detect male-produced pheromones with neurons housed in short basiconic sensilla. Neurons in the long trichoid sensilla do not respond to the male-produced pheromone, but do respond differentially to the thoracic froths produced by agitating male and female moths. The neuron producing the larger amplitude action potential (the A neuron) responds to female-produced froth and the neuron producing the smaller impulse (the B neuron) responds to male-produced froth. Here, we continue our investigations of these receptors by examining an additional range of stimuli including: intact animals of different ages, pupae, and molting fluid. These stimuli were selected in an attempt to determine the source of the chemical signals responsible for these dichotomous responses. The A neuron responds to odors swept from the surfaces of intact female moths whereas the B neuron responds to odors swept from male moths. A similar gender-specific pattern was also evident when pupae, the molting fluid produced by adult females, and as previously reported, thoracic froth and blood, were used as stimuli. Newly emerged moths seemed as potent, as stimuli, as older animals. Thus, the active release of thoracic froth is not required to produce an active stimulus animal. Sex attractant pheromones are generally released by active processes, such as gland eversions, compressions, or airings, which are environmentally and physiologically controlled. The stimuli that evoke the gender-specific responses reported here differ from these classic pheromone signals in that they appear to be present during many stages of life and do not seem to be temporally modulated by behavioral context. At present, the ecological significance of these gender specific responses is unclear. However, a number of behavioral conditions could be postulated in which it would be useful for a moth to monitor the sex, proximity, and density of conspecifics.

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Multiple Classes of Insect OBP Provide a Functional and Developmental Model for Olfactory Specificity.

RICHARD G. VOGT (University of South Carolina)

The insect olfactory epithelium consists of a mosaic of functionally defined and morphologically distinct olfactory sensilla, either sex-pheromone specific or general odorant sensitive. Early in adult development, maternal epithelial cells differentiate and divide to produce the cells of each sensillum; respective progeny become the sensory neurons and their glia-like support cells. These cells express genes for the processing of either sex-pheromone odorants or general odorants depending on the sensilla type, which is presumably determined in part by where the maternal cells were positioned in the early epithelium. Furthermore, while the neurons express transductory proteins including receptors etc., the closely related support cells express odorant degrading enzymes and odorant binding proteins (OBP).

Three classes of OBP are expressed in association with different classes of olfactory neuron: pheromone binding proteins (PBP) associate with pheromone specific neurons while general odorant binding proteins (GOBP1 and GOBP2) associate with the general odorant sensitive neurons (Vogt et al., 1991a,b). The insect OBPs represent molecular tools for studying the developmental regulation of olfactory specificity. These proteins allow us to ask what intrinsic factors determine regulation of expression with respect to sex specificity, sensilla and cell type, and time.

Focusing on temporal regulation, we have demonstrated that OBP expression is influenced by circulating levels of the steroid hormone 20-hydroxy ecdysone (20-HE). OBP first appears 36 hrs before adult emergence, during a declining phase of 20-HE levels. Antennae of different developmental stages were cultured for 20 hrs +/- a physiological concentration of 20-HE. Removal of tissue from endogenous steroid induced premature OBP expression. This study suggests that olfactory maturation is regulated by declining levels of steroid, and also that insect olfactory development in general may be hormonally coordinated.

Vogt et al., (1991a) *J. Neurobiology* 22,74-84; Vogt et al., (1991b) *J. Neuroscience* 11,2972-2984.

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Electroantennograms: Computer Analyses of Digitized Waveforms Reveal Differential Characteristics of Receptor Responses to Pheromone Components and Plant Odors

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The electroantennogram (EAG) is a summated slow potential which is recorded from an insect antenna upon stimulation with odorous stimuli. The EAG is similar to the electroolfactogram (EOG) recorded from the olfactory epithelium of vertebrates. Two parameters of EAGs often studied are: 1) magnitude of the depolarisation which is thought to be related to the relative number of responding receptors on the antenna, and 2) decay which may be related, in part, to inactivation or metabolism of odor molecules following stimulation. We report detailed investigations of several additional parameters of EAGs which may be useful in differentiating responses to similar compounds, and dose-related changes in these parameters. EAGs were recorded from the beet armyworm moth, Spodoptera exigua, in response to pheromone components, plant odors, and selected analogs. The parameters considered were "absolute" and "normalized" responses (i.e. depolarisations), "rise" and "decay" at several time intervals (absolute values and values relative to response magnitude), and "level" which measured the increase in the second half of the two second stimulation period relative to the last value in the stimulation period. "Level" was considered to be related to stimulus/receptor interactions and inactivation processes. Our results revealed that values obtained from receiving male moths for both "rise" and "decay" for essential pheromone components differed from those recorded for nonessential components of the female's pheromone emission. "Green leaf volatiles", which were effective behavioral modifiers in moths, elicited EAGs of similar magnitude to those elicited by inactive analogs, but could be statistically separated by differences in "rise". Variability of each of these parameters in dose-response studies will also be discussed.

*Research performed during USDA, ARS Fellowship to J. C. D. Address correspondence to J. C. D. at permanent address: USDA, ARS, BWRU, Mississippi State, MS 39762.

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Frequency Coding in Chemoreceptor Cells.

GEORGE GOMEZ, RAINER VOIGT, and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543).

The lateral filaments of the antennules of the American lobster *Homarus americanus* are important in distance orientation to chemical stimuli in turbulent aquatic odor plumes. To investigate the filter properties of lobster chemoreceptors that might be used in temporal information acquisition, lateral antennules were excised and situated in an open-faced chamber. Chemoreceptor-bearing sensilla were stimulated with 10 pulses of 10^{-4}M hydroxyproline delivered at frequencies of 0.5, 1, 2, and 4 Hz; simultaneous real-time recordings of the stimulus profile (using IVEC-5 and dopamine as a tracer) and the corresponding extracellular receptor responses (spikes) were recorded.

The majority of cells responded to individual stimulus pulses with a phasic spike burst. At stimulation frequencies of 0.5 Hz, receptor cell responses are phase-locked with the pulses and most cells show little cumulative adaptation. At stimulation frequencies of 1 and 2 Hz, half of the cells show reduced responses and increased response latencies while the rest remain phase-locked. At 4 Hz stimulation frequency, most cell responses approximate the response to a sustained square pulse. The averaged cell responses to various frequencies show that the population responds with distinct spike bursts to stimulation frequencies up to 2 Hz.

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Tuning Properties of Chemoreceptor Cells on the Antennae of the American Lobster.

RAINER VOIGT and JELLE ATEMA (Boston University Marine Program, Marine Biological Laboratory, Woods Hole, MA 02543)

The American lobster, *Homarus americanus*, uses its large ("second") antenna in social interactions (antenna whipping, antenna pointing), predator defense (pointing and tracking with antenna) and in food localization (pointing towards a source). While this organ has been considered as a largely mechanoreceptive organ its sensory structures resemble those described as chemoreceptive structures on the lateral (non-aesthetasc) and medial antennules ("first" antennae). In this study we determined the spectral tuning properties of chemoreceptor cells on the antenna with amino acids and other compounds known to be stimulatory to other organs of the American lobster.

Standard extracellularly recording techniques were use to record from single receptor cells. Receptor cells were identified with a mixture of 17 compounds each at 10^{-5} M: alanine, arginine, aspartic acid, glutamine, glutamic acid, glycine, hydroxyproline, leucine, lysine, methionine, proline, serine, taurine, valine, NH_4Cl, betaine, and glucose. Cells were tested with single compounds at 10^{-4} M and 10^{-7} M; stimulus response functions for excitatory compounds were determined in log steps from 10^{-4} M down to threshold.

When tested at 10^{-4} M 37 of 47 cells (78%) responded best to hydroxyproline, 2 to betaine, 4 to taurine and 4 to the mixture; at 10^{-5} M only two cells changed their best stimulus: from mixture to hydroxyproline. With increased concentration hydroxyproline best cells doubled their tuning breadth (H-metric) from 0.24 (10^{-4} M) to 0.50 (10^{-5} M). At both concentrations arginine or leucine were second best stimuli followed by lysine or betaine. Thresholds for hydroxyproline were between 10^{-6} M and 10^{-7} M.

These results indicate that the antennae of the lobster are a major chemosensitive organ dominated by a surprisingly homogeneous cell population narrowly tuned to hydroxyproline. This cell population resembles in its spectral tuning and threshold the hydroxyproline cell populations found on the lateral and medial antennules.

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Resistance to Osmotic/Ionic Stress in the Olfactory Receptor Cells of a Euryhaline Crustacean. RICHARD A. GLEESON[1], LORRAINE M. MCDOWELL[2], HENRY C. ALDRICH[2] and HENRY G. TRAPIDO-ROSENTHAL[1]. ([1]The Whitney Lab. and [2]Dept. Microbiol. and Cell Sci., Univ. of Fla.)

The effects of acute salinity stress on the chemosensory responses of olfactory sensilla (aesthetascs) were compared in the blue crab, *Callinectes sapidus*, a species that ranges from seawater to freshwater, and the spiny lobster, *Panulirus argus*, a stenohaline marine species. Multiunit responses to a general chemical stimulus (Tetramarin) were extracellularly recorded in perfused antennule preparations. Responses to this stimulus were sequentially measured in 100% artificial seawater (ASW), followed by 50% or 25% ASW, and again in 100% ASW. With a 50% reduction in the salt concentration of seawater, the chemosensory response magnitudes for both the crab and lobster are markedly reduced, and upon return to 100% ASW there is only partial recovery of the initial response. It is likely this partial recovery, in part, reflects osmotic/ionic "damage" to the exposed sensory dendrites within the aesthetascs. Recovery in the crab is significantly greater than that for the lobster. Exposure to 25% ASW followed by return to 100% ASW resulted in a complete loss of chemosensory activity in the lobster, whereas the level of recovery in crabs was comparable to that following an exposure to 50% ASW. Our EM studies revealed that although the aesthetascs of *C. sapidus* are similar in certain respects to those of marine species, they also have characteristics that correspond to a "freshwater type" sensillum. It is likely that the ability of the crab's olfactory receptor cells to withstand acute salinity stress is related to this hybrid morphology.

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Depths of Somata in Epithelia Reflect Connectional Status of Most Olfactory Receptor Neurons. PEGGY FARMER, MARIA CROWE, MATTHEW ENNIS, TILAT RIZVI, MICHAEL T. SHIPLEY & ROBERT C. GESTELAND (University of Cincinnati College of Medicine, Cincinnati, OH 45267).

Olfactory receptor neurons (ORNs) differentiate from stem cells close to the basal membrane, extending their dendrites to the epithelial surface and axons to the olfactory bulb. Thymidine-uptake studies show that as these ORNs mature and as new ORNs form, the somata of the more mature ORNs move superficially and their dendrites shorten. This has led to the hypothesis that the somata of ORNs whose axonal projections have reached their olfactory bulb targets lie well above the the basement membrane. To test this hypothesis we retrogradely filled ORNs by injecting 5 μl aliquots of a 1% solution of the conjugate wheatgerm agglutinin-horseradish peroxidase (WGA-HRP) into frog olfactory bulb. After 2-7 days, animals were sacrificed. Olfactory epithelia were removed, fixed and cut into 10-μM-thick coronal sections. In every fifth section the number of somata of filled ORNs and the total number of cell somata were counted. About half of the ORNs were filled. The fill rate was the same in dorsal and ventral portions of the epithelium and in 2-day and 7-day transport animals. Filled neurons constituted the same fraction of the total number of cell somata located in the central 60% of the epithelium. A small number of ORNs with somata in the superficial 20% of the epithelium were filled. The region constituting 20% of the epithelium in proximity to the basement membrane was nearly devoid of filled cells. We conclude that the somata of almost all ORNs that are connected with the bulb are located in the superficial 80% of the epithelium. Few ORNs in the deepest part of the epithelium have axons whose terminals have grown to reach the olfactory bulb. The uniformity of the number of back-filled ORNs over the entire epithelium allows another conclusion. Electrophysiological studies of spike activity in single ORNs all show active epithelial patches interspersed with extensive areas where there is no detectable activity. This can not be due to excitation properties which depend upon bulbar connectivity, since we show that connectivity does not vary significantly in different regions of the epithelium.

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Voltage-Sensitive Dyes Report Differential Odor Responses of Olfactory Receptor Neurons. ROBERT C. GESTELAND, JAN N. BROUWER & PEGGY FARMER (University of Cincinnati College of Medicine, Cincinnati, OH 45267).

Voltage-sensitive dyes appear capable of resolving several questions about stimulus-evoked responses of olfactory receptor neurons. Appropriate dyes allow selective staining of living olfactory receptor neurons and supporting cells. We have worked with styryl dyes RH414, RH 461 and RH 795 in three species of amphibia. With conventional epifluorescence microscopy of slices of olfactory epithelia, odor-evoked changes in fluorescence intensity as small as 0.1% can be reliably detected. These correspond to membrane potential changes of the order of a millivolt. For these dyes, depolarization diminishes fluorescence and hyperpolarization increases it. Video contrast enhancement followed by subtraction of the image before stimulation from the image following stimulation allows detection of changes not discernable with the unaided eye. Pseudocolorization of the difference image dramatically displays the cellular responses. The most significant result from the slice experiments is that when an odor depolarizes a cell (most likely a receptor neuron), a neighboring cell (probably a supporting cell) hyperpolarizes. We think that this is because the depolarized receptor neuron loses potassium to the extracellular medium. Potassium uptake by the supporting cells, which are thought to regulate the epithelial extracellular ionic environment, results in their hyperpolarization. Resolution of this technique is not adequate to resolve cell identities. Laser scanning confocal microscopy resolves this problem. The responses of several hundreds of cells in an *en face* epithelium can be studied individually and simultaneously. Sensitivity to fluorescence intensity changes is comparable for conventional and confocal fluorescence microscopy. Cell selectivity for odors is clearly evident. Different odors preferentially affect small fractions of the neuron population. The time course of the response varies among stimuli. A responding cell is inactivated for an extended period. Stimuli at low concentrations affect fewer cells than at higher concentrations.

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Modulation of Excitability in Frog Olfactory Receptor Neurons (ORNs). RAYMUND Y. K. PUN and ROBERT C. GESTELAND (University of Cincinnati College of Medicine, Cincinnati, OH 45267).

Transduction of odorant signals in the cilia of ORNs involves activation of a G-protein which leads to an increase in cAMP concentration. The cAMP then activates a non-specific cationic channel which induces current flow into the cilia and through the soma, depolarizing the cell membrane. When the threshold for an action potential is reached, spikes are generated and the signal is then transmitted to the brain. Previously we reported that in frog ORNs the voltage for half-inactivation ($V_{1/2}$) of the steady-state inactivation process for voltage-dependent Na^+ channels was about 20-30 mV more negative than the resting membrane potential. Thus at rest, few or none of the Na^+ channels are available to generate action potentials (Pun & Gesteland, *Pflügers Arch.* 418:504-511). We proposed that the excitability of voltage-dependent Na^+ channels of frog ORNs, like other voltage-dependent channels in excitable cells, can be modulated. We have investigated the effects of GTP, ATP, and their non-hydrolyzable analogues on steady-state inactivation of Na^+ channels in frog ORNs. We report here that the $V_{1/2}$ of steady-state inactivation is shifted to more positive potentials by GTP, GTPγS, and ATPγS. Electrophysiological experiments were performed on freshly isolated frog ORNs using the whole-cell voltage clamp technique. Outward currents were reduced or blocked by a Cs/TEA pipette solution. Nucleotides or their non-hydrolyzable analogues were added to the pipette solution. With no nucleotides, the $V_{1/2}$ was -77.6 ± 4.6 mV (n=8; mean ± SEM) with a slope factor of 10.5 ± 1.1. These values are similar to those previously reported. GTPγS (40-100 μM) and GTP (100 μM) each shifted $V_{1/2}$ to more positive potentials. With GTPγS and GTP, the $V_{1/2}$ values were -63.6 ± 3.5 mV (n=9) and -61.7 ± 3.1 mV (n=10), with slope factors of 8.3 ± 2.2 and 9.0 ± 0.7, respectively. The peak Na^+ current-voltage relation was not affected. ATPγS (100 μM) also shifted the steady-state inactivation process to more positive potentials, with a $V_{1/2}$ of -64.3 ± 1.7 mV (n=6) and a slope factor of 7.3 ± 0.3. On the other hand, ATP (2 mM) in the pipette solution had a less consistent effect, with 5 cells having $V_{1/2}$ more positive than -70 mV and 7 cells more negative than -70 mV. The mean $V_{1/2}$ was -76.6 ± 4.8 mV (n=12) with a slope factor of 10.3 ± 0.7. Our results indicate that odorant activation of G-proteins and/or phosphorylation processes may also modulate the voltage-dependent Na^+ channels and alter the excitability of the cell.

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Basal Conductance of Frog Olfactory Cilia. STEVEN J. KLEENE (University of Cincinnati College of Medicine, Cincinnati, OH 45267).

The conductance of isolated frog olfactory cilia in the absence of odorants and second messengers has been measured. It is possible to seal one cilium of an olfactory receptor neuron inside a recording pipette and excise it from the cell. In this situation, current measured at the recording pipette arises from two parallel sources. The first is current passing through the ciliary membrane itself. The second is a shunt current which passes through the membrane-pipette seal. In order to estimate the contributions of these two currents, I have assumed that the shunt current shows little ionic selectivity. Ion-selective currents are thus considered to flow through conductance pathways in the ciliary membrane. Currents that can be carried by large ions that do not generally permeate biological channels are attributed to the shunt. First, total current into the pipette was measured. Then cations on both sides of the ciliary membrane were replaced with choline. Current remaining at this point was attributed to the shunt, corrected for differences in free conductance between physiological and choline-replaced solutions, and subtracted from the total current. The remaining current was attributed to the ciliary membrane. In normal physiological solutions, each cilium has a conductance averaging 92 ± 22 pS (n = 20, range 7 to 301 pS) at the neuronal resting potential (-50 mV). The ciliary membrane is permeable to monovalent cations ($P_K > P_{Na}$) but not to Cl^-. In some cases, single channels of unit conductance 153 pS were observed. The specific membrane resistance of the ciliary membrane at resting potential is 2600 Ω cm^2. The conductance of the ciliary membrane implies a length constant for electronic conduction of about 160 μm, which is several times the length of an average frog olfactory cilium. Since the reversal potential of the basal conductance is near the neuronal resting potential, it should help to stabilize the ciliary potential at some cost to stimulus transduction efficiency.

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Odorant Responses of Olfactory Receptor Neurons in Dissociated Cell Culture: Analysis With Voltage Sensitive Dyes Combined With Immunocytochemistry. R. J. GRILL, P. FARMER, ROBERT C. GESTELAND and S.K. PIXLEY (Univ. of Cincinnati, Cincinnati, OH 45267-0521).

Dissociated cell cultures of nasal mucosal cells contain olfactory receptor neurons (ORNs) which respond to odorants with depolarization, as shown by patch clamp analyses (Pixley and Pun, Dev. Brain Res. 53:125, 1990). While powerful, patch clamp analyses are time-consuming and difficult to use for questions concerning populations of neurons. We have therefore chosen to analyze ORN odorant responses in culture with voltage sensitive dyes. Cells maintained in culture for 4-6 days were tested for uptake of several voltage sensitive dyes. The styryl dye RH461 was chosen because it gave bright fluorescence in the largest number of neurons with weak fluorescence in non-neuronal cells (cells identified by morphology). With RH461, depolarization results in a decrease in fluorescence intensity and hyperpolarization results in an increase. A field containing neurons was identified in 4-6 day cultures in a serum-free culture medium and the medium was then reduced to expose the cells. After recording a control image, a vapor phase odorant was presented to the cells and a second image was recorded (4 seconds after the first). The difference between the two images is the response. Some cells depolarized while others hyperpolarized. The cells were subsequently fixed and immunostained with an antibody to neuron-specific tubulin. In one case we have been able to determine that a depolarizing cell was a neuron and it was tightly adherent to an adjacent non-neuronal cell which had hyperpolarized.

If dissociated nasal cells are plated on polylysine coated glass only, then neurons die by about 6-7 days after plating, but if they are plated onto a monolayer of CNS astrocytes, then the neurons show much more robust survival (see Pixley, AChemS XIV). We have determined that neurons in both types of culture, at 4-6 days after plating, were responsive to several different odorants. Further analyses in conjunction with immunocytochemistry will allow us 1) to determine the percentage of neurons responsive to each odorant and 2) to identify the types of non-neurons which show activity during the odorant response.

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Effect of humidity on the loss of olfactory receptor neurons in unilateral naris closure mice. JOEL MARUNIAK and FRANK COROTTO (Biological Sciences, University of Missouri, Columbia, MO 65211).

In previous studies we have shown that after about two months of naris closure the rostral regions of the open-side olfactory epithelium show dramatic losses of receptor neurons. This suggests that continous exposure of the open side to all respiratory flows creates increased trauma to the olfactory epithelium on that side. We do not know what the specific nature of the traumatizing agent is but hypothesized that it might be, at least in part, the dryness of the inspired air. Thus this study investigated the effects, on the olfactory epithelia, of housing mice with unilateral naris closure in either high (≈65-100% RH) or low (≈8-35% RH) humidity rooms. Fifteen mice were housed in each room. High humidity was maintained by six steam humidifiers and low humidity was maintained by a single high capacity dehumidifier. To maintain humidity differences, fresh air turnover had to be somewhat restricted. After two months in these conditions, the two groups were sacrificed and their heads prepared for histological and immunohistochemical analyses. The numbers of receptor neurons, OMP-positive olfactory knobs and immature neurons were counted in rostral regions of all animals. The results were exactly opposite of what we expected. Naris closure mice in high humidity conditions suffered severe open-side losses of receptor neurons and OMP-positive olfactory knobs. Immature receptor neurons/globose basal cells were unaffected. In contrast, mice housed in the low humidity room showed no significant adverse effects. There is no possibility that the groups were mistakenly switched at some point in the experiment or during processing.

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The Effect of Age on Olfactory Physiology in Pigeons. M. W. Dahl and W. L. Silver (Department of Biology, Wake Forest University, Winston-Salem, NC 27109.).

Behavioral, anatomical, and psychophysical studies have demonstrated a decline in olfactory sensitivity with age. To our knowledge, few studies have examined the effect of age on olfactory physiology. In the present study we have examined the effect of aging on olfactory nerve responses in two groups of pigeons (1) one year of age and (2) eight years of age. Two odorants, benzaldehyde and phenethyl alcohol were presented to the nares of the pigeons via a computer-controlled, air-dilution olfactometer. Stimuli were delivered in an ascending concentration series and multi-unit, whole nerve responses were recorded using standard electrophysiological techniques. Concentration-response curves were then plotted for each pigeon and each odorant. The areas under the curves were calculated and statistically evaluated to determine whether significant differences exist in olfactory sensitivity between age groups. No statistically significant difference was found to exist between groups for benzaldehyde. Similarly, a significant difference in olfactory sensitivity between age groups for phenethyl alcohol could not be demonstrated, although a strong trend towards a decline in olfactory sensitivity with age for this compound is evident. It is speculated that the use of a larger sample size or the use of older pigeons would show a significant decline in olfactory sensitivity with age.

Developmentally Regulated Expression of Olfactory - Specific Genes

TALIA MARGALIT and DORON LANCET (Weizmann Institute of Science, Rehovot, Israel).

The detection of many odorants is mediated through specialized olfactory-unique forms of receptors, G protein, adenylyl cyclase and ion channel. In parallel, specific forms of biotransformation enzymes, cytochrome P450 and UDP glucuronosyl transferase (UGT) are present in olfactory mucosa and may mediate chemosensory signal termination. Morphological and electrophysiological studies of the developmental process in rat olfactory sensory cells indicate that the rat has a mature olfactory system before birth. Several parameters associated with the mature functional state appear between embryonic day 16 and 18 (E16-E18). These include the appearance of olfactory marker protein (OMP), synapse formation, tapering of olfactory cilia and selective responses to odorants. We studied the developmentally regulated expression the olfactory specific genes by quantitative Reverse Transcriptase - Polymerase Chain Reaction (RT-PCR). Assays were conducted on total RNA from embryos starting at day E14 and ending at postnatal day 35 (P35). All the genes are expressed before birth, but at different onset days. The first to be expressed is olfactory adenylyl cyclase which is already present at E14. $G_{olf\alpha}$ is expressed at E15 and olfactory receptors and olfactory cyclic nucleotide gated channel both start at E18. The olfactory biotransformation enzymes are expressed somewhat later: E19 for P450olf1 and E21 for UGT_{olf}. In all genes, the expression increases over several days until birth. Our data suggest that onset times of the molecular components of the transduction mechanism partly overlap with the morphological and functional changes. The correlation at E18 of functional maturation and of receptor and channel expression may be significant.

In Vitro Neurogenesis and Differentiation of Olfactory Receptor Neurons is Induced by CNS Non-Neuronal Cells. S.K. PIXLEY (Univ. of Cincinnati, Cincinnati, OH 45267-0521).

Dissociated nasal mucosal cells from newborn Sprague Dawley rats were grown on a polylysine-coated substrate (single cultures) or on top of monolayer cultures of partially purified astrocytes from newborn rat olfactory bulbs (co-cultures). In both single and co-cultures, olfactory receptor neurons (ORNs) positive for olfactory marker protein (OMP) disappeared by 4-5 days after plating. ORNs showing positive immunostaining with an antibody against neuron-specific tubulin (NST) were much more abundant than OMP-positive neurons. The number of NST-positive neurons was more than 4 fold greater initially in the co-cultures compared to the single cultures. NST-positive neurons disappeared from the single cultures by 7 days after plating, but they remained so numerous in the co-cultures that it was difficult to determine their number at 7 days. By day 15 after plating on astrocytes, NST-positive neurons had aggregated onto large clumps with radiating bundles of processes.

In the co-cultures, but not in single cultures, OMP-positive neurons re-appeared between 10 and 15 days post-plating. This suggests that contact with CNS astrocytes or some other non-neuronal element is the trigger for OMP expression. Co-cultures produced by using astrocytes from two other brain regions, the cortex and cerebellum, also generated OMP-positive neurons (with remarkably similar numbers). This suggests that there is no regional specificity of the regulatory signal for OMP expression.

Pulse labelling with tritiated thymidine showed that neurons were generated in the cultures after plating. Analyses of multiple tritiated thymidine pulses suggested that expression of neuron-specific tubulin by ORNs occurred within one day after the final cell division, but expression of OMP did not begin until 4-5 days after cell division. The average neuron expressed TUJ1 at 4 days after cell division and OMP at 7 days. All OMP-positive neurons were NST-positive. The timing of the in vitro events was very similar to that of regenerative events in vivo. This suggests that the co-culture of ORNs with astrocytes is an excellent model system for studying regulation of olfactory neurogenesis.

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Partial Purification of the Hydra Glutathione Chemoreceptor.

SUSAN L. BELLIS, G. KASS-SIMON and DENNIS E. RHOADS (University of Rhode Island, Kingston, RI)

Glutathione [Glu(Cys-Gly), GSH] is an activator of feeding behavior in the coelenterate, hydra. The association of GSH with putative hydra chemoreceptors triggers tentacle writhing and mouth opening, behaviors which facilitate the ingestion of prey. Recently, [^{35}S]-GSH has been found to associate with hydra membranes in a rapid, reversible manner (Bellis et al, 1991, Soc. Neurosci. Abstracts, 17:120). The dissociation constant (K_D) for [^{35}S]-GSH binding was evaluated as 3 uM, a value which is in good agreement with concentrations of GSH which stimulate feeding behavior. In the same study it was shown that membrane proteins which bind GSH could be detergent-solubilized in 10mM CHAPS/100mM KCl/10% glycerol without any adverse effect on binding activity. In the present study, detergent-solubilized proteins have been applied to a GSH-agarose affinity column. SDS-PAGE analysis of the proteins which associated with the column revealed five intensely-stained bands; a doublet at 27kD, and a triplet at 25kD. Additionally, two faint bands were observed; one at 48kD, another at 20kD. Four slices corresponding to the doublet, triplet, 48kD and 20kD bands were excised from polyacrylamide gels and injected into rabbits in order to generate antiserum against these proteins. Whole hydra were incubated with this antiserum and feeding behavior was monitored by measuring the duration of GSH-stimulated mouth opening, a well-established behavioral assay (Lenhoff, 1961, J. Gen. Physiol., 45:331-344). Preliminary results indicated that feeding behavior was inhibited by antiserum raised against the 25kD triplet. Antiserum raised against the other 3 protein fractions had no effect on GSH-stimulated mouth opening. We propose that the 25kD triplet contains a peptide(s) which either represents or comprises some portion of the hydra glutathione chemoreceptor.

Partial Purification of an L-Arginine Receptor from Catfish Taste Epithelium. D. LYNN KALINOSKI (Monell Chemical Senses Center), J.H. TEETER (Monell Chemical Senses Center and Univ. of PA), and J.G. BRAND (Monell Chemical Senses Center, Veterans Affairs Med. Center and Univ. of PA, Philadelphia, PA).

The channel catfish (I. punctatus) is a well characterized model for chemosensory research. Behavioral, neurophysiological and biochemical studies suggest that there are independent receptor sites for L-alanine and L-arginine in the catfish taste system. Biochemical studies have characterized binding of the taste stimuli L-alanine and L-arginine to a sedimentable membrane fraction (P2) from taste epithelium and have begun to characterize L-alanine-stimulated second messenger accumulation in taste tissue. Electrophysiological studies have identified an L-arginine-gated nonselective cation channel in purified membranes from taste epithelium. Recent studies have demonstrated that taste stimulus binding to fraction P2 could be selectively inhibited by the lectins DBA, Jacalin, PHA E&L and RCA I. Furthermore, the biotinylated lectins selectively labeled a few membrane glycoproteins by Western blotting to SDS-PAGE separated taste membrane proteins. We have taken advantage of this selective labeling of taste epithelial glycoproteins by lectins to partially purify and reconstitute an L-arginine receptor/channel. Fraction P2 from taste epithelium was extracted using 4% CHAPS. Extracted material was incubated with either PHA E or RCA I coupled to agarose for 90 mins at 10°C. The lectin-agarose was pored into a column, washed, and bound material eluted with either the specific hapten sugar or 1 M NaCl. Eluted material was either separated by SDS-PAGE or reconstituted into phospholipid vesicles by extensive dialysis. Phospholipid bilayers into which PHA- and RCA- purified vesicles had been incorporated frequently displayed reversible increases in cation conductance in the presence of micromolar concentrations of L-arginine. The conductance, ion selectivity and concentration dependence of the partially purified L-arginine-activated channels were similar to those observed with native membrane vesicles. SDS-PAGE followed by silver staining demonstrated the presence of several proteins including a prominent doublet of M, 55,000 in the RCA-purified material.

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Localization of PHA-E Lectin Binding (arginine receptors?) to Taste Buds on Catfish Barbels.
BARBEL BÖTTGER and THOMAS E. FINGER (Univ. Colorado Health Sci. Ctr. and Rocky Mountain Taste & Smell Center)

Phaseolus vulgaris Agglutinin lectin, subunit E (PHA-E) binds selectively to arginine, but not alanine receptors in the P2 fraction of catfish taste epithelium (Kalinoski et. al, AChemS XI). In order to examine the localization of PHA-E binding to intact tissue, mandibular barbels of channel catfish (*Ictalurus punctatus*) were exposed to biotinylated PHA-E lectin in phosphate buffered saline (100 µg/ml) after fixation of the tissue in 4% paraformaldehyde. Control barbels were exposed to a biotinylated antibody instead of biotinylated lectin. Lectin binding was visualized either with fluorescein-labeled strepavidin, or by means of the avidin-biotin-peroxidase method (Vector Labs, ABC kit). With either method of visualization, in whole mounts, the lectin binding sites appeared as round (15-20 µm diameter) patches at the apex of each taste bud along the length of the barbel. These patches appear coincident with the taste pore region. At higher magnification, the lectin binding within the taste pore region of each taste bud is not homogeneous, but appears granular or "pebbly". When taste buds are viewed in profile, small reactive extensions can be seen extending from the surrounding taste pore area. These may be especially long or thick microvilli, but their exact identification must await electron microscopic examination. In control preparations, little or no reaction product or fluorescence was seen; the apex of taste buds was never more reactive than the surrounding epithelium. In summary, PHA-E lectin binds to the apical region of every taste bud and within each taste bud to the vast majority, if not all cells that have apical processes in the taste pore.

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Isolation of Miraculin-Binding Proteins from Dorsal Epithelium of Tongue of *Rhesus* Monkey. MENGSHU WANG[1], GORAN HELLEKANT[2] and JOSEPH G. BRAND[1,3] ([1]Monell Chemical Senses Center; [2]Department of Veterinary Science, Univ. of WI; [3]Veterans Affairs Med. Ctr., Univ. of PA).

Miraculin is a sweet-inducing protein that is active primarily in simian primates (Brouwer et al., J. Physiol. 337: 221). Its mode of action is thought to involve a pH-dependent conformational change in the miraculin-receptor pair such that lower pH values will allow miraculin to stimulate the sweet taste receptor. Since miraculin is active in the micromolar range, it is possible that the proteins of the plasma membrane that bind miraculin may be partially purified via a miraculin affinity column. These "miraculin binding proteins" would then be candidates for a sweet taste receptor. Miraculin was covalently attached to CNBr-activated Sepharose CL-6B. *Rhesus* monkey dorsal lingual epithelial tissue was extensively washed with a "saliva rinsing buffer" and then with PBS buffer pH 7.4. The tissue was homogenized in a blender in HEPES buffer, pH 7.4, and the particulate fraction collected by centrifugation. The resultant pellet was further homogenized in a blender using HEPES, pH 7.4 containing 1% Triton X-100. The supernatant was collected by differential centrifugation and the centrifugate re-extracted and homogenized two more times. A final supernatant, collected after a 105,000 x g centrifugation, was run over the miraculin-affinity column and protein eluted with 0.1 M citric acid, pH 4.0. SDS-PAGE analysis of the eluated protein from the column showed four bands, Mr ~ 12,000, 18,000, 50,000 and 60,000. A cross-linking reaction between these miraculin-binding proteins and miraculin showed that the two higher molecular weight bands could be bound by miraculin. The Triton soluble proteins from monkey lingual tongue muscle extracts and the lingual epithelial tissue from the ventral side of the tongue did not contain proteins which bound the miraculin affinity column.

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Molecular Cloning of Taste Transduction Proteins
NANCY SPICKOFSKY, SUSAN McLAUGHLIN, PETER McKINNON, ROBERT F. MARGOLSKEE (Department of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110).

Our laboratory is studying the molecular and cellular biology of the mammalian taste transduction process. Our goal is to identify and characterize those proteins involved in the taste transduction process. For this purpose we made a cDNA library from taste cell enriched poly A^+ mRNA from the rat. This library served as the template for polymerase chain reaction (PCR) amplification and cloning of genes involved in taste cell transduction. Degenerate primers corresponding to conserved regions of G protein α subunits, G-coupled receptors, phosphodiesterase (PDE) and ion channels were used in the PCR with taste cell cDNA. We have detected specific PCR products from several of these reactions which are present in taste cell cDNA but absent from control non-taste lingual cDNA. Two different G protein α subunits are highly enriched for expression in taste tissue vs. non-taste lingual tissue, two other G protein α subunits are expressed in taste tissue, but completely absent from non-taste control tissue. We have isolated clones of a cAMP PDE and a G-coupled receptor from taste cell enriched cDNA: we are presently determining if these clones are specifically expressed within the taste cells.

Second Messenger Interactions in Taste: Effect of Arachidonic Acid on PIP2-PLC Activity in Catfish Taste Tissue. TAUFIQUL HUQUE and JOSEPH G. BRAND (Monell Chemical Senses Center, 3500 Market Street, Philadelphia, PA 19104) and JOSEPH L. RABINOWITZ (VA Medical Center, Philadelphia, PA 19104).

Previous studies from our laboratory have demonstrated the presence, in catfish taste tissue, of an active phospholipase C that degrades the membrane phospholipid PIP2 to form the putative second messenger IP3, which is then assumed to mobilize intracellular calcium. We have also shown that incubation of catfish gustatory epithelium with labeled lipid precursors leads to rapid incorporation of label into phospholipids, followed by appearance of label in fatty acids, implying the presence of phospholipases A1/A2 and formation of the putative second messenger arachidonic acid (AA). Studies in other tissues suggest that activation of phospholipases A1/A2 is less rapid than that of phospholipase C, and that there are interactions between the second messengers derived from the activities of phospholipases A1/A2 and C. Specifically, AA has been shown to activate phospholipase C and we have now studied this effect in catfish taste tissue. Homogenates of catfish barbel epithelium were incubated with 3H-PIP2, and PIP2-PLC activity was assayed by measuring release of 3H-IP3. AA was added as a liposomal suspension and its oxidation prevented by inclusion of the inhibitor BW755C. At concentrations of 10-1000 μM, AA enhanced basal PIP2-PLC activity in a dose-dependent manner, by up to 20-fold. When enzyme activity was measured in the presence of physiological levels of free Ca2+ (< 1 nM-12 μM) the stimulatory effect of AA was Ca-dependent at doses of 10-1000 μM. In the presence of the taste stimulus L-alanine plus GTPγS the stimulatory effect of AA was enhanced even more. The physiological significance of the Ca-dependent PIP2-PLC activating effect of AA may be that it provides a positive feedback mechanism for the generation of a second peak of IP3, followed by a second wave of intracellular calcium mobilization (the "calcium oscillation" phenomenon).

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In Vitro Uptake of ^{3}H Serotonin In Taste Buds of the Mudpuppy, *Necturus maculosus*
JOAN WELTON (Colo. St. Univ. and Rocky Mt. Taste and Smell Center)
STEPHEN ROPER (Colo. St. Univ. and Rocky Mt. Taste and Smell Center)

Neurotransmitters that act at synapses in vertebrate taste buds have not been identified unambiguously to date. We have recently shown that Merkel-like basal cells in *Necturus* taste buds possess a high concentration of serotonin (Welton, et al. submitted; Taylor, et al., this meeting; Roper, *J. Neuroscience*, in press, 1992), suggesting, as others have also speculated, this monoamine may be a neurotransmitter in taste buds. Two important criteria for identifying a neurotransmitter in a tissue are (1) the presence of a high affinity uptake mechanism for the transmitter candidate; and (2) a Ca^{2+}-dependent release mechanism that is triggered by depolarization. We have tested whether these two criteria are met for serotonin (5HT) in *Necturus* taste buds. ❶Lingual epithelium was dissected free and incubated with radioactive serotonin (^{3}H-5HT; 0.41 μM) in amphibian physiological saline (APS) for 15 minutes, rinsed with fresh APS, fixed with 2% glutaraldehyde, embedded in plastic, and sectioned at 2.0 μm. We used quantitative autoradiography to assay the uptake of ^{3}H-5HT. Sections which contained taste buds revealed that basal cells were heavily labelled with ^{3}H-5HT, indicating the presence of an uptake mechanism for the monoamine. In contrast, when lingual tissue was incubated with ^{3}H-5HT in the presence of 1-10 μM imipramine, a selective blocker of high-affinity 5HT uptake, the density of silver grains over basal cells was reduced to less than 15% compared with tissue where imipramine was absent. ❷In another series of experiments, lingual tissue was incubated in ^{3}H-5HT as before, but rinsed for 2 min. with APS that contained 40 mM KCl to depolarize basal cells. Under these conditions, label over basal cells was significantly less (52%; $p<.005$) than when the tissue was rinsed with normal APS. These data indicate that basal cells release ^{3}H-5HT when they are depolarized. ❸In a last series of experiments, lingual tissue was incubated in ^{3}H-5HT and rinsed with APS in which KCl had been elevated to 40 mM but $CaCl_2$ lowered to 0.2 mM. This was designed to test whether depolarization-induced release of ^{3}H-5HT was Ca^{2+}-dependent. Under these conditions, basal cells did not release ^{3}H-5HT: there was no significant difference between the labelling of basal cells when tissue was rinsed with control APS and when rinsed with high K^+/low Ca^{2+} APS. ❹Our results, then, indicate that basal taste cells take up 5HT via a high affinity, imipramine-sensitive pathway. Further, depolarization leads to 5HT release and this release is Ca^{2+}-dependent. These data are consistent with serotonin acting as a neurotransmitter in *Necturus* taste buds.

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Voltage Clamp Studies of the Amiloride-Insensitive Neural Response.
QING YE, GERARD L. HECK, JOHN A. DeSIMONE (Department of Physiology, Virginia Commonwealth University, Richmond VA, 23298-0551)

The appearance of an amiloride-insensitive component (AIC) in the neural response to sodium salts is both anion and salt concentration-dependent (Formaker and Hill,Amer J. Physiol. 255:R1002, 1988; Elliott and Simon,Brain Res. 535:9, 1990 ; Schiffman et al.,Physiol & Behav. 47:435, 1990). In the case of NaCl the AIC appears under zero current-clamp conditions at about 100 mM using amiloride concentrations of 10-100 μM. For nonchloride sodium salts an AIC appears only at higher salt concentrations, if at all. For example at 0.2 M, NaCl has an AIC whereas sodium acetate (NaAc) does not. The onset of the AIC for NaCl coincides with its anomalous voltage clamp response (AVCR). Below 100 mM NaCl, NaAc, and sodium gluconate (NaGlu) give equal neural responses at a given clamping voltage. Negative clamping voltages significantly enhance the salt response; positive voltages depress it. Above 100 mM, NaCl responses become greater than those of NaAc or NaGlu especially at positive clamping voltages, and NaCl responses cannot be eliminated by high positive voltages (AVCR). In earlier work we established the presence of a leaky, cation-selective paracellular shunt in the lingual epithelium (DeSimone et al., J. Gen. Physiol. 83:633, 1984; Ye et al. Science 254:724, 1991). We hypothesize that the AIC and the AVCR arise from passive penetration of NaCl through tight junctions. Evidence suggests that amiloride-inaccessible channels exist on the basolateral membrane. Their availability to Na could depend on salt shunt permeability. If the hypothesis is correct, the AIC will depend on NaCl trans-shunt electrochemical potential difference and the shunt ion exchanger fixed charge density. Tests to determine these dependencies are underway.

Ion Transport in Rat Tongue Epithelium: A Developmental Study.
A. MARK SETTLES AND SHEELLA MIERSON (University of Delaware, School of Life and Health Sciences, Newark, DE 19716, U.S.A.)

The responsiveness of the rat gustatory system to monochloride salts changes during development. Chorda tympani nerve responses to NaCl and to KCl in early postnatal rats are small relative to NH_4Cl; both become more potent stimuli as the animal matures (M.F.Ferrell, C.M.Mistretta, & R.M.Bradley, *J. Comp. Neurol.*, 198:37-44, 1981). This developmental increase is accompanied by an increase in amiloride sensitivity of the NaCl response (D.Hill & T.C.Bour, *Dev. Brain Res.*, 20:310-313, 1985). We measured ion transport properties of *in vitro* tongue epithelia from neonatal Wistar rats. When the tissue is mounted in an Ussing chamber, either open-circuit electrical potential difference or short-circuit current (I_{sc}) shows marked change as a function of age. With Krebs-Henseleit buffer on both sides of the tissue, I_{sc} is 4.7 ± 0.5 μA/cm² in the 11-13 day old animal, increasing to 11.2 ± 1.9 μA/cm² in the 3-month old rat (n = 7 & 6, respectively). The I_{sc} responses to both 0.5 M NaCl and to 0.5 M KCl are small in the neonatal rat. These responses are larger in the one month old animal (postweaning), and are larger still in the adult (3 months). Amiloride sensitivity of the NaCl response is greatest in the 3-month old. This study confirms that increased sensitivity of the rat gustatory system to NaCl with age reflects changes in the peripheral membranes.

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Properties of the Amiloride-Sensitive Salt-Taste Sodium Channel. HARRY WMS. HARPER (Duck Engineering Design, 500 E. 63rd St., New York, N.Y. 10021)

1) The inhibitory effect of amiloride on the sodium channel is use-dependent. Amiloride at .01 mM produces greater inhibition of the hamster *chorda tympani* response to 0.1 M NaCl when the stimulus sequence is [NaCl > NaCl + amiloride] than when the sequence is [H_2O > NaCl + amiloride].
2) Amiloride inhibition of NaCl responses occurs over the full range of NaCl concentrations examined (0.1 M - .005 M).
3) The amiloride-insensitive component of sodium salt responses may be due to responses mediated by receptor cells with apical channels permeant to potassium, rather than sodium, ions. These cells are driven by the liquid junction potential arising at the paracellular junctions of receptor cells (as proposed in the **Diffusion Potential Model** of salt taste transduction).
4) The response profile to the chloride salts of hydroxylammonium, hydrazinium, and methylammonium, is very striking. These cations are particularly interesting because: their electrical mobilities are similar, and hence their paracellular junction potentials will be similar, and would not account for any observed differences in responses; and, their permeability sequence in voltage-dependent, tetrodotoxin-sensitive sodium channels is well-known: hydroxylammonium > hydrazinium >> methylammonium (impermeant). In fact, their taste response profile is the same: hydroxylammonium > hydrazinium >> methylammonium (almost no response). However, salt taste is not sensitive to tetrodotoxin, and amiloride-sensitive sodium channels are not permeant to hydroxylammonium or hydrazinium. Thus, a new type of sodium channel may be involved in salt taste transduction.

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Hamster Chorda Tympani Responses to Sodium: Differential Anion Effects in H and N Fibers. BRADLEY G. REHNBERG, BRUCE I. MACKINNON, THOMAS P. HETTINGER, AND MARION E. FRANK (University of Connecticut Health Center).

Research on salt taste has focused on stimulatory aspects of cations. Beidler's work in the 1950's, however, showed that anions can strongly influence gustatory responses to sodium salts. Ye et al. recently supported a paracellular shunt model of "anion inhibition" in which anions with limited mobility across tight junctions in taste buds would effectively hyperpolarize basolateral regions of taste cells and thereby lower excitability (Science 254:724). We have demonstrated anion inhibition in the hamster by showing that the chorda tympani nerve responds more strongly to NaCl than to Na acetate over a wide range of concentrations. Iontophoretic presentation of Cl^- and acetate to the anterior tongue elicited no response in the chorda tympani suggesting that these anions are not directly stimulatory. Drugs (0.01, 1.0, and 100 uM anthracene-9-carboxylate, diphenylamine-2-carboxylate, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonate, and furosemide) which interfere with movements of Cl^- across epithelial cells were ineffective in altering chorda tympani responses to either 0.03 M NaCl or Na acetate. Anion inhibition related to movements of anions across epithelial membranes therefore seems unlikely. The chorda tympani contains a population of nerve fibers highly selective for Na^+ (N fibers) and another population sensitive to Na^+ as well as other salts and acids (H fibers). We found that N fibers respond similarly to NaCl and Na acetate, with spiking activity increasing with concentration (0.01-1.0 M). H fibers, however, respond more strongly to NaCl than to Na acetate. Furthermore, H fibers increase spiking with increases in NaCl concentration but generally decrease their responses to increasing concentrations of Na acetate. It appears that "anion inhibition" applies to taste cells innervated by H fibers but not by N fibers. We conclude that the anterior tongue of hamsters has two fundamentally different transduction mechanisms for the detection of Na^+. An apical Na^+ channel is used by taste receptor cells of N fibers, whereas H fibers may use a paracellularly-mediated, basolateral site of stimulation.

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Amiloride suppresses chorda tympani responses to NaCl in C57BL/6J but not 129/J mice. K. S. GANNON and R. J. CONTRERAS (The Florida State University, Program in Neuroscience, Tallahassee, FL 32306-1051)*

Results of behavioral taste testing indicate that 129/J mice exhibit a greater preference for 0.08 M NaCl compared to C57BL/6J mice (Beauchamp, AChemS Abstr., 1990; Gannon & Contreras, Soc. for Neurosci. Abstr. 1991). Since taste plays an important role in the control of NaCl ingestion, it was hypothesized that an underlying component of the above strain difference may reside in the gustatory system. To assess neural gustatory processing in 129/J and C57BL/6J mice, whole-nerve electrophysiological recordings were obtained from the chorda tympani in response to a concentration range of NaCl and KCl. Taste stimuli were presented before and after lingual application of 0.5 mM amiloride hydrochloride, a sodium channel blocker. Peak response (maximum response occurring within 2-s after stimulus onset) and tonic response (response magnitude 10-s after stimulus onset) data were normalized to 0.1 M NH_4Cl. Chorda tympani response magnitudes to NaCl in the absence of amiloride were similar for 129/J and C57BL/6J mice. However, following amiloride treatment, significant suppression of NaCl peak ($p<0.05$) and tonic ($p<0.001$) responses was seen for C57BL/6J mice but not for 129/J mice. Chorda tympani responses to KCl were not suppressed in either strain following amiloride application. The results indicate that the differential NaCl intake between 129/J and C57BL/6J mice may be mediated by differences in peripheral receptor mechanisms. Activity in salt-sensitive chorda tympani neurons in synaptic contact with taste receptor cells containing amiloride-sensitive sodium channels has been hypothesized to underlie the peripheral coding of NaCl intensity and quality. A relative paucity or lack of amiloride-sensitive sodium channels on taste receptor cells of 129/J mice may indicate that the perceived taste intensity elicited by NaCl is reduced compared to C57BL/6J mice. Compared to C57BL/6J mice, 129/J mice may consume more NaCl because it tastes less intense and more palatable.

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Stimulation of the Gerbil's Gustatory Receptors by the Super Sweetener Cyanophenyl methylbenzyl Guanidine Acetic Acid
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The purpose of this study was to determine how the gerbil responds to the artificial sweetener CYANOPHENYLMETHYLBENZYL GUANIDINE ACETIC ACID (CGAA). First, the gerbil's chorda tympani nerve responses were obtained to CGAA and sucrose. As in the human, CGAA was observed to be a more effective stimulant than sucrose in the gerbil (CGAA CR_{50} = $4x10^{-6}$M, sucrose CR_{50} = 0.035M). Next, conditioned taste aversion studies were conducted and it was observed that gerbils trained to avoid CGAA generalized an avoidance to sucrose and quinine, but not NaCl and HCl. Except for the bitter taste, these results indicate that for the gerbil CGAA tastes like sucrose as it does in the human.

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Multi-Fiber Taste Responses to Binary-Component Stimuli in the Hamster Chorda Tympani. BRADLEY K. FORMAKER & MARION E. FRANK (University of Connecticut Health Center, Farmington, CT)

Mammalian gustatory systems typically integrate multiple taste qualities during the ingestion of food. However, the majority of information obtained regarding gustatory function has been derived from studies using single-component taste stimuli. In order to investigate peripheral gustatory function using more "natural" stimuli, we recorded multi-fiber taste responses from the hamster chorda tympani nerve to single- and binary-component taste stimuli. Electrophysiological responses were recorded during chemical stimulation of the anterior tongue with a concentration series of either NaCl (0.05M - 0.5M), quinine.HCl (QHCl; 0.001M - 0.03M), sucrose (0.05M - 1.0M) or a binary combination of these stimuli. For example, each concentration of sucrose was seperately mixed with every concentration of QHCl and every concentration of NaCl. Mixture stimuli were combined so that the concentration of each component equalled its concentration when presented alone. NH_4Cl (0.5M) was periodically applied to the anterior tongue in order to assess the viability of each preparation. Preliminary results indicated that when expressed relative to the 0.5M NH_4Cl response, the response magnitude of a mixture was similar to the response magnitude of the more effective component in the mixture. Therefore, when combined in a binary mixture, each component of the mixture did not have an independent effect on the multi-fiber response of the chorda tympani. These results suggest that at the multi-fiber level, responses to binary combinations of qualitatively different taste stimuli are not predicted by an additive model of peripheral taste function.

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Astringent Compounds Suppress Taste Responses in Gerbil. SUSAN S. SCHIFFMAN, MARK S. SUGGS, and SIDNEY A. SIMON (Duke University).

Electrophysiological recordings were made from the whole chorda tympani nerve in gerbil to understand the interactive effect of astringent-tasting molecules with a broad spectrum of tastants including mono- and divalent salts, bitter compounds, acids, and sweeteners. The astringent tasting compounds were tannic acid (24 mM at pH's 2.9 and 5.5), aluminum ammonium sulfate (30 mM), aluminum potassium sulfate (10 mM) and gallic acid (30 mM). Hydrochloric acid (1 mM, pH 2.9) was also tested to control for acidity, since aqueous solutions of astringent-tasting compounds are acidic. Adaptation to tannic acid (24mM) at both pH 2.9 and 5.5 markedly inhibited responses elicited by salts, acids, sweeteners, and bitter-tasting compounds to about the same extent which suggests that tannic acid itself (as opposed to protons) may produce this inhibition. Chorda tympani responses to sweeteners were completely suppressed by tannic acid; responses to KCl, NH_4Cl, and urea were the least suppressed. The aluminum salts also inhibited the chorda tympani responses to all stimuli tested. Gallic acid, which is weakly astringent, had minimal effects on the chorda tympani responses to the test compounds. These data suggest that both tannic acid and the aluminum salts may inhibit a variety of transport pathways and receptors for a broad spectrum of tastants on the apical membranes of taste cells.

Functional Regeneration of Glossopharyngeal Nerve Through Micromachined Sieve Electrodes. ROBERT M. BRADLEY, RICHARD H. SMOKE, TAYFUN AKIN and KHALIL NAJAFI (Dept. Biologic and Materials Sciences, School of Dentistry, and Center for Integrated Circuits, College of Engineering, University of Michigan).

We report on the regeneration of the glossopharyngeal nerve through a sieve electrode consisting of an array of holes micromachined in a silicon substrate. The sieve electrode was implanted between the cut ends of the glossopharyngeal nerve in 28 rats. Several configurations of hole designs have been used, ranging from some with a large number (777) of small diameter (2 μM) holes and others with smaller numbers (500) of larger diameter (5 μM) holes. Some holes have electrodes associated with them so that it will be possible to record from axons that regenerate through the holes. After a period of at least 90 days rats with implanted sieve electrodes were reoperated and the nerve regeneration assessed. Based on gross examination of the implant and histological section of the distal nerve, nerve regeneration was successful in 20 of the implanted animals. Of these successful implants those with large numbers of small diameter holes were just as effective as those with larger diameter holes. Successful electrophysiological recordings were obtained in 9 animals. Responses to mechanical and thermal stimulation of the tongue were always obtained. Robust responses to chemical stimulation were obtained from 5 implants. These electrophysiological responses were similar to control recordings from unoperated nerves. In the animals in which successful regeneration had taken place taste buds were present in equivalent numbers in foliate papillae on both sides of the tongue. These experiments demonstrate that axons of the glossopharyngeal nerve will regenerate through an array of holes to support the functional regeneration of thermal, mechanical and taste receptors on the tongue.

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Enhancing Effects of Betaine on Taste Receptors of the Puffer, *Fugu pardalis*.
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We have shown that at least three different groups of taste receptor sites for alanine, glycine, proline, sarcosine, dimethylglycine and betaine are present in the palatal taste system of the puffer: (1) alanine sites for alanine, glycine and sarcosine,(2) proline sites for proline and dimethylglycine, (3) betaine sites for betaine and dimethylglycine. Among these potent stimuli, betaine not only stimulates the betaine sites but also acts as an enhancer for the alanine receptor sites. The purpose of this study is to characterize the enhancing effects of betaine on the amino acids responses.

Synergistic effects of betaine were studied by recording the whole nerve responses to various concentrations of alanine, serine, glycine or proline after application of 0.01 M betaine. A marked synergism was found between betaine and alanine, glycine, or serine. The prior application of betaine led to a shift of the response-concentration curves for alanine and serine to a lower concentration region(2-3 log units). Betaine had not siginificant effects on the threshold for glycine while it enhanced the glycine responses over the entire concentration range examined. The response to alanine over the concentration range from 0.00005 to 0.005 M tended to reach a saturation level as the concentration of betaine increased. No such synergism was found between betaine and proline.

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Development of Fungiform Papillae, Taste Buds and Epithelium in the Human Fetus R. XIAO & I. MILLER, JR., (Dept. Neurobiol. and Anat. , Wake Forest Univ., W-S, NC 27103)

Our objective is to study systematic changes in the size and shape of taste buds (tb), papillae, and the thickness of the epithelium during development. Tongue tips from N = 24 fetal specimens were sampled, ranging in age from 16 - 39 wks. Gestational ages were estimated from the fetal CHL. Sections were prepared for HE stain (paraffin) or tol. blue (plastic). Epithelial thicknesses, heights and widths of the fungiform papillae were measured with video-microscopy. Areas of tb were measured in sections containing the taste pore. In tb on tips of finger-shaped papillae, the buds were taller than they were wide. For tb located on the top of mushroom-shaped papillae, the width was longer than the height before 24 wks. The height of the tb increased as the epithelium grew thicker. Epithelial thickness on the tops of fungiform papilla increased as the fetus grew from 20 ± 6.1 μm (sd, N=5) at 18 wks to 99 ± 12 μm (N=2) at 39 wks. The shapes of fungiform papillae changed during development. The long, finger-like papillae had been reported to appear during gestation and regress after birth. We observed tb on these papillae, in contrast to the published account. The heights of papillae grew from 155 ± 27 μm (N=5) at 18 weeks to 603 ± 106 μm (sd, N=2) at 39 wks. The areas of the dermal cores of papillae increased about 6x from an avg. of 9.4 $x10^3$ μm^2 at 18 wks to 5. 7 x 10^4 μm^2 at 39 wks of gestation. The cross-sectional areas of tb increased about 2.2-fold (from 8.24 $x10^2$ μm^2 at 18 wks to 18.43 x 10^2 μm^2 at 30 wks). In one 38 wk fetus, over half of the fungiform tb contained pigment granules like melanin, which may be important for the origin of tb cells. Thus, tb grew taller, epithelial thickness grew 5x and the area of the central core increased 6x. Fungiform papillae grew taller, wider and some became elongated in the vertical or horizontal planes from 16 -39 wks of gestation.

Fetal specimens were obtained from West China Univ. of Med. Sci., Chengdu, PRC,and support come from NIH Grant CD 230.

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Immunohistochemical Evidence for the Presence of Amiloride-Sensitive Sodium Channels in the Taste Buds of Sodium-Restricted Rats. ROBERT E. STEWART and DAVID L. HILL (University of Virginia).

We have shown previously that rats exposed to low levels of dietary sodium (0.03% NaCl) from very early in gestation express greatly reduced chorda tympani sodium taste responses during adulthood, due to an apparent lack of *functional* amiloride-sensitive sodium channels in taste receptor cell membranes. Interestingly, normal, amiloride-sensitive sodium taste responses may be restored by repletion of dietary sodium, consumption of isotonic saline, or by some anesthetics. The present study was designed to test the hypothesis that amiloride-sensitive channels are present in a non-functional state within taste buds of sodium-restricted rats. We also sought to determine the developmental timecourse for the expression of amiloride-sensitive sodium channels in neonatal normal rats. Sagittal sections of anterior tongue from newborn (1 day old) and adult (45-90 days old) sodium restricted and control rats were labelled with an polyclonal antibody directed against purified amiloride-sensitive channel protein derived from bovine renal papilla in conjunction with an enhanced avidin-biotin complex method. Sections were counterstained and examined by light microscope. Adult control and sodium-restricted rat taste buds were highly immunopositive, and virtually all taste buds observed were labelled by the antibody. Remarkably, the poorly formed taste buds of 1 day old control rats were also labelled, at a time well before initial expression of neurophysiological sensitivity to amiloride. In contrast, taste buds of neonatal sodium-restricted rats showed less intense channel-like immunoreactivity, and occasionally demonstrated labelling of individual taste cells. These results lend support to the idea that the recovery and perhaps the onset of taste system amiloride sensitivity is due to the activation of quiescent amiloride sensitive sodium channels already extant in taste receptor cell membranes.

We are indebted to Dr. Dale Benos, University of Alabama at Birmingham, who generously supplied the anti-channel antisera. This work was supported by NIH grants HD007323 and DC000407.

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Development of Anterior Tongue Taste Buds in Rats Deprived of Dietary NaCl. JESSICA L. CANOS, ROBERT E. STEWART AND DAVID L. HILL (Dept. Psychology, Univ. Virginia).

We have demonstrated that dietary sodium restriction begun early postconception has profound influences on the functional development of peripheral and central taste neurons. Furthermore, such dietary manipulations affect the terminal field organization of chorda tympani neurons in the nucleus of the solitary tract (NTS) and affect the dendritic organization of relay neurons in the NTS. A fundamental gap in our understanding the role that these environmental influences have on the developing gustatory system relates to information about taste bud development in fungiform papillae. Therefore, the goal of this study is to examine the morphological characteristics of taste buds during development in rats deprived of dietary NaCl compared with those in normal rats. Pregnant mothers of rats in the deprived groups were fed a low sodium diet (0.03% NaCl) from day 3 post conception to the time when tongues were harvested. Control rats were always fed a sodium-replete diet (1.0% NaCl). At postnatal day 6, 10 or at adulthood (>60 days), rats were perfused with physiological saline followed with 10% neutral buffered formalin. Tongues were subsequently dehydrated and embedded in paraffin, and sagittal sections (12 μm) were cut and stained with hematoxylin and eosin. Preliminary findings from 4-6 tongues/group indicate that there is an age-related increase in the number of taste buds in deprived and control rats; however, there are no differences in taste bud numbers between the two groups at any age. Thus, based on taste bud numbers, there does not seem to be a developmental difference in the peripheral morphology of the gustatory system. We are currently examining whether other morphological characteristics in the taste bud may occur along with the functional and anatomical differences seen between groups.

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Ultrastructural Correlates of Development in Taste Buds. CYNTHIA CHURCH [1,2,3] and JOHN C. KINNAMON[2,3]. (University of Colorado, Boulder, CO 80309[1], University of Denver, Denver, CO 80208[2] and the Rocky Mountain Taste and Smell Center, Denver, CO 80262[3])

We have been studying the ultrastructural basis for development in rat circumvallate and fungiform taste buds in an attempt to correlate structure with function in this developmental system. We are examining ultrastructural features of taste buds from neonatal rats of the same ages used by Hill *et al.* (1982) in their physiological studies on development of the taste response. The occurrence of the following morphological features is being studied: the first appearance of specific cell types, the appearance of the taste pore, the formation of synaptic connections and the cell types with which they are associated, and the development of patterns of synaptic connectivity. Our preliminary results show that, when contrasted with neonates, adult taste buds show a greater range of cellular morphologies. Taste buds obtained from neonates are smaller and contain fewer cells, and the ultrastructural appearance of the taste cells is more homogeneous than from adults. Neuro-neuronal and efferent as well as afferent synapses are present in the taste buds of the neonates. Only afferent synapses are found in adults. Ultimately we plan to combine these ultrastructural studies with electrophysiological studies in order to better elucidate the relationship between structure and function in developing taste buds.

Hill, D.L., C.M. Mistretta and R.M. Bradley (1982) *J. Neuroscience.* 2:782-790.

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Sweet Taste In Two Malagasy Primates

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The relationship between phylogeny and taste is of growing interest. Here we present recordings from the chorda tympani nerve, CT, of two lemuriforme primates, the lesser mouse lemur (Microcebus murinus) and the mongoose lemur (Lemur mongoz), in addition to results of conditioned taste aversion, CTA, experiments in M. murinus and two-bottle preference, TBP, tests in L. mongoz. We used an array of taste stimuli including acesulfame-K, aspartame, D-glucose, dulcin, monellin, Na-saccharine, neohesperidin dihydrochalcone (NHDHC), stevioside, sucralose (TGS), suosan, tannin, thaumatin and xylitol and the "standards". Two new high potency sweeteners, an alitame derivative and superaspartame were included. The effect of gymnemic acid and miraculin was tested in M. murinus. All of the above compounds, except thaumatin, elicited a taste response. Thus, while monellin gave a unquestionable CT response, thaumatin did not, although we used a concentration of 60 mg/l versus 20 mg/l of monellin. The CTA results show that the animals generalized from sucrose to monellin but not to thaumatin. The intake of aspartame, 0.1 mM to 30 mM, was measured in L. mongoz with TBP. At no concentration did we see a preference, but a significant rejection of 10 and 30 mM aspartame ($p < 0.025$). Miraculin had no effects on the taste response to acids. Gymnemic acid did not selectively suppress the response to sucrose or other sweeteners. Thus the results with gymnemic acid and miraculin were similar to those obtained earlier in non-primates and other Prosimians. The results with thaumatin confirm the dichotomy between catarrhine and non-catarrhine species. They indicate that the sweet moieties on monellin and thaumatin are not identical, as has been assumed in some studies. Further, the results suggest that aspartame does not taste sweet to Lemuridae. This supports the conclusion that the sweetness of aspartame is limited to catarrhine species.

Membrane Properties and Transmitter Sensitivity of Merkel-like Basal Cells in *Necturus* Taste Buds

RONA J. DELAY, SUE C. KINNAMON and STEPHEN D. ROPER (Colorado State University and the Rocky Mountain Taste & Smell Center).

Over 60% of the observed synapses within the *Necturus* taste bud involve Merkel-like cells (Delay & Roper '88 J. Comp. Neurol. **277**:277-280). Not only do Merkel-like basal cells synapse with the sensory afferent nerve fibers, they also form synapses with the taste receptor cells. These synaptic connections suggest that Merkel-like basal cells may serve an interneuronal function in the taste bud. To test this hypothesis, we have examined the membrane properties of isolated Merkel-like basal cells and have tested their response to putative neurotransmitters using the giga-seal whole cell recording technique. Cells were held at -80mV and pulsed in 10 mV steps from -60 to +60 mV. After recording, the cells were fixed, photographed, and processed for electron microscopy. Light micrographs were compared with electron micrographs to locate the cell and confirm that it had the ultrastructural features of Merkel-like basal cells. Merkel-like cells had a voltage-dependent, transient inward Na^+ current followed by a sustained outward K^+ current. Therefore, we infer that Merkel-like basal cells are electrically excitable, like taste receptor cells. Merkel-like cells are small; the membrane capacitance of the isolated cells was <25 pF. This is compared with 50-75 pF for taste receptor cells in *Necturus*. Jain & Roper ('91 J. Comp. Neurol. **307**:675-682) showed that the plexus innervating *Necturus* taste buds was immunopositive for glutamate, and Taylor, Delay & Roper (this meeting) have shown that cells in the base of the taste buds are immunopositive for serotonin. We tested the response of isolated Merkel-like basal cells to focal applications of these two putative neurotransmitters. The majority of identified Merkel-like basal cells exhibited an inward current at -80mV in response to serotonin (100 μM), although a few cells showed no response. All the identified Merkel cells (11/11) responded to glutamate (5 mM) with an inward current. We are currently testing the response of this cell type to other putative neurotransmitters (GABA, ACH, aspartate) and to various neuromodulators (CCK, Sub P and CGRP).

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Monosodium Glutamate-Activated Channels in Mouse Taste Epithelial Membranes.

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Monosodium-L-glutamate (MSG) elicits a unique taste sensation in humans, called umami, which is potentiated by 5'-ribonucleotides. Although MSG taste receptors have been tentatively identified by ligand binding studies in bovine taste papillae (Torii and Cagan, BBA 627:313), little is known about their molecular properties. Ninomiya and Funakoshi have shown that mice can discriminate MSG from substances representing the classical four taste qualities (Comp.Biochem.Physiol. 92:365) and that the glossopharyngeal nerve, supplying the vallate and foliate taste buds, contains fibers differentially responsive to MSG (Comp.Biochem.Physiol. 92:371). To examine the possiblity that MSG taste receptors, like recently cloned subunits of the ionotropic glutamate neurotransmitter receptors, may contain an integral ion channel, we incorporated taste epithelial membranes from vallate and foliate papillae from C3H mice into phospholipid bilayers on the tips of patch pipettes. In a small percentage of the bilayers (< 15%), concentrations of MSG in the range that evoked neural and behavioral responses, elicited a specific, concentration-dependent and reversible increase in bilayer conductance. This conductance activated at about 0.5 mM, was half maximal at about 8 mM and saturated above 20 mM. Addition of 100 μM 5'-GMP, which alone had no effect on bilayer conductance, potentiated the response to MSG. With Ringer in the bath and pseudointracellular solution in the pipette, the MSG-activated conductance reversed between +15 and +60 mV, indicating that it was somewhat selective for Na^+ over K^+, although the channel was also permeable to Ca^{2+}. Single channel currents evoked by MSG had multiple conductance levels, ranging from 18 to over 150 pS. The high concentrations of MSG necessary to elicit a response and the potentiation by 5'-GMP are consistent with the observed channels participating in MSG taste reception.

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Characterization of amiloride-sensitive Na channels in isolated hamster fungiform taste buds.

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Amiloride-sensitive Na^+ channels present on the apical membranes of mammalian taste cells have been implicated in the detection of salty stimuli and, more recently, in the detection of acids using a non-invasive recording procedure (Gilbertson et al., *Soc. Neurosci. Abs.* 17:1216). To investigate the role of these amiloride-sensitive Na^+ channels in salt and sour taste transduction more directly, we have begun experiments to characterize these channels using both whole cell and perforated patch clamp techniques on fungiform taste buds isolated from hamster tongues. In an extracellular solution containing 140 mM NaCl, addition of 10 μM amiloride leads to reduction in a standing inward current, consistent with a decrease in Na^+ influx. This effect is accompanied by an increase in the input resistance of the cell and hyperpolarization of the cell's membrane potential. Replacement of Na^+ with N-methyl-D-glucamine ($NMDG^+$) mimics the effects of amiloride. To test whether proton permeability through amiloride-sensitive Na^+ channels contributes to the detection of acid stimuli, currents were recorded in taste cells in response to acid stimulation in a Na^+-free extracellular solution. In preliminary experiments, application of acid stimuli (3 mM citric acid, pH 4.5) at a holding potential of -80 mV leads to the development of a small inward current, which is inhibited by amiloride. This result is consistent with the intrepretation that in hamster, acid stimulation leads to an influx of protons through amiloride-sensitive Na^+ channels, causing depolarization of the taste cell. Acid stimulation also causes an increase in the input resistance of the cell which may be indicative of the effects of protons on other apical or basolateral ion channels.

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In situ recording from hamster taste cells: responses to sweet stimuli and cAMP. THOMAS A. CUMMINGS and SUE C. KINNAMON (Colorado State University and the Rocky Mountain Taste and Smell Center).

Cyclic nucleotides have been implicated in sweet taste transduction (Striem et al., Biochem. J. 260:121-126, 1989), yet their precise role remains unclear. Previous studies in our lab have shown that cAMP mimics a saccharin-induced block of K^+ currents in giga-seal whole cell recordings of isolated hamster taste cells (Cummings & Kinnamon, Chem. Senses 16:511, 1991). In this study we have used an *in situ* recording technique (Avenet & Lindemann, J. Membrane Biol. 124:33-41, 1991) to examine the effects of several sweeteners and cyclic nucleotides on taste cell activity in the intact hamster tongue. Stimuli were perfused through a recording pipette placed over the taste pore of a fungiform papilla and action currents reflecting taste cell action potentials were recorded. The control solution contained 30 mM NMDG-Cl and 5 mM HEPES, pH 7.4. The following stimuli (in mM) were added to this solution: NaCl (200), sucrose (200), NMDG-saccharin (20), 8-cpt cAMP (2), IBMX (0.1), and two high potency artificial sweeteners at 100 μM, NC-00044-AA and NC-00274-01 (The NutraSweet Company). Approximately 30% of the taste buds tested responded to NaCl and most of these also responded to the sweeteners. Sucrose and the high potency sweeteners induced a burst of action currents that adapted very rapidly. Saccharin elicited a similar response, however the response was usually less vigorous and some buds failed to respond. Long duration rinses (> 3 min) were required before the cells would again respond to the same sweetener. The responses to different sweeteners, however, exhibited little cross adaptation, suggesting that the different sweeteners bind to separate receptor sites. Since no permeant cations were required for the sweet response, the response likely involves a second messenger. The membrane permeant 8-cpt cAMP and IBMX elicited responses which mimicked those of the sweeteners, except they sometimes showed less adaptation. The responses to cAMP and IBMX did not cross adapt with those of the sweeteners, but responses to sweeteners were often potentiated after application of cAMP and IBMX. The data are consistent with the hypothesis that cAMP is the second messenger in a cascade that is initiated by a receptor-ligand interaction in the apical membrane of taste cells.

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Enhancement of Sodium-Epithelial Single Channel and Single Fiber Responses in Rat by the Antibiotic, Novobiocin. A.M. FEIGIN[1], Y. NINOMIYA[2], J.G. BRAND[1,3,4], M. KOMAI[1] B.P. BRYANT[1], M. WACHOWIAK[1] and J.H. TEETER[1,4] ([1]Monell Chemical Senses Center, 3500 Market St.; [2]Dept. of Oral Physiology, Asahi Univ. Sch. of Dent., Gifu, Japan; [3]Veterans Affairs Med. Ctr.; [4]Univ. of PA, Philadelphia, PA).

The transduction of salty taste involves the activity of sodium-transporting, amiloride-sensitive epithelial channels (DeSimone et al., Science 214: 1039). In the rat, the neural response to sodium ion is inhibited by the diuretic, amiloride, at both the whole nerve chorda tympani level and at the single fiber level. The antibiotic, novobiocin, stimulates Na transport in frog skin epithelium (Rick et al., Pflugers Arch. 411: 243). To investigate the pharmacological properties of epithelial sodium channels in taste cells, we studied the influence of novobiocin on stimulation by sodium chloride at two levels: (i) on plasma membrane vesicles (containing sodium channels) prepared from taste tissue from anterior tongue of rat and reconstituted into artificial lipid bilayers formed on the tips of patch electrodes; and (ii) on single fibers of the rat chorda tympani nerve. At symmetrical 110 mM NaCl, the conductivity of bilayers containing reconstituted sodium channels was decreased by amiloride and increased 2-5 times when 0.2-1.0 mM novobiocin was added. This effect of novobiocin was inhibited by amiloride (500 μm). Enhancement of the sodium response was also observed at the single fiber level. Sodium-sensitive fibers that displayed amiloride sensitivity showed enhanced activity to sodium in the presence of novobiocin, while more broadly tuned salt fibers - responding to sodium and potassium - that were not amiloride sensitive, did not show enhanced activity to sodium or potassium in the presence of novobiocin. These data demonstrate that the amiloride-sensitive sodium taste response is enhanced at both the single channel and single fiber levels by novobiocin, suggesting that salt taste can be enhanced by pharmacologically manipulating the peripheral receptor channels.

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G Proteins and P.I. Turnover in Bitter Taste Signal Transduction in Mice ANDREW I. SPIELMAN (New York Univ. Coll. Dent., New York), TAUFIQUL HUQUE (Monell Chemical Senses Center, Philadelphia), MAGED AYAD (New York Univ. Coll. Dent., New York), GLAYDE WHITNEY (Florida State Univ., Tallahassee) and JOSEPH G. BRAND (Monell Chemical senses Center, Philadelphia).

Sucrose octaacetate (SOA), a bitter stimulus, induced a significant increase in generation of IP_3 in taste tissue from a SOA-sensitive strain of mice (B6.SW), but was less effective in a SOA-insensitive strain (C57BL/6J). This response was GTP and Ca^{2+}-dependent, demonstrating that SOA signal transduction involves P.I. turnover and G proteins (Spielman et al., Chem. Senses, 16:585, 1991). The focus of this investigation was the nature of the GTP binding proteins involved in bitter (SOA) taste signal transduction. Intact circumvallate and foliate taste papillae from a congenic pair of mice (C57Bl/6J and B6.SW) were prelabelled with [^{3}H] myoinositol. Subsequently prepared tissue homogenates were stimulated with 10 μM SOA and 10 μM GTPγS in the presence of either 7.5 μg/ml of pertussis toxin (PTX) or 25 μg/ml cholera toxin (CTX). SOA and GTPγS-induced IP_3 production was completely abolished by PTX, but not CTX in both strains of mice. This demonstrates that SOA is mediated through a PTX-sensitive, CTX-insensitive G protein, perhaps of the Gi type. Furthermore, membrane preparations from the two congenic strains were separated on a 12.5% SDS-polyacrylamide gel, western blotted and probed for the presence of various G proteins. Using antibodies to the α subunit of Go, and Gi1-2, we have identified that both strains of mice contain GO and Gi1-2. However, the taste tissue of the SOA-sensitive strain contains at least twice as much Gi1-2 as the SOA-insensitive mice. Furthermore, the SOA-insensitive appears to contain an additional Go or Gi3, absent in the B6.SW strain. These observations point at a qualitative and quantitative difference at the G protein level between the congenic pair.

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Calcium Pumping and *Paramecium* Chemoresponse: Cloning the Pump Gene and Using Thapsigargin to Distinguish Plasma Membrane and Internal Pumps. NANCY ELWESS (University of Vermont, Dept. Zoology, Burlington, VT 05405).
JUDITH VAN HOUTEN (University of Vermont).

There appear to be at least two *Paramecium* chemosensory transduction pathways, one involving a membrane calcium pump and the other internal pH regulation. The characteristic hyperpolarization that follows stimulation with some attractants appears to be dependent upon a pump current, such as from a calcium plasma membrane pump. We have identified a major cell surface membrane-associated Ca-ATPase enzyme activity and a pump protein that are most likely one in the same. However, surface membrane preparations are complex structures containing more than plasma membrane as the potential sources of Ca-ATPase activity and the pump protein. Additionally, the role of the calcium pump in chemoresponse has been only indirectly supported (Biochim. Biophys. Acta 1029, 241-251 , 1990). Therefore, cloning the gene for the plasma membrane pump should provide oligonucleotide probes and sequences for the production of antibodies that will allow a more direct demonstration of the pump's location and role. Using polymerase chain reaction, we have cloned sequences from a family of ion pump genes. These sequences will be presented in addition to new pharmacological evidence for the assignment of the major Ca-ATPase to a pump protein of the plasma membrane. Previously, ER, mitochondria and dyneins had been eliminated as potential contaminating sources of the Ca-ATPase activity and the putative pump protein. This still leaves alveolar sacs as the only potential source of the Ca-ATPase activity other than the plasma membrane. These sacs are calcium sequestering organelles that form part of the complex cell surface membrane preparation. Thapsigargin, a specific inhibitor of ER calcium pumps, reduces the alveolar pumping of calcium but not the major Ca-ATPase activity of the surface membrane preparations. Therefore, the major Ca-ATPase activity must be from plasma membrane and most likely corresponds to the protein of 133kDa that forms the only major calmodulin-binding Ca-dependent phosphoenzyme intermediate (diagnostic of plasma membrane calcium pump). Thapsigargin may provide a useful means to study the function of the Ca-ATPase plasma membrane pump in the absence of alveolar function and better establish the calcium pump's role in chemosensory transduction.

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Gustducin: A Tasteful G Protein

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The guanine nucleotide binding proteins (G proteins) mediate signal transduction in olfactory, visual, and hormonal systems. The G proteins comprise a family of proteins which transduce an extracellular signal into an intracellular second messenger (e.g. cAMP, cGMP, IP_3). In the vertebrate taste cell, G proteins are involved in the transduction of both bitter and sweet tastants. Some bitter compounds raise the intracellular calcium concentration in rat taste cells, apparently via G protein induced IP_3. To identify and characterize those proteins involved in the taste transduction process, we have cloned G protein α subunit cDNAs from rat taste cells. Isolated rat circumvallate and foliate taste papillae were used to make polyA$^+$ RNA and a taste cell enriched cDNA library. Using degenerate primers corresponding to conserved regions of G proteins, the polymerase chain reaction (PCR) was used to amplify and clone taste cell G protein α subunit cDNAs. Hybridization with α subunit cDNA probes was also used to screen this library for G protein clones. One clone, α gustducin, is novel and distinct from other known G protein α subunits. *In situ* hybridization demonstrates that α gustducin is only present within taste buds, and apparently present within all taste buds of circumvallate, foliate and fungiform papillae. RNase protection demonstrates that α gustducin is absent from all other tissues examined. We propose that α gustducin is specifically involved in the transduction of bitter taste.

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Differential Localizations of Two Novel K$^+$ Channels of the *Shab* Subfamily in the Rat Circumvallate Papilla Epithelium.

P.M. HWANG and S.H. SNYDER (Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med.)

We have recently reported the identification of a novel K$^+$ channel belonging to the *Drosophila Shab* subfamily in a cDNA library constructed from rat circumvallate epithelial tissue (Hwang et. al., Neurosci. Absrr. 17, 1215, 1991). We designated it CDRK, indicating its original identification in the circumvallate papilla (C) and its prominent delayed-rectifier (DRK) properties upon electrophysiological recordings of the expressed channel. We subsequently cloned the full-length CDRK cDNA from a rat brain cDNA library and have fully characterized it. Since we wished to determine the localizations of CDRK and its only other rat homolog DRK1 cloned by others (Frech et al., Nature, 340, 642-645, 1989), synthetic peptide antibodies against specific regions of the two highly homologous K$^+$ channels were made and antibodies generated against them in rabbits. We now report the discrete immuno-localizations of CDRK and DRK1 proteins in the rat circumvallate papilla.

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Analysis of the protein composition of whole mouth saliva of inbred and wild mice.

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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 has 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 both ELISA techniques and electrophoresis, we compared the levels of the salivary proteins amylase , PRP, renin, NGF, and EGF, along with the total protein concentrations of the whole mouth salivas of five inbred strains of mouse and five outbred strains of mouse. Within a given inbred strain, levels of these proteins remained constant, while strain to strain comparisons indicate large variations in both individual and total protein concentrations. Extension of these data to wild mice was not always successful, as the data from ELISA studies did not always conform to those from electrophoretic and enzymatic analysis. This suggests that immunotechniques must be accompanied with another type of analysis to confirm the presence or absence of an antigen.

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Receptor-like Binding Sites in Monoclonal Antibodies for Intense Sweet Taste Compounds.

JERRY M. ANCHIN (Texas A&M University)
D. SCOTT LINTHICUM (Texas A&M University)

Knowledge-based computer-assisted molecular modelling of monoclonal antibodies is now feasible using a modelling protocol involving "canonical structures" from known crystallographic coordinates of emperically solved myeloma proteins and monoclonal antibodies. Modelling and energy minimization techniques permits the construction of interactive residues involved in ligand binding. In our study we examined the binding sites of monoclonal antibodies directed against intense sweet taste compounds derived from guanidines. The high potency of these sweetners is useful for the identification of sweet taste epitopes which bind the antibody. Some of these epitopes are probably important in sweetner-receptor interactions and our molecular modelling of the antibody binding sites has revealed structural features and chemical interactions which may be similar to those postulated for the receptor site model. Computer graphics models of eight different monoclonal antibodies were constructed using cDNA sequencing techniques, stucture-activity ligand binding immunoassays and computer-assisted modelling protocols previously developed for antibody binding sites. The study of antibody binding sites as a paradigm for taste receptor binding sites is an important concept in our understanding of ligand-receptor interactions. Detailed structure-activity relationships and molecular modelling studies of sweet taste ligands and receptors are important tools if valid comparisons are to be made.

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Direct Projection of Primary Vagal Gustatory Nucleus to the Oropharyngeal Motoneurons

K.C. DOCKSTADER and THOMAS E. FINGER (Univ. Colorado Health Sciences Ctr, Denver, CO.)

The vagal lobe (VL) in catfish is the primary gustatory nucleus in the medulla that is involved in the determination of whether potential foodstuff in the mouth will be ingested or rejected (spit out). The vagal oropharyngeal motoneurons (= nucleus ambiguus {NA}) that drive this motor behavior are situated ventromedial to the VL. The VL projects to the region occupied by the distal dendrites of the NA, however, whether any synaptic contacts exist between the secondary vagal axons and the distal NA dendrites is unknown. HRP was applied to the proximal stump of the cut vagal root to retrogradely label the neurons of the NA. In some cases, the vagal lobe was lesioned in HRP-labeled animals to produce terminal degeneration in VL output systems. A direct connection between the vagal gustatory nucleus and the oropharyngeal motor neurons would thus be indicated by the presence of degenerating axon terminals contacting an HRP-labeled dendritic process. HRP histochemistry shows that the NA motoneurons have large dendritic processes that arborize ventrolateral to the parent neurons. Morphometric analysis shows that on the average, the density of synaptic contacts on the proximal portion of these dendrites is one third that of the distal dendrites, i.e. in the vicinity of vagal gustatory terminals. There is, however no difference in the average length of synaptic appositions in the two regions. In non-lesioned animals, no degenerating terminals were found in the vicinity of the labeled dendrites. In VL-lesioned cases, electron dense degenerating axon terminals are found in synaptic apposition to HRP-labeled dendrites, suggesting that these motoneurons receive at least some direct reflex innervation from the VL. Thus the primary vagal nucleus projects directly to the motoneurons that drive the oropharyngeal muscles used in ingestive behaviors.

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Maintenance of Rat Agranular Insular Cortex *In Vitro*.

T.S. DONTA and J.A. LONDON (Dept. of Biostructure and Function, Center for Neurological Sciences, UConn Health Center, Farmington, CT.)

In order to understand the neuronal events underlying processing in the agranular insular cortex (AI), it is necessary to examine the interactions of cells in different layers. Combining optical recording techniques with organotypic cultures provides a way of doing so. As a first step towards this, we have adapted the organotypic roller tube culture method to slices of AI. This preparation results in thin sections of tissue which retain the cytoarchitectural organization that is typical of the organ from which the culture was derived. Very thin tissue is beneficial for optical recording resolution and for single neuron identification. Long-Evans hooded rats (4-7 days) were anaesthetized, their brains quickly removed and sectioned transversely at 350 um. Single sections of a hemisphere were bisected and the ventral halves containing AI were placed on glass coverslips. Coverslips were coated with poly-l-lysine to promote adherence, and a plasma-thrombin clot was used to keep the slice in place during the initial culture period. The cultures were examined for cytoarchitectural similarity to normal tissue, cell viability, and culture thickness. At 3, 4 and 10 days, cultures were stained with thionin and the vital membrane dye NK3041 to compare to normal D4 tissue. Cultures were similar to normal tissue in that the borders between layers 1 and 2, and between the intermediate (2,3) and deep (5,6) layers could be distinguished. Tissue viability was qualitatively assessed after application of trypan blue and NK3041, and by observing that process outgrowth was present around the perimeters of the cultures. With trypan blue, the majority of cells did not stain in the first 5 minutes, which indicated that most cells were alive. Using NK3041, which leaches into the cytoplasm from the stained membrane if cells are not healthy, it was noted that the vast majority of cells held the dye in their membranes. After 3 days, cultures had thinned to approximately 200 um. At 10 days, cultures were 100-150 um thick. We are encouraged by these preliminary results and feel that further analysis will indicate the suitability of this preparation for studies of circuitry.

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Intracellular Labeling of Neurons in the Secondary Gustatory Nucleus of the Channel Catfish.

CHARLES F. LAMB and THOMAS E. FINGER (Univ. Colorado Health Sci. Ctr., Denver, CO)

The superior secondary gustatory nucleus (nGS) of catfish receives input from both the facial (FL) and vagal (VL) lobes of the medulla. Efferents of the nGS project to several nuclei in the posterior part of the ventral diencephalon, including the nucleus lobobulbaris (nLB), which projects back to the FL and VL, and the nucleus centralis (nCLI) and nucleus diffusus (nDLI), which have reciprocal connections with the telencephalon. The axonal projections and dendritic morphologies of individual neurons in the nGS of the channel catfish were investigated in this study by intracellular injections of Biocytin (2% in 2M potassium acetate) into physiologically identified nGS cells. The nGS can be grossly divided into three regions - medial, central, and dorsolateral. The dendrites of a labeled cell typically could be seen densely arborizing over 300-600 μm within the region in which the soma was located, but seldom outside of that region. The projections of nGS neurons were consistent regardless of the location of the soma within the nucleus. Labeled axons traveled rostrally to the ventral diencephalon, where they turned caudad, sending collaterals to the nCLI, nDLI, and nLB as they continued posteriorly. The only exceptions were cells in the rostral face of the nGS which projected only to the rostrolateral portion of the nLB, a cell group that projects back to the VL. These results indicate that most nGS cells with ascending projections contact multiple target nuclei in the ventral diencephalon. Conversely, a restricted subset of nGS cells in the rostral portion of the nucleus (vagal taste recipient zone) project only to diencephalic cells that feed back to the VL.

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Concentration-Response Functions for Thirty Chemical Stimuli in the Hamster Solitary Nucleus.

HEATHER J. DUNCAN and DAVID V. SMITH (University of Cincinnati College of Medicine).

Studies of taste transduction have implicated amiloride-sensitive Na^+ channels in the response to sodium salts and apical K^+ channels in the response to acids (H^+). Input from amiloride-sensitive receptors appears to be restricted to NaCl-best cells in the chorda tympani nerve and in the nucleus of the solitary tract (NST). In order to evaluate the distribution of receptor sensitivities into NST neurons, we have chosen an array of 30 chemical stimuli that should be sufficient to examine the contributions of Na^+, H^+ and Cl^- receptor mechanisms to these cells. The array includes 8 sodium salts: NaCl, Na_2SO_4, $NaNO_3$, Na-acetate, Na-citrate, Na-tartrate, Na-ascorbate and monosodium glutamate; 2 lithium salts: LiCl and Li-acetate; 5 nonsodium salts: KCl, K-acetate, NH_4Cl, $CaCl_2$ and Ca-acetate; 5 acids: HCl, acetic, citric, tartaric and ascorbic acids, 5 sweet-tasting stimuli: sucrose, d-glucose, Na-saccharin, fructose and xylitol; and 5 bitter-tasting stimuli: urea, quinine-HCl, quinine-SO_4, denatonium benzoate and sucrose octaacetate. To determine concentrations of each of these stimuli that would produce equivalent responses in the NST, we recorded multiunit activity from the NST evoked by anterior tongue stimulation with five or more concentrations of each tastant. For a given concentration series, the integrated multiunit response to a standard stimulus (0.032M NaCl) was obtained between successive 1/2 log step concentrations of each stimulus. The peak of each integrated response was measured and tastant responses were expressed as a proportion of the mean of the responses to the NaCl standard presented before and after the tastant. Concentration-response functions were then determined and a concentration chosen for each tastant that produced a response equivalent to the standard. These data allow us to determine the dynamic range of hamster NST neurons to this broad array of stimuli and to select an equally effective concentration of each for single-unit studies.

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Differential Effects of Amiloride on Taste Neurons in the Hamster Solitary Nucleus.

SARAH C. NUDING & MARION E. FRANK (Center for Neurological Sciences and Dept. of BioStructure and Function, UCONN Health Center, Farmington, CT 06030)

When applied to the tongue, the sodium-channel blocker amiloride can completely suppress the NaCl responses of one class of chorda tympani neurons (N-units) in hamsters (Hettinger and Frank, 1990). The consequences of this N-unit peripheral inhibition on hamster solitary nucleus (NTS) taste-responsive neurons were measured. Thirty-three single units in the hamster NTS were recorded during tongue stimulation by three separate chemical solutions, (0.03 M NaCl, 0.1 M sucrose, 0.1 M KCl). After these normal stimulus trials, the tongue was rinsed for three minutes with 0.01 mM amiloride. Then the same series of stimuli was again presented, in the same order, but with each test solution made up in 0.01 mM amiloride. The responses of 24 % (8) of the units were extremely suppressed by the addition of amiloride to the test solutions. These amiloride-inhibited units showed the largest responses to NaCl and/or sucrose, 7 of which were greater than 20 spikes / second before amiloride. These results resemble data from the rat NTS reported by Giza and Scott (1991). 30 % (10) of the units, however, actually increased their firing rates to all three stimuli during amiloride presentation. These amiloride-released units were uniformly characterized by a small response to NaCl, which was never the best stimulus and was always < 5 spikes / second before amiloride. Fifteen of the units had a "mixed response" in that amiloride appeared to increase response rates to some stimuli but decrease rates to other stimuli. These "mixed" units generally had a moderate response to NaCl, (1.3 - 14.8 spikes / second above spontaneous), and NaCl was not their best stimulus. These data suggest that some NTS neurons transform afferent information, since amiloride-released units do not occur in the chorda tympani.

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The Effects of GABA in the Gustatory Portion of the Hamster NST: A Patch-Clamp Analysis of Cells in a Brainstem Slice.

HONGYANG LIU, MICHAEL BEHBEHANI and DAVID V. SMITH (University of Cincinnati College of Medicine).

Cells in the nucleus of the solitary tract (NST) of the rat contain the inhibitory neurotransmitter GABA and *in vitro* electrophysiological investigation in the rat has shown that several different physiological neuron types are influenced by GABA infusion. We have developed an *in vitro* brainstem slice preparation in the hamster, which we employ here to investigate the mechanisms of GABA inhibition in rostral NST neurons. We recorded the activity of cells in the rostral central (RC) or rostral lateral (RL) subdivisions of the NST using patch-clamp methods (12 cells) or conventional intracellular micropipettes (21 cells). In the patch-clamp experiment, the application of GABA evoked an outward current and an increase in membrane conductance. The reversal potential produced by GABA was close to the Cl^- equilibrium potential. The effects of GABA on the Cl^- channel were suppressed by bicuculin methiodide (BICM), a $GABA_A$ antagonist. Intracellular recording revealed that GABA caused hyperpolarization of the membrane, decreased the impulse frequency and decreased the membrane resistance. An additional 91 cells were recorded extracellularly from the rostral NST. Application of GABA produced dose-dependent inhibition in 57 cells, excitation in 12, and had no effect on 22 of the cells. In the 69 cells for which GABA was effective, the responses to the $GABA_A$ antagonist BICM and to the $GABA_B$ agonist Baclofen were tested. GABA responses in 56 cells were blocked by BICM. Another 11 cells were responsive to Baclofen. Perfusing the slice with low-Ca^{++}, high-Mg^{++} saline, which effectively blocks synaptic release mechanisms, resulted in prevention of the BICM blockade in 17 of 25 cells. These data show that the majority of GABA-evoked responses are mediated by $GABA_A$ receptors and that the response to GABA occurs via a postsynaptic mechanism. However, some of GABA's effects are mediated through $GABA_B$ receptors.

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Inhibition of Neuronal Responses in the Hamster Gustatory Cortex.

R.G. WEHBY and J.A. LONDON (Center for Neurological Sciences, Department of BioStructure and Function, University of Connecticut Health Center)

Our laboratory is characterizing the electrophysiological responses of the gustatory cortex of the golden Syrian hamster (*Mesocricetus auratus*). Anesthesia was induced with pentobarbital and then maintained with urethane. Neuronal responses were recorded using multi-unit extracellular micropipettes filled with an electrolytic solution. Taste stimulants were flowed over the anterior tongue. Taste stimulants tested were NaCl (0.1 M), KCl (0.3 M), sucrose (0.3 M), or a search solution consisting of a mixture of the three at the same final concentrations. Stimulant-elicited responses were evaluated in comparison to prior baseline activity. Following stimulus application the tongue was rinsed with water. All responses were successively replicated at least twice in each animal. We report here observation of several different types of inhibitory phenomena. (1) Inhibition of baseline activity by application of individual tastants: application of either sucrose (three animals), KCl (two animals), or NaCl (one animal) resulted in a decrease in baseline activity. (2) Inhibition of baseline activity by application of the search solution (one animal): in this animal, application of each component tastant individually did not alter baseline activity, but application of the search solution resulted in a decrease in baseline activity. (3) Long term suppression of activity normally elicited by search solution application (one animal): application of search solution first elicited robust activity. Then after NaCl was applied, subsequent application of search solution failed to elicit any responses for a period of about 5 - 10 minutes. Following this period search solution again elicited responses. Neither KCl nor sucrose elicited or inhibited any responses. These results demonstrate that gustatory processing has inhibitory as well as excitatory components.

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Excitatory Neuronal Responses in the Hamster Gustatory Cortex.

J.A. LONDON and R.G. WEHBY (Department of BioStructure and Function, Center for Neurological Sciences, Univ. CT Health Center)

This laboratory is describing the activity and location of excitatory taste responsive units in the gustatory cortex. Male, golden Syrian hamsters (*Mesocricetus auratus*) were initially anesthetized with pentobarbital and maintained with urethane. Neuronal responses were recorded using multi-unit extracellular glass microelectrodes. Taste stimulants were applied to the anterior tongue. The stimuli consisted of NaCl (0.1 M), KCl (0.3 M), sucrose (0.3 M), or a search solution consisting of a mixture of these three component stimuli at the same final concentrations. The tongue was rinsed with water for at least 1 minute between stimulus applications. Responses were replicated at least twice in each animal. Stimulant-elicited responses were evaluated in comparison to baseline activity. Locations where activity was recorded were marked with horseradish peroxidase. Several types of excitatory responses were recorded. (1) Stimulus-specific units: the activity of sucrose-specific units, NaCl-specific units and KCl-specific units was recorded. Two of the KCl-specific units responded in a bursting fashion when the stimulus was applied, and also fired a burst of spikes when the KCl was washed off. (2) Non-specific units: several units responded with an increase in activity to the search solution and to all component stimuli. (3) Off-response units. Two units in two different animals were recorded which exhibited no increase in activity when search, KCl, NaCl or sucrose were flowed over the tongue, but exhibited a burst of activity when either search or KCl were washed off. Examination of the locations of unit types indicates that similar response units are clustered. KCl-sensitive units appear in a region of the cortex separate from NaCl- and sucrose-sensitive units. The non-specific units appear scattered throughout the cortex. The KCl off-response units are located in close association with sucrose-sensitive units. Preliminary evidence suggests that neurons in the gustatory cortex exhibit several response patterns, and that some neuron types with similar or related response patterns can be clustered in the same region of the cortex.

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Responses of Single Hamster Parabrachial Neurons to Taste Mixtures: Mutual Suppression between Sucrose and Quinine. MARK B. VOGT and DAVID V. SMITH (University of Cincinnati College of Medicine).

In order to characterize CNS taste mixture responses, we are recording responses of single neurons in the hamster PbN to mixtures of sucrose and QHCl at different concentrations. Based on the responses of 29 neurons to lingual stimulation with a sucrose concentration series (0.001M, 0.01M, 0.10M and 1.0M) presented alone and mixed with 0.1M QHCl, two-thirds of all neurons displayed sucrose suppression (mixture response at least 5 imp/s < response to sucrose alone) to one or more mixture concentrations. The frequency and magnitude of sucrose suppression was greatest for those neurons most responsive to sucrose, and was greater for the mixtures containing 0.10M sucrose than for those containing other sucrose concentrations. In addition, one-third of these neurons showed QHCl suppression (mixture response at least 5 imps/s < QHCl response), and these were almost exclusively a subset of the neurons that displayed sucrose suppression. Based on the responses of 14 neurons to stimulation with a QHC concentration series (0.00032M, 0.0032M, 0.032M and 0.1M) presented alone and mixed with 1.0M sucrose, mixture responses were generally equivalent to the response to the more effective component alone. The low incidence of sucrose and QHCl suppression in these 14 cells appears to be related to the relative sucrose-insensitivity of neurons that are responsive to QHCl. A factor analysis was performed on the across-neuron correlations for both studies. In each case, two factors emerged which correlated highly with the patterns evoked by QHC and sucrose, respectively. The mixtures loaded differentially on these two factors to the extent that one stimulus dominated the response, either by being the more effective component or by suppressing the other response. Overall, these data are consistent with the mutual suppression between sucrose and quinine seen in human psychophysical studies.

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INFLUENCE OF MONOSODIUM GLUTAMATE ON RESPONSES OF CORTICAL TASTE NEURONS IN MONKEY. Y. Miyaoka and T.C. Pritchard. Dept. of Neuroscience and Anatomy, The Pennsylvania State Univ. College of Medicine, Hershey, PA 17033.

Previous research in this laboratory has shown that the taste preference of Old World monkeys for sucrose is reduced if it is dissolved in 0.03 M monosodium glutamate (MSG) rather than distilled water (DW). The present experiment examined the neural correlates for these behavioral data in an alert, female rhesus monkey. The activity of 65 taste neurons in insular and opercular cortex was measured during oral stimulation with sapid stimuli. The following stimuli were dissolved in DW and presented to the monkey with a plastic syringe (volume = 0.3 ml): 1.0 M sucrose (Suc), 0.03 M sodium chloride (NaCl), 0.3 M glycine, 0.1 M proline, 0.03 M polycose, 0.1 M malic acid, 0.003 M hydrochloric acid, 0.001 M quinine hydrochloride, and 0.03 M MSG. All but the last four stimuli also were dissolved in 0.03 M MSG and tested a second time. 1.0 M Suc also was tested in combination with 0.03 M NaCl. The criterion for a gustatory response was 3 sec of neural activity that exceeded the average response evoked by DW by 1.96 S.D. Stimulation with DW had a negligible effect upon the firing rate of these neurons (spontaneous = 2.5 $\pm$ 2.8 spikes/sec; water = 2.8 $\pm$ 2.7 spikes/sec). The average evoked response to 1.0 M Suc in sucrose-best cells decreased from 4.3 to 0.1 spikes/sec when the solvent was changed from DW to MSG. The effectiveness of sucrose was reduced by 3.4 spikes/sec when the solvent was changed from DW to NaCl. In NaCl-best cells changing the solvent from DW to MSG reduced the effectiveness of 0.03 M NaCl from 2.5 to -0.5 spikes/sec. The lower evoked responses to binary mixtures containing MSG correspond to the monkeys' reduced taste preference for these same stimuli. The lower responses of these cortical neurons to the Suc/NaCl mixture, however, was unexpected because aldulteration of sucrose solutions with NaCl does not affect the monkeys' taste preference. The data from this experiment suggest that the preference of Old World monkeys to binary taste stimuli is not directly related to the firing rate of cortical gustatory neurons.

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Cyclic Spontaneous Activity in Taste-responsive Units in the Parabrachial Pons of the Rat. SCOTT MONROE and PATRICIA M. DI LORENZO (SUNY at Binghamton)

In studies of electrophysiological responses to taste stimuli recorded from various part of the nervous system, measures of response magnitude generally exclude the spontaneous or baseline firing rate of the unit or fiber. This procedure is based on two assumptions: 1) that there is no regularity in spontaneous activity, i.e. it contains no discernible information, and 2)that the spontaneous rate of activity does not predict response magnitude. However, if there were systematic variations in the spontaneous activity of taste-sensitive neuronal elements, then it would be possible to hypothesize that there might also be a systematic relationship of spontaneous activity to response magnitude. As a first step in this investigation, spontaneous activity was recorded from taste-responsive neurons in the parabrachial nucleus of the pons (PbN) in rats that were anesthetized with urethane. A fifteen min period of activity was analyzed in five sec bins. Each time series was examine for cyclic characteristics using a Fourier analysis. The resulting cumulative variance distribution (in the frequency domain) was then compared with the distribution expected from a random process using a Kolmogorov-Smirnov test. If the results showed that fluctuations in spontaneous activity were not random ($p < 0.05$) the time series was spectrally analyzed to identify specific cyclic patterns. Linear trends were removed prior to spectral analysis. Spontaneous activity was classified as cyclic if a peak in the activity spectrum exceeded the 95% confidence limits of the spectral estimates of random data. Preliminary analyses revealed that 8 of 16 taste-responsive PbN units showed significant cyclic spontaneous activity. These units showed single peaks in the spectral analysis ranging from 0.11 - 4.33 cycles/min with a mean of 1.24 cycles/min (+/-0.63 s.e.m.). The observation of cyclic spontaneous activity did not appear to be related with the time of day that the unit was recorded. Further analyses will focus on the possible relationship of cyclic spontaneous activity to the response characteristics of these units.

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An Investigation of the Mechanisms Responsible for the Intrinsic Firing Pattern of Neurons in the Gustatory Zone of the Nucleus Tractus Solitarius. FABIEN TELL and ROBERT M. BRADLEY (Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan).

Neurons of the rostral nucleus tractus solitarius (NTS) are not physiologically homogeneous. Four basic neuron groups have been distinguished based on passive membrane properties, responses to an intracellular current pulse, and the repetitive firing response to prolonged intracellular current injection preceded by membrane hyperpolarization. Group I, II and III neurons respond to long depolarizing current pulses with a regular train of action potentials. Group IV neurons respond to depolarizing current pulses with a short burst of action potentials followed by prolonged spike inactivation. When the neurons are depolarized from different levels of membrane hyperpolarization the pattern of discharge of Group I and II neurons is altered. Group I neurons produce irregularly occurring spikes. A long delay occurs in the initiation of the spike train in Group II neurons. Membrane hyperpolarization has the least effect on the firing pattern of Group III neurons. We have investigated some of the possible ionic mechanisms underlying these intrinsic firing patterns using channel blockers in rat brainstem slices of the gustatory zone of the NTS. Recordings were made from neurons at 32°C with patch electrodes containing in mM, K-gluconate 130; $MgCl_2$ 1; EGTA 10; HEPES 10; ATP 2; $CaCl_2$ 1. Superfusion of 4-aminopyridine (4-AP; 1-5 mM) reduced the input resistance and increased the spike duration of all the neurons tested. The delay in spike initiation of Group II neurons was significantly reduced or eliminated by 4-AP application. Superfusion of tetrodotoxin (TTX; 1 μM) inhibited spike generation in all neuron groups. In Group IV neurons, although the action potential burst was eliminated by TTX, a small depolarizing hump remained that was increased by membrane hyperpolarization. These results suggest that Group II neurons posses an early transient outward A current, (I_{KA}), that is deactivated at rest. Membrane hyperpolarization removes this deactivation allowing I_{KA} expression during depolarization. The burst of action potentials in Group IV neurons might involve a low amplitude TTX-resistant depolarizing component.

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In Vitro Patch Clamp Analysis of Substance P Effect on Neurons in the Rostral Nucleus Tractus Solitarius. LIMEI WANG, MICHAEL KING and ROBERT M. BRADLEY (Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan).

Substance P (SP) has been shown to have excitatory effects on neurons in the caudal nucleus tractus solitarius (NTS) of rats (Jacquin et al., Brain Res. 502:214-222, 1989). Using whole cell recordings in brain slices of the rat medulla we have examined the effect of SP on neurons in the rostral NTS. Neurons in coronal slices of rat rostral NTS were recorded at 32°C with patch electrodes containing in mM, K-gluconate 130; $MgCl_2$ 1; EGTA 10; HEPES 10; ATP 2; $CaCl_2$ 1. Recordings were made from 43 neurons with stable resting membrane potentials and a spike overshoot. Superfusion of SP (1.7×10^{-6} or 3.7×10^{-6} M) resulted in membrane depolarization of 20 neurons usually accompanied by an increase in membrane resistance. The neurons either increased their firing rate or became spontaneously active with the depolarization. Membrane hyperpolarization by SP was also produced in 3 neurons associated with decreased membrane resistance and an inhibition of action potential production. SP had no effect on the remaining neurons. Of the 23 neurons responding to SP, X also were effected by GABA (1×10^{-3} M) which decreased the input resistance and hyperpolarized the membrane. These studies indicate that SP may have a role as an excitatory neurotransmitter in the rostral, gustatory portion of NTS. Moreover, because some rostral NTS neurons respond to both SP and GABA, complex synaptic interactions are possible during the processing of afferent sensory information.

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The Prenatal Critical Period for Chorda Tympani Terminal Fields in the Rat NTS. ROBIN F. KRIMM AND DAVID L. HILL. (Department of Psychology, University of Virginia, Charlottesville, VA 22903)

Sodium restriction on or before postconception day 8 produces a suppression in the response of the chorda tympani nerve to sodium salts, while responses to nonsodium salts remain unaffected. Placement on a sodium replete diet after postnatal-day 28 results in complete recovery of the peripheral nerve response; however, alterations in the size and distribution of the chorda tympani terminal field are permanent. Therefore, there must be a critical period, during which placement on the sodium replete diet after sodium deprivation is effective in reversing the alterations in chorda tympani terminal field. The present study was designed to determine the time frame of this critical period during development. The terminal field size and distribution was measured in deprived rats placed on the sodium replete diet on postconception day 15 (PC15-day repleted), the day of birth (birth-repleted) or on postnatal day 28 (28-day repleted) and in control rats. For visualization of the terminal field, HRP was placed on the cut nerve and the tissue was processed with a modified TMB technique after 24-hour survival. Preliminary results indicate that the terminal field size of PC15-day rats and birth-repleted rats is enlarged, corresponding in size to terminal fields of 28-day repleted rats. For all three groups, the observed increase in terminal field volume is primarily due to rostral to caudal expansion and not due to expansion of terminal field width. These results indicate that the critical period for the effects of sodium deprivation on the chorda tympani terminal field is before postconception day 15. Experiments are in progress to examine terminal field size and distribution in deprived rats placed on a sodium replete diet on postconception days 9 and 12 in order to more precisely define the boundaries of the critical period.

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ARTIFICIAL REARING DURING EARLY POSTNATAL DEVELOPMENT ALTERS THE ORGANIZATION OF GUSTATORY RECIPIENT ZONES WITHIN THE NUCLEUS OF THE SOLITARY TRACT. *PHILLIP S. LASITER* (Florida Atlantic University) and *JAIME DIAZ* (University of Washington).

Previous studies have shown that damage induced to fungiform papillae of the anterior tongue at postnatal day 2 (P2) alters both pre- and postsynaptic development of gustatory recipient zones within the rostral nucleus of the solitary tract (NST). The present study was conducted to determine whether or not artificial rearing (AR) manipulations, which reduce normal orochemical stimulation during early postnatal development, would be sufficient to produce alterations in anatomical development of the rostral gustatory NST, similar to that observed following receptor damage. Two groups of Long-Evans hooded rats were examined. One group received normal rearing with a lactating dam from birth to weaning (mother reared; MR). A second group of animals received artificial rearing via intragastric cannulae between the ages of P4 and P12, and were thereafter returned to lactating dams until the age of weaning. Following weaning and maturation to adulthood (P49), the organization of gustatory afferent terminal fields in the NST was examined using fluorescent tracing procedures which permit the simultaneous visualization of gustatory afferent terminal fields arising from the seventh and ninth cranial nerves. Results show that AR manipulations between the ages of P4 and P12 produce alterations in development of gustatory afferent terminal fields in the NST which are essentially similar to those observed following early postnatal receptor damage. These results confirm previous suggestions that orochemical stimulation during a limited portion of rats' postnatal life is essential in inducing normal development of primary afferent terminal fields in the gustatory NST.

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Decrease of Discrimination Ability for Sucrose Taste After Bilateral Sectioning of the Greater Superficial Petrosal and the Chorda Tympani Nerve. S.HARADA, H.KOHORIYAMA and Y.KASAHARA (Department of Oral Physiology, Kagoshima University Dental School, Kagoshima 890, Japan)

The greater superficial petrosal (GSP) nerve innervates taste buds on the soft palate in mammals. Electrophysiological recordings from the GSP nerve revealed that the GSP produces robust responses to sweet substances in the rat and the hamster. Behavioral experiment in the rat also suggested the importance of the GSP for discriminating sucrose solutions. To confirm this function of the GSP, in this experiment, effects of bilateral sectioning of the GSP and/or the chorda tympani nerves on discriminating sucrose taste were examined through conditioned taste aversion paradigm. Licking behavior was recorded in male hamsters. Once the animal was conditioned to avoid 0.1 M sucrose solution by i.p. injection of 0.5 M LiCl, the animal avoid to lick sucrose solution at higher concentration above 0.03 M. The degree of the aversion was significantly decreased when the GSP and/or the CT were sectioned bylaterally. The order of the sectioning effects were GSP+CT > GSP >= CT > Sham. These results suggest that the GSP plays an important role for mediating sweet taste information, which is consistent with neurophysiological data in the hamster.

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Conditioned Aversion to Lick-paired Electrical Stimulation of the Nucleus of the Solitary Tract in the Rat. PATRICIA M. DI LORENZO AND GERALD S. HECHT (SUNY at Binghamton).

Students of sensory physiology have long been aware that electrical stimulation of sensory nerves can produce a sensory experience appropriate to the modality of the nerves that are stimulated. However, despite nearly two hundred years of work it is still not possible to reproduce meaningful perceptions with artificial stimulation alone. Using the gustatory system as a model, the possibility that the temporal pattern of stimulation might enhance that meaningfulness of an artificial stimulus, e.g. electrical pulses, was studied. The first test was to examine whether an electrical pulse train, constructed to mimic the temporal pattern of the neural response to a natural tastant, could serve as a conditioned stimulus in a conditioned aversion paradigm. An array of microwires were implanted unilaterally into the nucleus of the solitary tract (NTS), the first synaptic relay in the central pathway for gustation. Following recovery from surgery, animals were placed on a 20 hr per day water deprivation schedule and trained to lick water from a drinking spout for 20 min per day in an experimental chamber. On the training day, animals received a 1 sec electrical pulse train delivered through the NTS electrodes paired with licking of water. Electrophysiological responses to sucrose in NTS units recorded from anesthetized rats were used as templates for the temporal pattern of pulses in the trains. Following this session, the NTS-CA group (n=7) received an injection of LiCl (.15 M, 1% b.w.). Training days were administered every third day until the number of licks paired with NTS stimulation fell below 200. Thereafter, NTS stimulation sessions continued, but LiCl injections were not given. Animals in the NTS-CONT group (n=4) received NTS stimulation every third day, but were not injected with LiCl. Results showed that all animals in the NTS-CA group acquired an aversion to lick-paired NTS stimulation. With repeated extinction sessions all animals in this group resumed their pretraining lick rate associated with the NTS stimulation. Rats in the NTS-CONT group showed no decrease in their lick rate across several stimulation days. The observation that behavioral reactions to lick-paired NTS stimulation can change with experience suggests that this type of stimulation constitutes a meaningful sensory experience. How critical the temporal pattern of the pulse train might be to support this type of phenomenon is the subject of continuing investigations; however, preliminary data suggest that it may be an essential feature.

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Hypoglossal Neural Activity During Taste-Elicited Rejection Responses in the Awake Rat

L.A. DINARDO and J.B. TRAVERS (Ohio State University, Columbus, OH, 43210)

Behavioral and electromyographic (EMG) studies indicate that the lingual musculature participate in the ingestion and rejection of taste stimuli (Grill and Norgren '78; Travers and Norgren '86). The role of single hypoglossal motoneurons (mXII) in producing the appropriate response, however, is unknown. This study examined the characteristics of single mXII cells during ingestion (licking and swallowing) and rejection (gaping) of gustatory stimuli in a chronic preparation to determine if cells are more specialized for one response over the other. Licking bouts were initiated by intra-oral infusions of water, sucrose, and sodium chloride, while gapes were induced by quinine monohydrochloride (QHCL). Comparison between neural activity and behavior was accomplished by recording single mXII cells simultaneously with EMG recordings from 3 oro-pharyngeal muscles. The temporal characteristics of mXII cells were determined by cross-correlating unit activity with rhythmic jaw opening (anterior digastric EMG). The temporal re-ordering of lingual muscle activity during gapes was observed at the single motoneuron level as phase shifts in the cross-correlations. Phase shifts elicited by QHCL were confirmed in a sample of mXII cells in which videographic records of lingual movements were directly compared to patterns of neural activity. Neural activity was quantified by counting the number of spikes per lick, swallow, and gape for 32 mXII cells. Cells were characterized as either excitatory or inhibitory during swallows or gapes if the number of spikes deviated by 25% or more from the number of spikes per lick cycle. Both excitatory and inhibitory responses were evident during gapes (excit. = 66%; inhib. = 22%) and swallows (excit. = 22%; inhib. = 6%) suggesting specialization in the motoneuronal pool. Nevertheless, a scatterplot of the number of spikes during gapes versus swallows showed no consistent relationship. Thus, although subsets of mXII cells specialized for different components of ingestion and rejection were evident, excitation or inhibition of a single cell during ingestion did not predict a cell's response during rejection.

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Self-Reported Illness from Chemical Odors in Young Adults without Clinical Syndromes or Occupational Exposures

IRIS R. BELL, GARY E. SCHWARTZ, JULIE PETERSON and DIANE AMEND (University of Arizona)

The present survey on young adult college students at the University of Arizona investigated the prevalence of self-reported illness from the smell of five common environmental chemicals: pesticide, automobile exhaust, paint, new carpet, and perfume. Fifteen percent of 643 students reported feeling ill from four out of five chemicals, whereas 20% reported little or no illness from any of the substances. Ratings of illness from pesticide correlated weakly but significantly with ratings for the largest number of individual symptoms (9 out of 11); daytime tiredness and daytime grogginess both correlated at high levels of significance with illness ratings (on a five-point scale) for four of the five chemicals. The chemically sensitive (CS) group included significantly more women (79%) than the chemically nonsensitive (NS) group (49%); women overall were more chemically sensitive than men ($p<.01$), even with the significant covariate of depression. Ratings of chemical sensitivity correlated only weakly with scores for depression ($r=0.16$), anxiety ($r=0.08$), and trait shyness ($r=0.18$). On stepwise multiple regression with chemical sensitivity score as the dependent measure, shyness accounted for 5.8% of the variance, while depression, anxiety, sense of mastery, and repression did not enter the equation. Histories of physician-diagnosed hay fever, but not asthma, were more frequently in the CS (16%) than in the NS group (5%). Without the confounds of chronic illnesses or specific treatment programs, these data are similar to patterns described clinically for a subset of patients with multiple chemical sensitivities (MCS), including previous data on increased nasal resistance in MCS. The findings also suggest a limited relationship between degree of self-reported chemical sensitivity and trait shyness, possibly on the basis of limbic hyperreactivity. Psychological variables did not otherwise account for any of the variance in self-rated illness from chemical odors in this nonclinical sample.

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Treatment of Olfactory Loss with Amantadine - An Open Label Trial.

ALAN R. HIRSCH, M.D. (Smell & Taste Treatment and Research Foundation)
JACK G. ARANDA (University of Illinois College of Medicine)

Despite that the D2 agonist Quipirole impairs olfactory ability in rats, since Dopamine is one of the olfactory neurotransmitters and neurologic diseases with low Dopamine have impaired olfactory ability, we hypothesized a Dopamine agonist might improve olfaction in those with reduced ability. We provided Amantadine, a Dopamine agonist, to six hyposmic and four anosmic patients. Average age was 47.6 with a range of 27 to 76 years. Both sexes were represented equally and duration of loss ranged from five months to 19 years and four months with an average of eight years and one month. Etiologies included six post viral, one sinusitis, one posttraumatic, one toxin induced and one of idiopathic origin. Four had previous medication treatments including Prednisone, Zinc and Lecithin. All underwent unilateral olfactory threshold testing by the method of Amoore to phenol, pyridine, cineole, lactone, phenone and carbinol. More than half of subjects had abnormal unilateral threshold testing to pyridine and cineole. Absolute thiophane threshold testing as by the method of Amoore revealed average threshold of 24.5 decismells on the right nostril and 16.5 decismells on the left nostril. All underwent UPSIT with an average score of 21.4 (range 10 to 38). All also underwent neurologic and psychiatric testing and none were found to have a disease associated with a known Dopamine deficiency. Patients were treated with 100 mg of Amantadine for three months and if tolerated increased to 100 mg b.i.d. for an additional three months. RESULTS: Three dropped out due to medication side effects, and of the remaining seven one hyposmic patient of traumatic origin reported olfactory improvement.

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Phosphatidyl Choline for Olfactory Problems
ALAN R. HIRSCH, M.D., F.A.C.P. (Smell & Taste Research and Treatment Foundation)
DARIN D. DOUGHERTY (University of Illinois at Chicago College of Medicine)

We hypothesized that acetylcholine deficiency may cause olfactory dysfunction. And since phosphatidylcholine can increase acetylcholine in the brain, we tested our hypothesis by giving oral doses of PhosChol, 9 gm/day, to each of ten anosmic or hyposmic patients in an open label pilot study. After three months of treatment, four of the ten said they had improved. Therefore, we proceeded with a second study: a double blind trial of the drug against placebo with 20 anosmic or hyposmic patients. However, after three months of treatment, neither experimental subjects nor controls showed measurable positive response or significant change. Further, any subjective changes did not correlate with measured changes, either among experimental or control subjects. The conflicting results between our pilot study and subsequent double blind experiment may be accounted for in various ways: too few subjects can bias results toward false negatives. Patients who dropped out of the experiment because they disliked the taste (smell) of their oral doses may have skewed the results negatively, a positive response to treatment making them more sensitive to a taste they disliked. The placebo may have unknown effects of preventing further deterioration. Or the dosage of PhosChol may have been too small; a larger dosage may be found to yield positive results. Patients' noncompliance is a possibility since blood levels of acetylcholine were not measured. And finally, olfactory tests other than those we used might have revealed a positive response.

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Subjective Improvements in Smell Dysfunctions: Relationships to Etiology and Olfactory Diagnosis. B.J. COWART[1,2], E. ROBEN[1], I.M. YOUNG[2] AND L.D. LOWRY[2] ([1]Monell Chemical Senses Center; [2]Jefferson Medical College)*

One hundred-one patients, all of whom had been found by the Clinic of the Monell-Jefferson Chemosensory Clinical Research Center (MJC) to suffer from an olfactory dysfunction, were interviewed to determine whether they had experienced any improvement in their olfactory symptoms. Follow-up interviews took place at least 8 months after the initial evaluation, and at least 16 months after the onset of the patient's olfactory symptoms. Overall, 34.7% of these patients reported that their symptoms were improved at the time of interview. However, despite the fact that smell dysfunction secondary to nasal/sinus disease is the form of olfactory disorder most amenable to treatment, only 24% of these patients reported subjective improvement, as compared to 61% of upper-respiratory infection patients and 38% of head trauma patients. Subjective improvement was even more strongly related to the type/degree of dysfunction than to etiology. At MJC, patients receive an olfactory diagnosis of either anosmia, hyposmia or dysosmia (the latter requiring both a complaint of odor distortions and a discrepancy in odor threshold and identification test performance). Only 9.1% of anosmic patients reported improvement compared with 44.4% of hyposmic patients and 71.4% of those diagnosed as primarily dysosmic ($X^2 = 26.76$, $p < .00001$). Even with the exclusion of anosmic patients, the difference between patients diagnosed as hyposmic and those diagnosed as dysosmic is statistically significant ($X^2 = 3.89$, $p < .05$). This finding lends support to the diagnostic criteria we have developed, suggesting possible prognostic significance.

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The Value of Laboratory Tests in the Nasal Dysfunction Clinic. ALFREDO A. JALOWAYSKI, TERENCE M. DAVIDSON (UCSD Medical Center, San Diego) and CLAIRE MURPHY (San Diego State University and UCSD Medical Center, San Diego)

Clinical evaluation of patients presenting with nasal dysfunction depends on a careful clinical history and physical examinaiton, supported by laboratory data. In this presentation we put emphasis in evaluating, for patients with complaints consistent with allergic rhinitis (AR), the concordance with 2 or more tests: nasal cytogram (NC), total IgE (TIgE), specific IgE (SIgE), and olfactory function (Olf). Participants in this investigation comprised a subset of 170 patients who presented to the UCSD Nasal Dysfunction Clinic in San Diego, CA. All patients had NC, 98% had TIgE, 62% had SIgE, and 62% had all three tests. The concordance with 2 or more of the tests were as follow: in 8% (8/106) of the tests all were positive in support of AR; 18% (19/106) had 2 of 3 tests positive; in 28% (30/106) only one test was positive and in 45% (48/106) all three tests were negative. Individually, TIgE was positive in 22% (23/106), SIgE in 35% (37/106) and NC in 29% (31/106) of the patients. The concordance of TIgE with NC is as follows: 68% (113/167) both tests were either negative or positive and in 32% (54/167) they were discrepant. The concordance of eosinophils with basophilic cells within the NC was as follow: 9% (16/170) both cell types were present, supporting the possibility of allergic disease; in 19% (33/170) only one type of cell was present; and, in 71% (121/170) both cells were absent. Within the group of patients, 19% (33/170) were referred for olfactory testing. Normosmia was present in 12%, hyposmia in 52% and anosmia in 36% of the patients studied. In conclusion, we find the battery of tests which include NC, TIgE, and SIgE to result in similar percentages potentially indicative or supportive of allergic disease.

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A Comparison of the Forced-Choice Ascending Method and the Up-Down Staircase Method for Olfactory Threshold Determination in a Life-span Sample STACY MARKISON, DAYNA WILNITE (San Diego State University), TERENCE M. DAVIDSON, ALFREDO A. JALOWAYSKI (UCSD Medical Center) and CLAIRE MURPHY (San Diego State University and UCSD Medical Center)*

Precise measurement of threshold is crucial if the effects of significant variables on olfactory sensitivity are to be characterized. This is particularly important in a longitudinal study where the same individual is studied over time and very small effects are followed over an age span. We set out to determine whether the forced-choice ascending method or the up-down staircase method for determining threshold for the olfactory system would produce identical data or whether one method would prove more reliable than the other. The forced-choice ascending method employed a criterion of five correct choices at a given concentration level for threshold determination. The up-down staircase threshold was computed from the last four of five reversals. A concentration series of butanol was prepared in dionized water, with the strongest concentration 4%. Each of nine successive dilutions was a third the previous one in the series. In each method, the subject chose which of two samples smelled stronger. Stimuli were sniffed from a squeezable, polyethylene bottle with a pop-up spout inserted into the nostril. Each nostril was tested separately for each subject. A total of 50 subjects, distributed over the ages 30-75 years, participated. Both males and females were tested. Subjects were given complete ENT examinations, including a history, nasal cytology, rhinomanometry, and nasal endoscopy, and only those considered to be normal in this regard met the criterion for participation in this study. Cognitive status was assessed and fell within the normal range, with the exception of one subject at risk for dementia. T-tests for matched samples showed no difference between the two nostrils for either method, thus the average threshold was used in the data analysis. Analysis of Variance with repeated measures was used to analyze the results. Data analysis showed the forced-choice thresholds to be less sensitive than the up-down staircase thresholds, $p < .0001$. In addition, standard errors were smaller for the up-down staircase procedure. The advantages and disadvantages of both methods will be discussed.

*Supported by NIH grant #AG04085 to CM

Treatment of Olfactory Loss with Amitriptyline

ALAN R. HIRSCH, M.D. (Smell & Taste Treatment and Research Foundation)
JULIE G. VANDERBILT (University of Illinois College of Medicine)

Two million Americans suffer from chemosensory disorders but an effective treatment for smell dysfunction has yet to be established. Studies suggest that modulation of noradrenergic and cholinergic neurotransmitters may be useful for this purpose. We attempted to modulate the noradrenergic system in those with olfactory dysfunction with amitriptyline, an antidepressant thought to increase amount of CNS interneuronal norepinephrine and serotonin. Eleven patients with complaints of olfactory loss of at least one year duration were treated with 10 mg of amitriptyline every night for three months. Two patients reported improvement in olfaction, seven described no change and two dropped out. This response rate may be attributed to the placebo response, an increase in norepinephrine and serotonin in the olfactory pathways or amitriptyline's antidepressant effects.

Olfactory Function in Children with Down's Syndrome.

DONALD A. McKEOWN, RICHARD L. DOTY, ANDREW MESTER, RICHARD E. FRYE & IVY SIMS (Smell and Taste Center, Dept. Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA); DANIEL P. PERL (Neuropathology Division, Mt. Sinai Medical Center, New York NY USA).

Down's syndrome is characterized by abnormalities of chromosome 21 and, later in life, with neuropathology similar to that observed in Alzheimer's disease. In the present study, we administered tests of odor detection, discrimination, and identification to 20 children with Down's syndrome, and compared their test scores to those from 20 mentally retarded and 20 non-mentally retarded controls matched on the basis of Peabody Picture Vocabulary Test scores. Upper airway otolaryngologic examinations and anterior rhinomanometry studies revealed no abnormalities in nasal airway function. No significant differences among the three groups in any of the test measures were observed. These findings suggest that young Down's syndrome patients do not evidence marked olfactory dysfunction.

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Olfactory Thresholds in Down's Syndrome. RANI NIJJAR (San Diego State University), SAMUEL JINICH (SDSU/UCSD Joint Doctoral Program), LAURA SPRINGER (SDSU), AND CLAIRE MURPHY* (SDSU and UCSD)

Patients with Down's Syndrome who have reached the age of 40 almost invariably show neuritic plaques, neurofibrillary tangles, and loss of choline acetyltransferase in the neocortex and the hippocampus, in the same areas that patients with Alzheimer's disease show them; and these patients also have abnormal amyloid protein just as the Alzheimer's patients do. Since the olfactory system is particularly vulnerable in Alzheimer's disease, both anatomically and functionally; studying olfactory sensitivity in Down's Syndrome may contribute to our understanding of both Down's Syndrome and Alzheimer's disease. As a first step we set out to measure olfactory sensitivity. A total of 46 subjects participated: 23 with Down's Syndrome (Mean age = 30.13 yrs old), and 23 Controls (Mean age = 30.17 yrs old). Cognitive status was assessed using Dementia Rating Scale (Mean score = 102.65) for Down's Syndrome and (Mean score = 141.43) for Controls. We assessed threshold for butanol using the forced-choice ascending method with a criterion of five correct choices at a given concentration level for threshold determination. The concentration series of butanol was prepared in deionized water, with the strongest concentration 4% and each of nine successive dilutions one third the previous one in the series. Stimuli were sniffed from squeezable, polyethylene bottles with pop-up spouts which could be inserted into the nostrils. Each nostril was tested separately for each subject. The subject's task was to choose which of two samples smelled stronger. T-tests for matched samples showed no differences between the two nostrils for either group, thus the average threshold was used in the data analysis. Analysis of Variance showed the thresholds of the Down's patients to be significantly higher than those of the controls, $p < .0001$. Thus, older Down's Syndrome patients do exhibit significant olfactory impairment. The implication of this finding will be discussed.

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Evaluation of the Chicago Smell Test in a Normal Population.

ALAN R. HIRSCH, M.D. (Smell & Taste Research and Treatment Foundation)
DANIEL R. CAIN (University of Illinois)

Olfaction is not frequently tested in the clinical setting. This is partially due to the difficulty in administering such tests. In order to overcome this, we devised a portable, re-usable, easily administered test of olfactory and trigeminal function. This test consists of two olfactory stimuli (isoamyl isovalerate and isoamyl acetate) and one trigeminal stimulus (menthol valerate) placed in a plastic pen-like dispensing device. Fifteen English speaking University of Illinois college student volunteers of each sex (with an average age of 24.3 years) without a history of chemosensory complaints and not currently pregnant, breast feeding, with asthma or an upper respiratory infection were tested. All inhaled each odorant and were asked if they could detect an odor as well as identify the odor if detected, and all also underwent pyridine threshold tests as by the method of Amoore. As per pyridine threshold testing, none were anosmic; all were able to detect pyridine at 55 decismells as compared to blank control. All were able to detect the three odorants and identify at least one of the odorants correctly. CONCLUSION: This test appears to be a valid discriminator of anosmia in a normal population. Studies of this in pathological states is warranted.

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Are Weber Ratios for Salty and Sour Stimuli Influenced by Age?

LALEH J. RAZANI, STACY MARKISON (San Diego State University), MAGDALENA M. GILMORE (Monell Chemical Senses Center), TERENCE M. DAVIDSON, ALFREDO A. JALOWAYSKI (UCSD Medical Center) and CLAIRE MURPHY (San Diego State University and UCSD Medical Center)*

Gilmore and Murphy (Perception and Psychophysics, 1989) found suprathreshold discrimination ability for bitter to be more affected by the aging process than discrimination ability for sweet. In the present study, young and elderly subjects' suprathreshold discrimination abilities for sodium chloride and citric acid were compared in order to further explore whether age-associated changes in human taste perception are quality specific when assessed at suprathreshold levels. Using the method of constant stimuli, we set out to determine Weber Ratios (Wrs) for sodium chloride and citric acid in subjects from two differenct age ranges, 30-50 years and 70-80 years. These subjects are participants in a longitudinal study of the chemical senses in aging, which includes ENT examinations, including a history, nasal cytology, rhinomanometry, nasal endoscopy and oral pharyngeal examination. Three standards for the citric acid (.0015, .003, .006) were prepared. Also three standards for the NaCl (.1, .2, .4 M) were prepared. Six comparison stimuli were prepared to match each of the standards. The six comparison stimuli for each standard were .70%, .82%, .94%, 1.06%, 1.18%, and 1.30% of the standard concentration. In sessions designed to provide a self-paced atmosphere sensitive to the elderly's ability to perform to capacity when not under time pressure, subjects were asked to compare the standard concentration with the six comparison stimuli at each session. Mean Wrs for the two age groups were compared separately for each sttimulus. Analysis of Variance was used to analyze the data. The results showed a significant age difference for citric acid, $p < .05$. There appears to be a trend towards the elderly having higher Wrs at the lower concentrations. The difference in the Weber ratios for sodium cloride for young and elderly was not statistically significant, $p < .10$. The results of this study indicate that the magnitude of age-associated changes in the human taste system is quality-specific and may be dependent on different underlying mechanisms.

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Efficacy of Group Therapy in the Treatment Approach to Chemosensory Disorders.

ALAN R. HIRSCH, M.D. (Smell & Taste Treatment and Research Foundation)
JONATHAN M. SCOTT, Ph.D. (Michael Reese Hospital)
SUSAN H. KOCH (University of Illinois College of Medicine)

Disease-oriented group therapy has never been described specifically for those suffering from chemosensory dysfunction. In order to assess its efficacy, four respondents to a study announcement underwent short-term time-limited group therapy for four weekly sessions, each of one and a half hours duration, with two trained psychotherapists. All subjects were women, age 28 to 39 years old (average 33.25) who suffered from hyposmia (2), hypogeusia (3), phantosmia (3), phantogeusia (1) for one to 14 years with an average of six years. Three were currently undergoing individual psychotherapy. Patients individually completed the Beck Depression Inventory and 25 questions about their feelings concerning their chemosensory dysfunction using a visual analog scale one month prior to, immediately prior to, and immediately after treatment. The one month pretreatment changes functioned as a nonintervention control. All subjects felt the experience was beneficial and wished to continue beyond four sessions. Significant improvement compared to control month included: Beck Depression Inventory (average - 2.25), Analog Scales of Depression (2 of 4), anxiety (7 of 7), anger (3 of 3), acceptance (4 of 7) and perceived empathy (2 of 4). Issues which appeared to worsen during the group therapy month included life satisfaction, emotional acceptance of loss and annoyance with lack of perceived empathy from others including physicians. Overall, disease-oriented group therapy appears to be beneficial to those suffering from chemosensory dysfunction.

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Human odor perception by multidimensional discrimination from remembered patterns

M. KENDAL-REED &
D.A. BOOTH (University of Birmingham, UK)

Our approach to studying the cognitive processes in olfaction is to map the discriminative spaces within which individuals respond when recognising familiar smells in odorant mixtures and judging their strength relative to remembered normal intensity. We are exploring the hypothesis that chemically complex odors of familiar materials are recognised by patterns of stimulation to receptors that are approximately simulable by a small number of volatile compounds. Thus we attempt to reproduce the recognisable smell of a material by mixtures of some of the compounds present in the typical headspace and/or compounds that strongly evoke notes that have been described as important contributors to a material's smell. The JNDs of each of the compounds are then calculated from the intensity ratings of the recognised smell (Booth, 1988). These JNDs enable us to model the combination of signals from these compounds as a multidimensional discrimination space (Booth *et. al*, 1991). This approach is being tested first on the aroma of a fresh fruit that has a complex headspace composition, and by addressing Cain's (1980) suggestion that the smell of leather is a mixture of fishy, goaty and oily notes. Our approach also suggests an interpretation that we are testing of the claim by Laing *et. al* (1983, 1984) that a familiar aroma from one compound can totally suppress the recognition of another familiarly smelling compound.

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A Comparison of Item and Order Processing in Olfactory and Verbal Short-Term Memory.

THERESA WHITE (Oxford University)

Memory for items in a series can be analysed into two components: Item information and order information. The question of whether item and order information arise from different sources in a serial memory task was investigated in two recognition tasks. These two tasks were performed in both the olfactory and verbal modalities. The exploration of olfactory memory has been limited by difficulty with stimulus preparation and appropriate presentation apparatus. Accordingly, a presentation device which controlled the rate of selected stimulus delivery was developed. A comparison was made between item and order information by examining the shape of the serial position curve associated with each one in olfactory and verbal modalities. The results indicated that in the olfactory modality with a short list length (5) and long inter-stimulus interval, neither task showed evidence of a primacy effect, but both tasks in both modalities exhibited a strong recency effect. The results were similar in the verbal modality when inter-stimulus interval was short, and the list length was long (10 items). A slower presentation rate in the verbal modality gave rise to a primacy effect, as well as a recency effect. Both modalities showed a difference in slope in the latter halves of the two curves. A model of retrieval is explored which assumes two related mechanisms for dealing with item and order information. The existence of a separate olfactory short-term store is discussed. This store could be qualitatively similar to the verbal store, but with a more limited capacity.

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Investigation of the Relationship Between Olfactory, Visual and Verbal Processing of Odours Using a Suppression Paradigm.

JUDITH PERKINS (University of Ulster at Jordanstown, N Ireland)

In contrast to non-scientific writers, who emphasise the role of odours in subjective human experience, the scientific literature has traditionally presented olfaction as a minor human sense. As a result, until relatively recently, psychologists have neglected to examine the contribution of the olfactory system to human psychological function. Pioneering work during the 1970's (for example Engen and Ross, 1973) supported the Proustian view that olfactory memory was unique, independent and impervious to interference. More recent studies, however, question this view (for example, Lyman and McDaniel, 1986). This current paper presents some research which investigates the relationship between olfactory, visual and verbal processing of odours, using a suppression paradigm (cf Baddeley, 1986). Recognition and recall memory for odours was tested under a range of experimental conditions which manipulated the availability of visual and/or verbal encoding resources. Results suggest that a complex relationship exists between olfactory memory and memory in other modalities, which can in part be expressed in terms of Paivio's Dual Coding Theory.

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Orthonasal and Retronasal Olfactory Sensitivity and Rated Food Preferences in Elderly Females. VALERIE B. DUFFY[1,2], ANN M. FERRIS[1], AND WILLIAM S. CAIN[2,3] ([1]University of Connecticut, Storrs, CT; [2]John B. Pierce Laboratory, New Haven, CT; and [3]Yale University, New Haven, CT)

As part of a longitudinal study to investigate the influence of measured and perceived olfactory dysfunction on food intake and food behaviors in elderly females (65 or older), we examined the relationship between measured orthonasal (butanol threshold and odor identification - Cain et al, 1989) and retronasal (flavor threshold - Duffy et al, 1991) olfaction and rated food preferences (an interviewed food preference questionnaire with 87 items and a 5-point hedonic rating scale). The olfactory measures (table 1) correlated significantly with each other ($r=0.31$, $p=.01$), however, as we previously reported, some individuals have better functioning in one domain than the other.

TABLE 1. SMELL SENSITIVITY MEASURES IN ELDERLY FEMALES

	n of Ss	$\bar{x} \pm$ Std.	Range
Orthonasal	73	4.28±1.90	0 - 7.00
Retronasal	69	2.52±1.89	0 - 6.00

Differences in olfactory sensitivity were associated with differences in the pattern of rated food preference. Orthonasal function correlated significantly with preference ratings for 13% of the foods; peaches/apricots, strawberries, oranges, grapefruit, cauliflower/brussel sprouts, cabbage, tuna fish, pasta with sauce, pizza, hot dogs and flavored yogurt, and a lower preference rating for cream. Retronasal sensitivity correlated jointly with orthonasal sensitivity only in the rated preference for peaches/apricots, but did correlated significantly with preference ratings for 9% of the foods: onions, winter sqaush, hamburger, dark breads, horseradish, black pepper and vinegar. Some of these food possess a strong primary taste component (e.g. yogurt, brassica vegetables, citrus fruits, hot dogs) or a trigeminal component (e.g. onions, horseradish, black pepper, vinegar) which may become more apparent and less pleasing to an older person with olfactory dysfunction. Further analysis of the data will examine if the olfactory sensitivity relates to food intake, if the perception of olfactory dysfunction influences the relationship of olfaction to nutrition, and finally if the differences in orthonasal and retronasal olfactory sensitivity persist in other nutritional outcomes.

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Dietary Complications of Taste and/or Smell Disorders. RICHARD D. MATTES (Monell Chemical Senses Center), BEVERLY J. COWART, DONNA M. KENNY (Monell Chemical Senses Center).

There is wide variability in dietary responses to taste and/or smell disorders and a limited ability to identify individuals at high risk for dietary complications. To address this issue, patients undergoing clinical and psychophysical evaluation at the Monell-Jefferson Chemosensory Clinical Research Center complete a dietary questionnaire and 3-day diet record. To date, data are available from 328 patients and 79 control subjects. Analyses have failed to reveal significant differences in nutrient adequacy ratios between patients with disorders of: a) taste, smell or both senses, b) long versus short duration or c) differing etiologies. However, patients who present to the clinic with a chemosensory complaint, but have no confirmed abnormality, experience a significant elevation of body weight subsequent to the onset of their purported problem. In addition, analyses involving only those patients who report alterations in dietary behavior related to their chemosensory problem reveal significant differences in body weight between those who indicate that they increase or decrease their intake. Regression analyses indicate that subject reports of altered appetite best predict percent change in body weight in each diagnostic group (i.e., problem of taste only, smell only, taste and smell) where age, gender and problem duration are the other independent factors. These preliminary observations suggest questions regarding shifts of ingestive behavior may hold greater clinical utility for identifying patients at nutritional risk than findings related to the nature of their chemosensory abnormality.

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Changes in food cravings over the lifespan.
MARCIA LEVIN PELCHAT (Monell Chemical Senses Center). CHRISTOPHER BLAKELOCK (Monell Chemical Senses Center).

We define a food craving as an intense desire or longing to eat a particular food. It is important to understand food cravings because of their influence on snacking behavior and on compliance with dietary restrictions. There has been a great deal of recent interest in the study of cravings, but little attention has been focused on the elderly. We interviewed 98 young (18-35 y.o.) and elderly ($\geq$ 65 y.o.) subjects about foods they had craved in the previous year. Young subjects were significantly more likely than elderly subjects to report cravings. Subjects on restricted diets were more likely to report cravings than were subjects on unrestricted diets. Because dietary restrictions have both nutritional and sensory consequences, a future goal will be to examine separately, the influences of sensory and nutritional deprivation on cravings. Men of both ages craved entrees (e.g. steak, pizza) more frequently than sweets and young women craved sweets more frequently than entrees. However, elderly women craved sweets and entrees with about equal frequency. This finding is consistent with the view that ovarian hormones exert an influence on cravings. Cravings were not correlated with particular moods, places or activities, but showed strong variation with time of day for subjects in both age groups.

Hedonic Ratings of Sucrose Solutions Before and After Exercise

H.P. BRAMLEY (Philadelphia College of Osteopathic Medicine, Philadelphia, Pa.) AND D.E. HORNUNG (Biology Department, St. Lawrence University, Canton,N.Y.)

This study examined the effect of the level of exercise on the hedonic ratings of sucrose solutions by members of a men's intercollegiate cross country team. The runners rode a stationary bike for 40 minutes at either 75% or 85% of their reserve maximum heart rate. Athletes used the method of magnitude estimation to rate the pleasantness or unpleasantness of a chilled, unsweetened lemonade solution alone and mixed with 5 concentrations of sucrose (2,4,8,16,32% wt/vol) before and after the exercise. Blood glucose levels were measured before and after the exercise. For the 85% group there was a significant drop in the blood glucose levels and an accompanying increase in the liking of the sucrose solutions. The 75% group showed neither a significant change in blood glucose levels nor a change in the hedonic ratings for the sucrose solutions. One interpretation of these results is that a change in hedonic ratings of sucrose will be seen only if a continuous exercise is severe enough to decrease the blood glucose level.

Perception and Pleasantness of Sweetened Foods as Related to Diet in Type II Diabetic Males.

LISA M. HARTFIEL, BEVERLY J. TEPPER (Rutgers University) and STEPHEN H. SCHNEIDER (Robert Wood Johnson University Hospital).

Previous studies examining sweet taste in diabetes have used laboratory solutions instead of actual foods. This study examined sweet taste perception and pleasantness for 2 foods in 13 normal weight, well-controlled Type II, diabetic males (age 65.8±5.4) and 11 age- and weight-matched, healthy controls. Using magnitude estimation scaling, subjects rated the sweetness intensity of a series of fruit beverage (Koolaid) and applesauce samples sweetened with 5 concentrations of sucrose: 1.5-24%, fructose: 1-18%, or aspartame: 0.025-4%. These concentrations bracket those used in commercial products. Subjects also evaluated the pleasantness of the samples using a 15 cm line scale. Samples were evaluated in 3 morning sessions after an overnight fast. Blood samples for glucose determinations were collected at each session. Magnitude estimates of sweetness intensity for both foods were similar in diabetics and controls, regardless of sweetener type. Both groups displayed similar pleasantness curves for the applesauce samples, but diabetics consistently gave lower hedonic ratings to the beverage samples. Seven-day food records revealed that diabetics consumed sucrose-sweetened foods significantly less often, but non-nutritive sweetened foods 10 times as often as controls. Thus, total dietary sweetness (i.e., sucrose, fructose and non-nutritive sweetener) was higher in diabetics than controls. Dietary sweetness level was positively correlated ($r = +.61$) with peak preference for the beverage samples in diabetics but not in controls. No relationships between fasting glucose levels and either perceived intensity or pleasantness of the samples were found. These preliminary findings suggest that diabetics experience changes in pleasantness for sweetened beverages which appear to be related to their desire for dietary sweetness rather than changes in taste perception.

Reconstitution of the Olfactory Epithelium and Re-innervation of the Olfactory Bulb after Methyl Bromide Lesions.

J.E. SCHWOB and S.L. YOUNGENTOB (Depts. of of Anatomy and Cell Biology, Physiology, and the Clinical Olfactory Research Center, SUNY Health Sci. Ctr, Syracuse, NY 13210)

In adult animals, the olfactory epithelium has a well-known capacity to recover from direct injury by generating a new population of sensory neurons. However, the degree to which the epithelium reconstitutes and the bulb is reinnervated is still somewhat poorly characterized. We have been examining the recovery of the primary olfactory projection in adult rats following lesions of the olfactory epithelium induced by a single inhalation exposure to methyl bromide gas (330 ppm X 6 hr). The rate of basal cell proliferation was determined at various time points after lesion. A variety of immunohistochemical markers for immature (e.g., anti-GAP-43) and mature (e.g., anti-OMP) olfactory neurons and their axons have been used. In addition, the status of the axonal projection of the newly born neurons onto the bulb was assessed (1) by anterograde transport of WGA-HRP, to identify when the bulb had been reinnervated, (2) by immunohistochemical staining with the monoclonal antibody RB-8, to selectively mark axons that derive from the ventrolateral olfactory epithelium, (3) by retrograde transport of fluorescent microspheres from the bulb. We find that the epithelium recovers in thickness by 2 weeks after lesion as a consequence of accelerated basal cell proliferation, but at this time it contains mostly immature neurons. The normal balance between immature and mature olfactory neurons is not struck until 6 weeks post exposure. By comparison, reinnervation of the bulb begins as soon as 1 week after MeBr, and is largely complete by 4 weeks. The newly formed axonal projection recapitulates the restricted spatial distribution revealed by the RB-8 antibody in normal rats. That is, after recovery, axons from the ventrolateral epithelium re-innervate the ventrolateral bulb, and those from the dorsomedial epithelium re-innervate the dorsomedial bulb. In conclusion, the primary olfactory projection has the capacity for both substantial recovery after MeBr lesions and the recapitulation of the quadrant-to-quadrant pattern of the axonal projection. Furthermore, the time course of anatomical reconstitution can be correlated with behavioral recovery in companion experiments described in the accompanying abstract (Youngentob and Schwob, this meeting).

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Recovery of Mucosal Inherent Activity Patterns Following Methyl Bromide Induced Lesions of the Olfactory Epithelium.
S.L. YOUNGENTOB, P.F.KENT, J.E. SCHWOB, M.M. MOZELL, and E. TZOUMAKA. (Departments of Physiology, Anatomy and Cell Biology, and the Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse NY 13210).

One aspect of the functional recovery following peripheral olfactory lesion would be the restoration of odorant-induced neurophysiological activity at some time after damage. Stated differently, does the neurophysiological activity of the peripheral olfactory system differ from "normal" when the olfactory receptors are reconstituted en masse rather than turn over in the usual piecemeal and staggered manner? To address this issue we have examined the recovery of mucosal inherent spatial activity patterns in the rat (see also Youngentob et al., this meeting) following lesions that induce the cycle of neuronal degeneration, epithelial reconstitution and axonal reconnection with the bulb. Twenty-four Long-Evans rats were allocated to the 7 day, 6 week or 3 month survival group and then lesioned by exposing them to 330 ppm MeBr gas for 6hrs. An additional twenty-four animals served as age matched controls (8 per time period). Using optical recording techniques and a voltage sensitive dye (di-4-Anepps), we monitored the fluorescence changes at 100 contiguous sites in a 10 x 10 pixel array on the olfactory mucosa of each rat's septum (Experiment 1) and medial surface of the turbinates (Experiment 2) in response to five odorant stimuli. The odorants were 2-propanol, citral, carvone, ethyl acetoacetate and propyl acetate. For each time period the recorded spatial activity patterns of lesioned animals were compared to those of age matched controls. Data will be presented describing the degree to which the inherent spatial activity patterns are restored following epithelial recovery.

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Recovery of Odorant Identification Following Methyl Bromide Induced Lesions of the Olfactory Epithelium.
S.L. YOUNGENTOB and J.E. SCHWOB. (Departments of Physiology, Anatomy and Cell Biology, and the Clinical Olfactory Research Center, SUNY Health Science Center, Syracuse NY 13210).

Several investigators have demonstrated that the olfactory epithelium has a remarkable capacity to recover anatomically following experimentally induced lesions. However, there is a paucity of information regarding the degree of functional recovery. Therefore, the present study was undertaken to assess functional recovery, as defined by psychophysical techniques, following lesions that induce the cycle of neuronal degeneration, epithelial reconstitution and axonal reconnection with the bulb. Long-Evans rats were trained to criterion (>90% correct) on a five odorant identification task (Youngentob et al., Physiol Behav. 47:1053-1059;1990) and then lesioned by exposing them to 330 ppm MeBr gas for 6 hrs. Ten experimental animals were allocated into either the 3 day survival or full behavioral recovery group (FBR). An additional five animals were allocated to either the odorant relearning control group (N=3) or stability of task control group (N=2). For each lesioned animal the anatomical state of the olfactory epithelium was evaluated relative to behavioral performance on the odorant identification task. The results of this study demonstrated for the first time that the reconstituted olfactory epithelium supports the coding of odorant quality. Furthermore, restoration of behavioral function is supported by less than complete anatomical recovery.

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Recovery of the Olfactory Epithelium and Functional Reinnervation of the Olfactory Bulb after Nerve Transection. RICHARD M. COSTANZO and NANCY L. KOSTER (Department of Physiology, Virginia Commonwealth University, Medical College of Virginia, Richmond, VA 23298-0551).

The olfactory system is an ideal model for the study of regeneration and repair of sensory systems. Olfactory receptor cells are susceptible to injury either by direct contact with the environment (i.e., chemicals, viruses, and neurotoxins) or indirectly via retrograde degeneration following injury to the olfactory nerves (i.e., head trauma). Experimental models to study recovery following chemical exposure or nerve injury have been used to investigate the remarkable capacity of the olfactory system for regeneration. Following injury, olfactory neurons are replaced by new cells that grow axons and reestablish connections with the olfactory bulb. The extent to which these reconnected axons can support function is a question currently under investigation. In our studies, we have examined the recovery of the olfactory system following nerve injury. We have developed flexible, teflon instruments that can be passed along the cribriform plate to achieve complete transection of olfactory nerve fibers. This results in a retrograde degeneration of olfactory neurons located in the nasal epithelium. Maximum degeneration occurs 3-4 days after transection. During subsequent recovery, replacement neurons grow axons that project back to the olfactory bulb and restore sensory connections. We have observed the morphological recovery of the olfactory epithelium and reconnection of axon projections to the olfactory bulb. Electrophysiological recordings from second order cells in the reconnected bulb demonstrate that replacement axons are capable of transmitting olfactory information. Bulb unit responses increase with odor stimulus concentration indicating that replacement receptors can encode stimulus intensity. These results suggest that the olfactory system is capable of recovery from nerve injury and has the capacity to restore functional connections with the olfactory bulb.

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Recovery Mechanisms in the Olfactory System of Vertebrates. Pasquale P.C. Graziadei (Department of Biological Science, Florida State University, Tallahassee, FL 32306).

The olfactory system possesses the unusual capacity of recovery after injury thanks to the presence, even in adult animals, of a disposable population of sensory neurons. These neurons, which are located in the nasal epithelium, are normally replaced through life by a continuous process of turnover and they can also be replaced when their total degeneration has been experimentally induced.

It is of primary importance to understand why these neurons are replaceable (while all other neurons of the vertebrate nervous system are not) and it is still not clear if their morphological replacement can assure the full recovery of function and behavior. Unclear is also if complete anatomical recovery is essential to assure a complete behavioral recovery.

Several experiments in different laboratories have suggested that behavioral proficiency can be reacquired even when only partial anatomical recovery is obtained. Indeed, the system seems to be endowed with considerable redundancy which, coupled with its regenerative capacity, allows the organism to minimize the traumatic effects of life (and of our experiments).

A series of transplantation experiments, where the entire olfactory bulb has been substituted with an embryonic one, demonstrate the capacity of anatomical recovery that can lead to functional recovery. This experimental paradigm, which manipulates the CNS first relay station, offers another example of the usefullness of the system for the study of this sensory pathway in the understanding of brain recovery after damage.

In the discussion several putative mechanisms in the recovery of the system will be outlined and many examples of the plasticity and redundancy of the system will be presented.

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In Vitro Patch Clamp Analysis of GABA Effects on Neurons in the Gustatory Zone of the Nucleus Tractus Solitarius. LIMEI WANG and ROBERT M. BRADLEY (Dept. Biologic and Materials Sciences, School of Dentistry, University of Michigan).

Based on whole cell recordings in slice preparations of the rostral nucleus tractus solitarius (NTS) in rats, we have shown that neurons can be separated into four groups based on their repetitive discharge patterns. Group I, II and III neurons respond to long depolarizing current pulses with a regular train of action potentials. Group IV neurons respond to depolarizing current pulses with a short burst of action potentials followed by prolonged spike inactivation. Membrane hyperpolarization alters the pattern of discharge of Group I and II neurons, and results in irregularly occurring spikes in Group I and a long delay in the initiation of the spike train in Group II. Membrane hyperpolarization has the least effect on the firing pattern of Group III neurons. Because hyperpolarization usually results from inhibitory synaptic activity we examined the effect of the inhibitory neurotransmitter GABA on rostral NTS neurons, using whole cell recording in rat brainstem slices. Neurons in coronal slices of rat NTS were recorded at 32°C with patch electrodes containing in mM, K-gluconate 130; $MgCl_2$ 1; EGTA 10; HEPES 10; ATP 2; $CaCl_2$ 1. Superfusion of GABA resulted in membrane hyperpolarization and reduced input resistance, which was a direct action on the postsynaptic membrane since it could be elicited when synaptic transmission was blocked. Increasing concentrations of GABA (2.5×10^{-4} to 1×10^{-3} M) resulted in a systematic increase in the level of membrane hyperpolarization and decrease in the input resistance. At the higher concentrations of GABA the hyperpolarization was sufficient to inhibit action potential generation. The mean reversal potential for this effect was -55 mV (± 15 SD) suggesting the involvement of Cl^- ions. Although GABA affected all neuron groups not all the neurons in each group were influenced by GABA. These studies indicate that GABA has an important role as a neurotransmitter in the rostral NTS and that inhibitory activity influences the firing properties of NTS neurons.

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NADPH-Diaphorase Activity in the Gustatory Zone of the Nucleus of the Solitary Tract in the Hamster. BARRY J. DAVIS (Univ. Alabama at Birmingham)

Both histochemical and immunocytochemical methods have been shown to be powerful tools for studying the biochemical properties of brain regions and individual neurons following initial, formative classical cytoarchitectonic, connectional and Golgi analyses. In the olfactory bulb, cell specific markers have been used quite effectively and a potpourri of neuroactive substances have been associated with several classes of neurons. Less is known about the biochemical organization of the brainstem relay nuclei that process taste information. While the function of NADPH is unknown, it co-localizes in some neurons that also express specific neuropeptides or monoamines. NADPH-staining identifies types of neurons that appear morphologically similar but yet are biochemically diverse. NADPH-staining is distributed throughout the gustatory NST and involves neurons that range in somal area from 60-500 μm^2. We regularly saw a mixture of small ovoid and larger multipolar or fusiform NADPH-stained neurons. More large neurons were stained in the ventral aspects of the gustatory zone. Dendritic staining was generally restricted to short segments of primary dendrites. A dense concentration of what appeared to be staining of terminal or preterminal axons was also observed. While NADPH-staining in the gustatory NST was substantial, the more caudal, non-gustatory NST contained significantly more stained somata and a denser accumulation of terminal staining. The distribution of NADPH-staining in the gustatory zone will be compared to that of substance P-like and tyrosine hydroxylase-like immunoreactive somata.

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Neuromodulator-Induced 'Switching' Between Swimming and Pheromone-Initiated Courtship Motor Outputs in the Blue Crab. DEBBIE WOOD AND CHARLES DERBY (Georgia State University)

Neuromodulation is a mechanism by which neural systems can achieve flexibility in their patterns of motor outflow. We are studying two rhythmic behaviors in the blue crab (*Callinectes sapidus*): courtship behavior by the male, and escape swimming. Courtship behavior is evoked by a pheromone released by female crabs. Both courtship and swimming result from rhythmic waving of the 5th legs. Courtship also contains a distinctive postural component. EMGs reveal that courtship can be distinguished from swimming because the motor program for courtship has a lower frequency of the rhythmic waving, a unique phase relationship between the 5th legs, and a rotational stationary component. A behavioral assay has revealed that proctolin can initiate the rhythmic component of courtship behavior and dopamine can initiate the postural component. In a reduced preparation, rhythmic outputs from motor nerves have been recorded which resemble either fictive courtship or fictive swimming. Electrical stimulation of any of 3 discrete regions of the oesophageal connectives evokes rhythmic motor outflow from motor nerves similar to escape swimming. Bath-applied proctolin shifts this motor pattern to one resembling the courtship motor pattern. One of these discrete neural regions also contains units that respond when pheromone is applied to the olfactory organ, as well as to mechanosensory and visual stimulation. Bath-applied dopamine and proctolin lowers the threshold dose for the proctolin effect on rhythmic output. Bath-applied octopamine diminishes the output of the rhythm when applied alone or when co-applied with proctolin but not when applied with proctolin and dopamine together. These results suggest that specific combinations of neuromodulators can influence the nature of olfactory-evoked motor output.

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Plasticity of Primary and Secondary Olfactory Projections in Goldfish. J.S. STEWART and P.C. BRUNJES (University of Virginia).

Our recent investigations relate to reorganization of the primary and secondary olfactory projections in the goldfish following various perturbations to the system. We previously reported that receptors from the anterior half of the olfactory rosette (containing the mucosa) project mainly to the medial half of the bulb, and receptors in the posterior half of the rosette terminate within the lateral half of the bulb (Stewart & Brunjes, 1991, AChemS). In this experiment, olfactory rosettes (N = 34) received ablations by microcautery to one of four regions: medial, lateral, anterior, or posterior. After a 4 week survival period tissue was collected and fixed, and crystals of DiI were applied to a single rosette region which corresponded to the lesion site. After 3 weeks, bulbs were sectioned and examined, revealing an absence of the previously documented organization of the primary projection into discrete halves. In order to investigate reorganization in secondary projections, we removed the left olfactory bulb. Six weeks later animals (n's = 5 experimental and 6 controls) received injections of ^{3}H-proline (15 μCi/animal) into the right bulb. Grain counts were taken within 6 different forebrain nuclei in both the right and left hemispheres of each animal. Using data from control animals as a standard, grain density data for 4 of the 6 nuclei examined in bulbectomized animals revealed right/left differences suggestive of a reorganization of afferent input from the remaining olfactory bulb to those regions. We conclude that both the primary and secondary olfactory projections in goldfish are capable of reorganization following insult, even in the mature animal.

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Functional and Morphological Regeneration of Peripheral and Central Olfactory Structures in Adult Goldfish

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Behavioral investigations were made with shock-free discrimination trained animals to show whether a functional (FR) or a "specific" (SR) regeneration (e.g. discrimination of amino acids preoperatively learned) takes place. Neuroanatomical investigations (mainly HRP) were made with behaviorally tested animals immediate post op. or long term after functional regeneration. Functional or specific regeneration was observed with different time courses post op: olfactory nerves (SR 10 days), olfactory nerves and crossing bulbs (FR), rostral bulbectomy (SR 10 days), caudal bulbectomy (FR 6-7 weeks), dissection (FR 6-7 weeks) or crushing (SR 4 weeks) olfactory tracts, dissection of lateral olfactory tracts (SR 10 days), dissection of medial olfactory tracts (delayed deficit, thereafter normal). No functional recovery and no neuroanatomical connections after bilateral total bulbectomy. No behavioral deficit after dissection of anterior commissure.

It is, therefore, evident that in adult lower vertebrates functional and neuroanatomical regeneration is apparent even after lesions in central olfactory pathways, and that the regenerated structures are able to inform the animals about the different stimuli they have learned.

From neuroanatomical data taken immediately after specific regeneration it is evident that even with a small number of regenerated fibers and also with reduced neuronal nuclei, adequate behavior can be recorded.

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Tyrosine Hydroxylase Activity and Immunoreactivity Decline Slowly Following Unilateral Naris Closure In Adult Mice. HARRIET BAKER and KIMBERLY MOREL (Cornell Univ. Med. Coll. at The Burke Medical Research Institute).

Tyrosine hydroxylase (TH), the first enzyme in the catecholamine biosynthetic pathway, is expressed in dopamine (DA) neurons intrinsic to the main olfactory bulb. Peripheral afferent dennervation of the olfactory bulb produced by chemical destruction of receptor cells results in a profound reduction in TH activity within the DA neurons (to 40%, 18% and 6% at 7, 15 and 30 days post-lesion, respectively; Nadi et al. Brain Res. 1 365, 1981). Recently we have shown that unilateral naris closure in adult mice also results in large reductions in TH activity, immunoreactivity and mRNA. These changes, measured 2-6 months after closure, occur without apparent loss of afferent innervation. The experiments described here investigated the time course of the decrease in TH immunoreactivity and activity following adult naris closure. Our hypothesis was that the continued presence of afferent innervation might slow the rate of disappearance of TH expression as compared to the effects of chemical deafferentation. Immunohistochemical analysis indicated a small, but perceptible, loss of TH expression within perikarya and glomerular processes of the ipsilateral bulb at 7 days post-closure. Larger reductions were observed in both perikarya and processes 2 weeks after closure with almost complete loss of TH immunostaining at 2 months. Biochemical analysis supported the apparent slow loss of TH expression. TH activity ipsilateral to the closure (expressed per mg protein) was reduced to 50% and 24% of the contralateral bulb at 7 and 14 days, respectively. These data indicate that a surprisingly small difference exists in the rate of loss in TH expression following closure as compared to chemical deafferentation. The maintenance of TH expression over a prolonged period (up to 2 weeks) in both paradigms is unexpected given the rapid regulation of TH following other perturbations. These data suggest that functional as opposed to structural integrity of the receptor afferent innervation of the olfactory bulb DA neurons is critical for maintenance of TH expression.

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Contributions of Neurogenesis and Cell Survival to the Net Loss of Olfactory Bulb Granule Cells that Follows Naris Closure in Adult Mice. FRANK COROTTO, JEFF HENEGAR AND JOEL MARUNIAK (Univerisity of Missouri - Columbia).

In adult mice, naris closure leads to atrophy of the ipsilateral olfactory bulb and a decrease in the number of its granule cells (Henegar and Maruniak, Brain Res. in press). This net loss of granule cells may result from reduced neuronal survival, reduced neurogenesis or both. Here we will assess the contributions of each of these possibilities. While adult granule cell neurogenesis is well established in rats, to our knowledge the phenomenon has not been established in mice. We hope to demonstrate olfactory bulb granule cell neurogenesis in adult mice by injecting animals with bromodeoxyuridine (BrdU) followed by a 30 day survival period and subsequent immunoflourescent detection of the label in semi-thin 1-2 µm paraffin sections. To examine the effects of naris closure on granule cell neurogenesis, we injected animals with BrdU to label S-phase stem cells and sacrificed them 1 h. later. Then we counted the number of labelled cells in the subependymal layer extending from the lateral ventricles into the olfactory bulbs since granule cell neurogenesis is thought to occur throughout this region. We found a highly significant difference in the number of BrdU-labelled nuclei between the open and closed sides in and caudal to the olfactory bulbs. If we confirm that granule cell neurogenesis persists in adult mice, then this difference in cell proliferation presumably reflects a difference in neurogenesis. Since a net loss of granule cells could also come about by reduced cell survival, we are presently counting pyknotic nuclei to determine whether or not naris closure affects the rate of cell death in the olfactory bulb granule cell layer.

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Morphological features of developing dendrites of putative mitral cells of the rat main olfactory bulb. SHIGERU TAKAMI & PASQUALE P. C. GRAZIADEI (Department of Biological Science, Florida State University, Tallahassee, FL 32306).

In the main olfactory bulb (MOB) of many mammals, each mitral cell sends a single apical dendrite into the glomerular layer (GL) to form a single glomerular arbor which receives synaptic inputs from olfactory receptor axons. A light microscopic Golgi observation on the putative mitral cell dendrites in the MOB of developing rats will be reported here. Sprague-Dawley rats 21 days of gestation (E21), 0 day old (P0), P1, P3, P7 and P14 were used. For E21 rats, the heads were immersed overnight in a mixture of glutaraldehye (GA) and paraformaldehyde (PFA) containing buffer and the brains were successively dissected out. At other ages, rats were transcardially perfused with Ringer's solution and then with a mixture of GA and PFA. Brain blocks including MOB and the acccessory olfactory bulb were processed by using one of the following methods: rapid Golgi, Golgi-Kopsch (G-K), and a modified G-K. Afterwards, the brain blocks were embedded in resin and 100 µm thick sagittal sections were made by using a sliding microtome. The modified G-K method was most sucessful in the present study. In E21 rats, the glomerular arbors of putative mitral cells could not been seen. Instead, the putative mitral cells expanded several stem dendrites directed to the GL. Some branches of these dendrites reached and entered the GL. The same pattern was also seen in P0 and P1. In some putative mitral cells at these stages, the branches of dendrites reached the surface of the GL and the dendritic branches spread out as far as 300 µm apart. Some putative mitral cells in P0 and P1, however, had single apical dendrite similar to those in adult animals. At P7, the morphological features seen in the putative mitral cells acquired the characteristics of adults. The present results suggest that the majority of mitral cells of rats mature in the first week after birth. The process of maturation seems to consist in the dramatic reduction and reorientation of their preliminary branching dendrites which initially project to large areas of the GL spanning over a diameter of some 300 µm.
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Morphometry and Cellular Dynamics of Denervated Fungiform Taste Buds in the Hamster. SCOTT D. OLIVER (Math/Science Div., Shawnee State Univ., Portsmouth, OH) and MARK C. WHITEHEAD (Anatomy Div., UCSD, La Jolla, CA).

Denervation of gustatory papillae, for most species, results in a virtual disappearance of taste buds. This is not the case for hamster fungiform papillae, which contain taste buds that survive denervation. To characterize these taste buds, in this study, counts and measurements were made of all buds on the anterior 3 mm of the hamster tongue at 36 or 91 days after resecting the chorda/lingual nerve. Taste bud numbers were, at both time periods, unaffected by denervation. However, bud dimensions were affected with denervated buds 25-30% smaller than control ones. Counts of taste bud cells suggest that decreases in bud size may result from shrinkage but not a loss of cells. Tritiated thymidine autoradiography was used to evaluate whether denervation influences the mitotic activity or the migratory pattern of bud cells. For every animal, the average number of labelled cells per bud was slightly lower on the denervated than the control side of the tongue. However, when labelled cell positions were evaluated at .25, 3 and 6 days after thymidine, the distances from the sides of the bud increased at increasing times after injection for both the innervated and the denervated buds. Stem cells were located laterally or basally in the bud. Labelled cells that migrated into the centers of the buds were few and seen only at the 6 day post-injection time in both control and experimental buds. The moderate effects of denervation on taste bud sizes and mitotic activities may indicate a generalized atrophy. Remarkably intact were taste bud numbers and the migratory patterns of cells, features of anterior tongue taste buds that, in the hamster, are relatively invulnerable to denervation.
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Changes in distribution of tenascin correlate with gustatory papilla and taste bud development and morphogenesis in fetal sheep. L. F. HAUS and C. M. MISTRETTA (School of Dentistry and Center for Human Growth and Development, Univ. of Michigan, Ann Arbor, MI 48109).

The extracellular matrix molecule, tenascin (TN), appears in mesenchyme tissue that surrounds differentiating epithelia in a temporally regulated manner that suggests a role in the morphogenesis of various organs. However, TN distribution has not been studied during development of taste papillae and buds. We localized TN with immunocytochemistry in developing fungiform and circumvallate papillae from fetal sheep using a rabbit anti-human polyclonal antibody against tenascin (Telios). Early stages of papilla formation were examined in tissue from 60 and 65 day fetal sheep (term = 147 days). Advanced stages of papilla development, with associated taste bud multiplication, were studied in 100 and 110 day tissue. In fungiform papillae, TN staining at 60 days is absent or very weak in the dermal core of the papilla, but is homogeneously distributed in mesenchyme subjacent to anterior tongue epithelium. By 65 days TN is apparent in diffuse rivulets throughout the papilla core. In the more developed, 100 day papilla, TN stains intensely in the apical papilla core and in a horizontal band in the reticular layer of the dermis parallel to the epithelium. At 110 days TN is highly localized in the apical papilla core under gustatory epithelium, and is virtually absent elsewhere. Within circumvallate papillae, TN is distributed more densely in the papilla walls than in the central (taste bud-bearing) papilla at 60 days. By 65 days TN is distributed evenly in walls and central papilla. The distribution then changes again, and at 100 and 110 days there is more TN staining in the central papilla and very little in the walls. In both fungiform and circumvallate, TN staining becomes progressively restricted to gustatory regions of the papillae during periods of significant growth, alterations in shape, and taste bud multiplication. A role for the matrix molecule TN in taste papilla morphogenesis and taste bud proliferation is suggested.

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Nerve Growth Factor-Receptor is exhibited in non-gustatory nerves of the developing rat tongue but is absent in the gustatory nerves. ALBERT I. FARBMAN, JUDITH BUCHHOLZ (Dept. of Neurobiology, Northwestern Univ., Evanston, IL) and JOSEPH-PASCAL MBIENE (Dept. of Biology & Materials Science, Univ. of Michigan School of Dentistry, Ann Arbor, MI.)

Each fungiform papilla on a mammalian tongue is innervated by two sensory nerves: 1) the chorda tympani (CT), which innervates taste buds, and 2) the lingual, a branch of the trigeminal nerve, which provides somatosensory innervation to the papilla but is essentially excluded from the taste bud. This innervation pattern is evident during early development and maturation. It is possible that the two nerves develop contacts with cells that provide specific trophic factors required for survival of the contacts. Trigeminal neurons are known to be dependent on Nerve Growth Factor (NGF) for their survival. We examined the developing rat tongue for the presence of the NGF-receptor by an immunohistochemical method. Trigeminal nerves and their perigemmal terminals contained NGF-receptor-like immunoreactivity whereas CT nerves within taste buds did not. Moreover, in the region of the circumvallate papilla, the non-gustatory nerves of the glossopharyngeal nerve exhibited NGF-receptor-like immunoreactivity but gustatory nerve terminals within the taste buds themselves did not. The results suggest that NGF may be one of the determinants of innervation specificity in the region of the taste buds by attracting non-gustatory nerves to tissue surrounding taste buds, whereas taste buds may produce different trophic factor(s).

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Epidermal growth factor (EGF) enhances the rate of cell division in olfactory epithelium in vitro. ALBERT I. FARBMAN, SHUBHIK DEBBURMAN AND JUDITH A. BUCHHOLZ (Dept. of Neurobiology, Northwestern University, Evanston, IL 60208)

Vertebrate olfactory sensory cells are unique neurons because they are continually replaced throughout the lifetime of the animal. We have shown previously that the rate of cell division (and, concomitantly, cell death) in the olfactory epithelium can be up- or down-regulated by experimental manipulation (Farbman et al., 1988, J. Neurosci., 8:3290; Carr & Farbman, 1992, Exp. Neurol., in press). This implies the existence of factors that regulate both mitotic rate and the rate of cell death in the olfactory system. We have devised an *in vitro* method to evaluate the efficacy of agents in regulating mitosis in olfactory sensory epithelium. Organotypic cultures of olfactory mucosa from 18 to 20 day rat fetuses were grown in a serum-free medium supplemented with 10 to 250 ng/ml of EGF for 3 days. Controls had no EGF. The cultures were then exposed to medium containing [^{3}H]-thymidine for 1 hour, fixed and prepared for autoradiography. Results showed an increase in the number of [^{3}H]-thymidine-labeled cells in the olfactory epithelium of the cultures related to the dose of EGF. The highest doses elicited an increase of more than twofold. It is possible, then, that EGF receptors in olfactory epithelium are linked to the mechanism regulating mitosis.

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Olfactory Receptor Neurons Express Luteinizing Hormone Releasing Hormone (LHRH) during Development

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Both LHRH neurons and olfactory receptor neurons (ORNs) are derived from the olfactory placode. Using an antibody to LHRH (LR-1, gift of Dr. Robert Benoit) and the avidin-biotin-HRP technique (Vectastain), we examined the temporal and spatial distribution of LHRH expression in cells of the developing chick olfactory epithelium. No cells were immunostained in control sections incubated in LHRH antisera preabsorbed with LHRH (Peninsula) (0.1μg/ml). Both darkly staining and lightly staining LHRH cells are found within the olfactory epithelium of embryonic chicks. However, the darkly and lightly staining cells have different temporal and spatial distributions within the olfactory epithelium. Darkly staining LHRH neurons are found in the olfactory epithelium from embryonic day 4 (E4) to E9. Lightly staining LHRH neurons are found from E7 to at least E12 and possibly later. Some of the darkly staining cells are found in clusters in the rostral-medial portion of the olfactory epithelium, in close proximity to the respiratory epithelium. Some of these cells resemble olfactory receptor neurons. The cells in the rostral-medial epithelium are continuous with a tangentially oriented cell-stream extending caudally within the olfactory epithelium. These cells appear to exit the olfactory epithelium through a gap in the basal lamina and continue their migration within the medial half of the olfactory nerve. Many lightly stained LHRH cells are observed in both the medial and lateral halves of the olfactory epithelium. All of these cells have a radial orientation and closely resemble olfactory receptor neurons, i. e., they possess a process which extends towards the lumen. Structures resembling dendritic knobs can be observed on many of these dendrites. The lightly staining LHRH neurons of the olfactory epithelium do not assume the tangential orientation of migrating cells seen with darkly stained cells in the medial epithelium. In addition, the lightly stained cells are not found in the olfactory nerve. One interpretation of these results is that the darkly staining LHRH cells represent neurons destined to migrate to the brain while the lightly stained LHRH cells may be olfactory receptor neurons which express LHRH transiently during development. Additional experiments are needed to confirm this interpretation. This work was supported by NIH grant NS30047.

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Squeeze-Bottle Test Kits for Specific Anosmia: Demonstration and Applications.

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This odor-threshold test system has been in clinical use since 1987. The 60-ml polypropylene squeeze-bottles contain 13 ml of odorized (test) or odorless (blank) light mineral oil absorbed in 1.6 g of polypropylene felt. The subject squeezes the headspace air toward the nostrils, and decides which bottle has the odor. A descending concentration series in quarter-log steps is used to determine the subject's threshold, which is expressed in "decismel" units that are closely analogous to the familiar decibel scale for hearing. Earlier research by this author and his associates has delineated eight classes of specific anosmia. For each of these a single pre-eminent compound has been identified that exhibits the greatest degree of olfactory defect for specific anosmics among its tested chemical homologs and analogs, and usually the lowest odor threshold concentration for normal observers. Five of these compounds can be conveniently presented in mineral oil solutions in the squeeze-bottle format, permitting up to 100 threshold tests and a shelf-life of six months or more. They are isovaleric acid, androst-16-en-3-one, pentadecalactone, l-carvone and 1,8-cineole. Their specific anosmic frequencies in the population range from 3% to 47% (subjects that cannot smell the compound at 25 dS or about 16 x the normal mean threshold concentration). Several more compounds revealing different classes of specific anosmia will also be demonstrated. One intriguing application of this test method would be to seek correlations between specific anosmia phenotypes and transmembrane receptor genotypes. It could also provide a logical basis for blending the corresponding putative "primary odors" in flavor research, for an understanding of air and water pollution, and in seeking clinical associations.

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A Comparison of Experienced and Naive Subjects for Olfactory Acuity and Variability in Detection Threshold Testing

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Although sensory panels are widely used to measure the olfactory properties of perfumes in household and personal products, there are still many aspects of human olfactory perception which are poorly understood. In particular it is not known why olfactory sensitivity varies between subjects, nor is the extent of that variation well understood. Also, the effect of previous olfactory testing experience on acuity and consistency of performance has not been established. This work compares olfactory acuity and variability of naive and experienced subjects. Detection threshold testing of single odorants was carried out on each of 5 successive days by 9 naive subjects and by 9 subjects with long experience of functioning as a perfume quality profiling panel. Six odorants were studied in all. Experienced panellists did not appear to have generally greater acuity than naive subjects, suggesting that acuity, at least to the stimuli tested, appears to be inherent, with little scope for development. Day-to-day variability within subjects was as great as inter-subject variability, stressing the importance of repeat testing to obtain mean values for each panellist. Even allowing for variability the results suggested real differences in acuity between panellists, with some scoring consistently above average and some scoring consistently below. Variability within subjects tended to be lower for experienced panellists.

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Perceptual And Verbal Components Of Short-Term Odor Memory.

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In a previous experiment on odor memory for familiar and unfamiliar odors, subjects performed a same / different task on two odors separated by intervals of 8, 20, 40 and 100 sec. The results showed significant forgetting after 20 sec, with poorer memory performance for unfamiliar odors. Reaction times increased significantly as interval length increased (Schab, de Wijk and Cain, AChems 1991). In an additional experiment using shorter intervals, memory for familiar odors was tested with both perceptual (odors) and verbal (odor labels) test stimuli. Thirty-three subjects participated in a same / different task in which odors were followed either by odors or odor labels after 2, 6, 18 or 60 sec. Afterwards, subjects sought to identify all 72 test odors. Memory performance was highest for correctly identified odors, irrespective of whether it was tested with perceptual or verbal stimuli (8.1% and 9.5% errors, respectively). Memory performance for incorrectly identified odors was significantly lower, especially if tested with verbal stimuli (13.6% and 25.6% errors). Memory performance for incorrectly identified familiar odors was essentially equivalent to memory performance for the unfamiliar odors used in the first experiment. Although memory performance was only marginally affected by interval ($p=0.07$), reaction times increased significantly ($p < 0.001$). The results suggest that even very short-term odor memory entails both perceptual and verbal information.

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Viewing Time and Liking of Slides in the Presence of Congruent and Incongruent Odors.
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Ninety subjects (45 women and 45 men) between the ages of 18-35 participated individually in the lab study. Twice, subjects viewed a set of 24 slides; six were pictures of chocolate items, six were pictures of babies, and twelve were pictures of other things. While subjects viewed the slides the room was scented with chocolate odor, baby powder odor or no odor, depending on the condition to which they had been randomly assigned. Pilot testing had found that both chocolate and baby powder scents were rated as pleasant, both scents were easily recognizable and neither scent was confused with the other. Pilot testing had also found that people rated the chocolate slides as being congruent with the chocolate scent but incongruent with the baby powder scent and vise versa. During the first viewing, subjects looked at each of the slides for as long as they liked while, without their knowledge, the computer recorded the amount of time they looked at each slide. During the second viewing, subjects rated each slide on three 9-point scales: how pleasant the slide was, how interesting the slide was, and how easy it was to imagine oneself in the slide. The computer timed how long subjects spent answering the questions for each slide. After the second viewing, subjects completed questionnaires concerning their mood, health and perceptions of the environment. Following this, they were taken into another room where they completed a questionnaire concerning the smell of the testing room and answered questions about scents they sniffed in plastic bottles.

This research was supported in part by a grant from the Fragrance Research Fund.

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The Effect of Olfactory Stimuli on the Time Spent by Costumers in Textile-Department-Stores.
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(Psychological Laboratory, University Utrecht, The Netherlands)

This study investigated the relationship between the presence of odorized air in textile-department-stores and the time costumers spent in these stores. Three stores in different city's, total more then 7,500 m^2 salesfloor, participated and were assigned to one out of three conditions; two odour conditions and one control. Each week the stores were exposed to another condition so after nine weeks each store had received all conditions three times. Well known concentrations of the odorous substances were injected into the airconditioning system at certain pulsfrequencies. During a nine week period, more then 4100 costumers were observed and, among other variables concerning shopping behavior, the time subjects spent in the store was registered. The results indicate that the presence of odorized air results overall in an increase in shopping-time, and is most effective in the afternoon and evening. It therewith counteracts the general tendency of the costumers to spend less time per visit as the day progresses. This effect is also dependent on the age of the costumers.

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A Laboratory Test of the Proustian Phenomenon: Memory for Odors with Emotional and Neutral Associations.
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(Trinity University).

The well-known Proustian phenomenon, in which odors bring back vivid and emotional memories, is difficult to study because of the idiosyncratic nature of such memories. We attempted to study the Proustian phenomenon in the laboratory by creating associations between odors and emotional or neutral scenes. Odors were paired with photographs that portrayed neutral or emotional events (e.g., a toothpaste scent paired with a photo of person brushing their teeth or a person having a tooth drilled by a dentist). In one condition, subjects were asked to make up a story about themselves participating in the scene. In a second condition, subjects were asked to describe the appropriateness of the odor to the scene. In a second phase, all subjects rated additional odors for pleasantness (no photo present). One week later, subjects took part in a yes-no odor recognition test. Odor recognition performance was affected to various degrees by pairing an odor with a photograph, the emotional tone of the photo, and the level of personal involvement in describing the scene. Memory processes involved in the formation of odor-emotional memory links and possible relevance to the Proustian phenomenon are discussed.

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The Emotional Nature of Odor-Evoked Memories
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Memories evoked by odors have long been characterized by their distinctive emotional and personal salience, although little empirical evidence exists to substantiate this claim. Two experiments were conducted, with 32 subjects in each (16 male, 16 female), to test whether memories associated with odors differed from memories associated with words in terms of their emotional quality. The experimental paradigm was the same in both studies; each was conducted in two phases, study and test, separated by a 48 hour interval. Subjects were told that the purpose of the experiment was to investigate the effects of odors on the perception of art. In an incidental learning procedure on the study day, subjects smelled 8 odors, and were told the names of 8 odors (word condition) while they viewed 16 different emotionally evocative visual stimuli (paintings). Cue (odors and words) and painting combinations were completely crossed and counterbalanced across male and female subjects. Two days later, in the same lab setting, memory for the paintings and the feelings that had been associated with them were tested by cued recall. Results of these experiments together revealed that when paintings were correctly recalled with an odor cue, the descriptions were significantly more emotional than when they were recalled with a word cue. In addition, more emotions were listed and more intense feelings evoked (irrespective of painting recall) when the triggering cue was an odor. Subjects were also significantly more confident that the feelings brought back by the cues on the test day were those they had experienced on the study day when the triggering cue was an odor as compared to when it was a word. These experiments thus provide convincing empirical evidence that memories evoked by odors are distinguished from memories evoked by other cues, such as words, by their emotional quality.

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Effects of androstenone dissolved in ethanol or light mineral oil on the assessment of men by women considering ratings of pleasentness and oral contraception

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102 female students took part in the double blind study wich was conducted by two female experimenters. Aqua dest. (control) or 5alpha-androst-16 en-2-alpha-on dissolved in a) pure ethanol or b) light mineral oil was applicated on the upper lip of each subject prior to testing. Each subject rated 5 photographs of men (random order) of differnt levels of attractiveness (estimated before in a separate study) concerning their attractiveness and other attributes on 30 bipolar adjective scales and on an attractiveness scale. The subjects state of the mood was measured prior and after testing by a standardized test. The subjects were tested for anosmia to androstenone. Concerning the assessment of men we found a significant difference between the osmic and the anosmic women. The osmic women evaluate men significantly different when they assess the odour of androstenone as pleasent, neutreal or unplesent. The use (respectively no use) of oral contraceptives has a significant influence on the evaluations. The assesment of men is connected with the perceived pleasentness of androstenone. The assessments show significant differences when androstenone is dissolved in light mineral oil or in ethanol. Androstenone in light mineral oil shows a stronger impact on the evaluation of men. This effect is related to the ratings of pleasentness. There is a significant interaction between the menstrual cycle and the ratings of pleasentness.

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Individual Differences in the Perceived Intensity and Quality of Specific Odors Following Self- and Cross-Adaptation. ROBERT J. O'CONNELL (Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545), DAVID A. STEVENS and LUCIA M. ZOGBY (Clark University, Worcester, MA 01610).

We continue to explore the individual differences within a set of subjects classified by their ability to detect and identify the odor of the diastereoisomeric ketone, *cis*-4-(4'-t-Butylcyclo-hexyl)-4-methyl-2-pentanone (pemenone) which shares with 5α-androst-16-en-3-one (androstenone) a pronounced urine-sweaty type odor. We previously determined intensity ratings and quality reports for suprathreshold and threshold concentrations of these compounds and several other materials described as urinous or which were said to exhibit specific anosmias (O'Connell *et al.*, *Chemical Senses* 14:293-302, 1989; Stevens and O'Connell, *Chemical Senses* 16:57-67, 1991). Principal-component analyses of these data revealed significant relationships between near-threshold and suprathreshold intensity scores for pemenone, androstenone and several of the other odorants and a corresponding clustering of the odor descriptors used to characterize these materials by subjects judged to be anosmic for the urinous note. Here, we attempt to dissect further the web of interactions which give rise to perceived odor quality by evaluating the effects of pemenone adaptation on the perceived intensity and quality of pemenone, androstenone and four other odors. Eighteen human subjects including both pemenone osmic and anosmic (N=8) individuals were tested. Following a 2 minute adaptation period during which subjects sniffed the test odorant, pemenone or the diluent, subjects judged the intensity and quality of each compound in the test sequence. For each odorant, intensity ratings under the different adapting conditions were compared by ANOVA. Self-adaptation was universal but cross-adaptation by pemenone was found only for androstenone. For most of the subjects osmic for androstenone, it retained its urinous quality following pemenone adaptation, but for most of the anosmic subjects the quality of androstenone changed after adaptation. This suggests that the urinous odor quality for these individuals is more diverse and multidimensional than in osmics. These, and a number of other findings which collectively provide clues about quality perception and the defects which give rise to specific anosmias are described.

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Do Similar-Smelling Odorants Stimulate the Same Olfactory Channels? Evidence from Psychophysical Studies.
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Psychophysical studies of odor discrimination, olfactory cross-adaptation, and maintenance of and recovery from cross-adaptation offer insights into whether similar-smelling odorants stimulate the same olfactory channels. Odorants sharing the same olfactory channel(s) should not be discriminable, should show mutual, symmetrical cross-adaptation, and should display similar patterns of recovery from cross-adaptation. Deviations from this predicted pattern may reflect channel differences, e.g., in the population of receptors stimulated. Two pairs of similar-smelling odorants were used. In triangle discrimination tests, eight subjects were unable to discriminate between 5α-androst-16-en-3-one (androstenone) and a synthetic functional analog, 4(4',4'-dimethylcyclohexyl)-2(R)-methyl-cyclohexanone. Further, these urinous compounds, when matched for perceptual intensity, displayed mutual, symmetrical cross-adaptation and similar patterns of maintenance and recovery following cross-adaptation. These results suggest that androstenone and the analog may be utilizing the same olfactory channel(s). Conversely, in the triangle tests, the same subjects reliably discriminated between two synthetic musks, Galaxolide® and Thibetolide®. Tests using equivalent intensities of the two musks also produced asymmetric cross-adaptation; adaptation to Galaxolide resulted in more complete cross-adaptation to Thibetolide than vice versa. Recovery from adaptation was similar for both musks, but more depressed following adaptation to Galaxolide compared to adaptation to Thibetolide. These data suggest that the channels stimulated by Thibetolide may be a subset of the channels for Galaxolide. Psychophysical evidence may provide a measure of the degree to which odors share olfactory channels.

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An Examination of Priming With Odors
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Priming is the facilitative effect of a previously experienced stimulus on subsequent processing with that same stimulus in the absence of conscious recollection of the previous stimulus. The present study examined priming with odors. Experiment 1 compared identification latency and accuracy for 30 odors. Ten of these odors had been presented previously along with their appropriate names in a rating task (odor-name condition), another ten had been presented in name form only (name-only condition), and the remaining ten were new (control condition). Results showed significant facilitation on identification accuracy and latency for the odor-name condition over the name-only condition and for the name-only condition over the control condition. In Experiment 2, subjects participated in an ascending detection threshold procedure for nine odors. Three of the stimuli had been presented earlier in suprathreshold concentration along with their names. Three other stimuli had been presented in name form only. The remaining three stimuli were new. No differences were found across the three conditions. Experiment 3 used a similar procedure with three exceptions: identification thresholds rather than simple detection thresholds were tested, six rather than nine stimuli were used, and the threshold measurement procedure was employed in both priming and testing sessions. Both the odor-name and name-only conditions showed facilitation relative to the control condition. However, the odor-name condition was not different from the name-only condition, suggesting that the facilitation observed was primarily name priming. In Experiment 4, finally, subjects first rated 24 stimuli for intensity. Twelve stimuli were presented in the odor-name condition. The remaining 12 were presented in name form only and subjects were instructed to image the odors and make their ratings based on the images. Later subjects rated the pleasantness of 36 odors (the 24 stimuli of Session 1 and 12 new stimuli). The latency to make pleasantness ratings served as the response measure. No differences were found between the three conditions. Taken together, these experiments provide little support for the existence of priming in odor memory.

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A Metric for Odorant Hedonics

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It is generally recognized that odorant perception is a multidimensional event with hedonics being an inescapable psychophysical attribute for all odorants. The Odorant Confusion Matrix provides a unique opportunity to evaluate this proposition for odorant identification. The off-diagonal responses of the OCM by 50 normal controls (25 male, 25 female) were transformed into a ratio-scaled measure of dissimilarity utilizing information transmitted. Multidimensonal scaling of this dissimilarity matrix yielded a two-dimensional solution. The purpose of this study was to determine possible verbal descriptors for each of the two dimensions. To this end, a series of iterative experiments were conducted utilizing bipolar word pairs of the semantic differential. The semantic differential responses by 5 observers to 76 word-pairs were factor analyzed (with verimax rotation) to yield seven factors of three word-pairs each. A seven-category scale was used. The three word-pairs comprising each of the seven factors were then evaluated for their test-retest reliability. A 100 mm line was used and the data analyzed to the nearest mm. RMS differences between test and retest revealed that only those word-pairs associated with the first factor of the previous experiment were reliable. As a consequence, an addtional 10 word-pairs associated with the first factor were selected from the original pool of 76 and their test-retest reliability evaluated. These results yielded 10 word-pairs which loaded .90 or greater (without rotation) on the 10 odorants of the OCM and had a high test-retest reliability (r=.953) for ten observers (5 male, 5 female). The RMS differences between test and retest were comparable to those found for the 3 word-pairs of first factor in the second experiment. Males correlated highly with females (r=.963) and were associated with Dimension I (r=.780) of the OCM. Verbal descriptors for Dimension II, to date, have not been found. Nevertheless, a high group test-retest reliability (r=.993) is interpreted to indicate that the 10 resultant word-pairs are a viable metric to specify hedonic values for odorants.

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Chemosensory Event-Related Potentials (CSERP) in the Investigation of Interactions Between the Olfactory and the Somatosensory (Trigeminal) Systems

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The aim of the study was to investigate the interaction of the olfactory and somatosensory systems in the perception of chemical stimuli. Stimuli were chosen so as to selectively activate the olfactory (hydrogen sulphide, H2S), and trigeminal (carbon dioxide, CO2) nerves. In addition, carvone was included as a stimulus with mixed properties. 30 healthy volunteers participated in the experiments. Subjects rated the intensity of each of the stimulants when presented alone and as a component of binary mixtures. CSERP were obtained from 5 recording positions. Analysis of the intensity ratings indicated that there was no difference between the 3 stimulants when used as single components. In binary mixtures intensity estimates of H2S were suppressed by CO2 and carvone. In addition, while estimates of CO2 were suppressed by carvone estimates of the latter were enhanced in the same mixture. These results were interpreted within a "dual encoding" framework whereby the mixed stimulus benefits from a more extensive neuronal representation within both, the somatosensory and olfactory system. CSERP data confirmed earlier findings with regard to the topographic distribution of amplitudes, i.e., if the olfactory system had been activated largest amplitudes were observed at position Pz, whereas activation of the trigeminal nerve produced largest amplitudes at Cz. Moreover, the suppression of CO2 estimates by carvone was reflected in a corresponding suppression of the CSERP amplitudes. In addition, when CO2 was mixed with H2S or carvone there was a decrease in the CSERP latency indicating interactions of both sensory systems in the time domain.

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Conscious and Unconscious Odor Registration in the Elderly

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Psychophysiological research in vision and audition suggests that stimuli below levels of conscious experience can be registered in the EEG, and recent research suggests that this may apply to olfaction as well (Lorig, Herman, Schwartz and Cain; 1990). In the present experiment, 19 channels of EEG were recorded from 54 subjects (16 males and 38 females) between the ages of 60 and 70 while they smelled pairs of flasks presented sequentially. One of the flasks contained concentrations of isoamyl acetate predissolved in dipropylene glycol and then diluted in 50 ml water. The concentrations increased sequentially from subthreshold (3 concentrations) through threshold (4 concentrations) to suprathreshold (4 concentrations). The other flask contained just the solvents (the control condition). The order of experimental and control flasks was counterbalanced across subjects. At the end of each trial, subjects guessed which flask contained the odor and rated their confidence and perceived intensity. EEG was amplified and analyzed using the NeuroSearch-24 EEG system. EEG was sampled at 128 Hz, spectral analyses were performed, and alpha power was displayed in topographic maps. Correct responses were 92% for the suprathreshold trial and 52% (chance) for the subthreshold trial. When subjects smelled the suprathreshold concentration, significant EEG alpha frequency blocking was observered in anterior, mid central and posterior regions, whereas when subjects smelled the subthreshold concentration, significant alpha blocking was observed only in the mid central and posterior regions. These data support the hypothesis that older adults can be sensitive to odors at subconscious levels, and that conscious perception may involve the frontal regions as originally hypothesized by Luria (1969).

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Effect of the solvent polarity on the sweet taste of small carbohydrates: Sweetness intensity and persistence in ethanol-water mixtures.

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Since the stimulus-receptor interaction at the origin of the sweet sensation takes place in water (saliva), all factors modifying water structure play an important role in determining the intensity and duration of this sensation. After the effects of the temperature and viscosity, shown in a previous paper (Portmann et al., 1991), we now study the role of the polarity of the solvent modified by ethanol on the sweet taste chemoreception. Intensity/time responses of some sugars (D-glucose, D-fructose, sucrose and xylose) and polyols (sorbitol, xylitol) in ethanol-water mixture are assessed. In all cases, sweetness intensity and persistence decrease when the ethanol concentration increases from 10 to 30% in the tasting medium. Comparison of the sweetness response of D-glucose, D-fructose and sucrose at different concentrations (from 2.3 to 9.2% w/v in water and 5% ethanol solutions) shows that persistence is more affected by the presence of ethanol than intensity. These sensory results can be explained in the light of solution properties of sugars in the binary solvent, e.g apparent specific volume. The compatibility of the solute seems to decrease with increasing ethanol concentration. Therefore, in concentrated ethanol solution, the accession of the sugars to the deeper layers of the taste bud may be hindered. Mixing ethanol with water enhances the structure of the water. This is opposed to the ease of ion (Na^+/K^+) transport across the receptor membrane; hence the perceived sweetness response is decreased.

M.O. PORTMANN, S. SERGHAT , M. MATHLOUTHI, (1991) Food Chemistry, accepted.

Solution Properties and Tastes of Maltooligosaccharides

ROSELINA KARIM and GORDON G BIRCH (University of Reading, UK)

Maltooligosaccharides in the form of corn syrup solutions have been studied for average chain length (DP) in relation to solution properties and taste in order to assess the sweet taste response in terms of average size of the stimulus molecules presented. Average apparent molar volumes of the solutes increased perfectly linearly with increasing DP whereas apparent specific volumes decreased with increasing DP up to about DP 4 when they appeared to reach a limiting value. This showed that the more highly ordered (higher DP) maltooligosaccharides packed better into water structure. All apparent specific volumes were central in the range for sweetness and varied only from 0.60-0.64 cm^3g^{-1}. Spin-spin relaxation times (T_2 values) also decreased with increasing DP of solute as the proportion of ordered protons in the solutions increased. These studies begin the quantification of the ordered state of the hydrated sweet stimulus molecules from which a correlation with sweet taste response may be attempted. Early sweetness studies show that sweetness is not much affected by increasing chain length of the maltooligosaccharide molecule which may be interpretable as an elongated channel approach of stimulus to the receptor.

Failure To Discriminate Chemicals That Elicit A Similar Taste Quality: Glucose vs. Fructose.

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Consider the hypothesis that within the gustatory system the number of distinct neural signals, arising from a distinct number of receptor classes, is restricted to a small number. If this hypothesis is true, then it should be possible to find a suitable *mixture* of appropriately chosen stimuli, or 'primaries' that is indistinguishable by taste from any gustatory stimulus. We have undertaken a series of experiments to test this hypothesis, determining whether a set of laws for matching (analogous to Grassman's laws of metameric color matching) are obeyed. As a first step, experiments were conducted to see if matches could be obtained between two monosaccharides: glucose and fructose. The experiments consisted of a series of two alternative forced-choice (duo-trio) trials comprised of three sequential sip & spit exposures in which the subjects had to state whether the 1st or 3rd stimulus was different from the 2nd. Across sets of trials, the concentration of one sugar was held constant while the concentration of the other sugar was varied semi-randomly. The order of the solutions within the three cups was counter-balanced across trials. For all subjects (n=4), we found a characteristic concentration of glucose (~0.4M) that was indiscriminable from 0.2M fructose; glucose concentrations that were higher or lower than the match point were discriminable following an inverted bell-shape function. Next, subjects were asked to discriminate between the two sugars after the concentration of fructose was reduced to half its previous value. The subjects failed to discriminate for a concentration of glucose that was also half its previous value. Thus, the match between these compounds was maintained at a fixed concentration ratio. Finally, equal amounts of sodium chloride (NaCl) were added to both test solutions and the discrimination function was redetermined. Although, the NaCl altered the taste of the solutions, the subjects still could not discriminate between the two. We surmise that the tastes elicited by glucose and fructose are indistinguishable for a specific concentration ratio because they act identically upon the relevant receptor mechanisms and give rise to indistinguishable neural signals.

This research was supported by NIH DC 00882 and training grant DC 00014-13.

Thresholds and Preferences for Monosodium Glutamate (MSG) with and without Inosine-5-monophosphate (IMP) in Foods for Young and Elderly Subjects.

SUSAN S. SCHIFFMAN, ELIZABETH A. SATTELY-MILLER, INGRID A. ZIMMERMAN, BREVICK G. GRAHAM, and ROBERT P. ERICKSON (Duke University)

The taste of glutamate salts (called "umami") is unique and different from the common basic tastes of sweet, sour, salty, and bitter. The purpose of this study was to determine the detection and recognition thresholds for umami taste in nine foods: corn, peas, carrots, ground chicken, ground turkey, cube steak, tomato soup, chicken broth, and onion soup. In addition, the concentrations of maximum preference for umami taste in each of these foods was also determined. The meats and vegetables contained 5 grams of solids and 1 1/2 grams of liquid. Thresholds and preferences for umami taste in foods were determined for MSG alone or in combination with either 1mM IMP or 0.5mM IMP. For young subjects, the detection thresholds for MSG without IMP in nine foods ranged from 0.16% MSG in carrots to 4.47% MSG in tomato soup. Recognition thresholds for umami taste in young subjects ranged from 0.41% MSG in carrots to 8.17% MSG in ground chicken. Preference curves for MSG (or MSG plus IMP) were U-shaped, and the maximum preference ranged from 0.3% MSG to 4.79% MSG for most foods. In soups, MSG with IMP was almost always preferred over MSG alone when the concentrations of MSG were the same. This is due to the fact that IMP synergizes the umami taste, and the MSG-IMP combination has a stronger umami taste. The threshold values and concentrations of maximum preference were elevated in elderly subjects.

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Psychophysical Functions and Taste Quality Profiles for Fifteen Organic and Inorganic Salts.

NICOLETTE J. VAN DER KLAAUW, VALERIE BALL and DAVID V. SMITH (University of Cincinnati College of Medicine).

The taste qualities of salts have been shown to be concentration dependent and to vary with cation and anion composition; most of these data have been obtained on inorganic salts. The present investigation employs direct scaling methods to investigate the psychophysical functions and taste quality profiles of both organic and inorganic salts. Fourteen subjects provided magnitude estimates of the total intensity of 8 sodium salts: NaCl, Na_2SO_4, $NaNO_3$, Na-acetate, Na-citrate, Na-tartrate, Na-ascorbate and monosodium glutamate (MSG); 2 lithium salts: LiCl and Li-acetate; 5 nonsodium salts: KCl, K-acetate, NH_4Cl, $CaCl_2$ and Ca-acetate; and sucrose, citric acid, and quinine hydrochloride (QHCl) across a 2-log-step range of concentrations. Subjects were instructed to divide each total estimate among the appropriate qualities (salty, sweet, sour, bitter or other). The 90 stimuli (18 tastants x 5 concentrations) were presented via dorsal flow to the anterior portion of the tongue at 34°C. Prior to each of 3 sessions, subjects were presented with 0.1 M sucrose, 3.2 mM citric acid, 0.1 mM QHCl, and 0.1 M NaCl as examples of the four taste qualities; this NaCl presentation served as a modulus. At concentrations that produced similar total intensity, the saltiness of these stimuli ranked from NaCl > LiCl > KCl > NH_4Cl > Na_2SO_4 > Na-tartrate > Na-citrate > Na-ascorbate > MSG > Li-acetate > other stimuli, which were salty to less than 1/2 the subjects. At these matched concentrations, $CaCl_2$ and Ca-acetate were about half as bitter as 0.1 mM QHCl; at higher concentrations, KCl, K-acetate and $NaNO_3$ also became quite bitter. The acetate salts of sodium, lithium and potassium were less salty and more sour than their chloride counterparts. These data provide taste quality profiles for a variety of salts across concentrations and allow an evaluation of the growth of total intensity and of each taste quality with concentration.

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Effects of amiloride on human taste responses to NaCl: Time-intensity and taste quality descriptor measures. B. P. HALPERN, S. T. KELLING, J. DAVIS, K. M. DORRIES, A. HAQ, AND J. S. MELTZER (Cornell University, Ithaca NY 14853-7601).

The K^+-sparing natriuretic and mild diuretic amiloride is used to probe for NaCl taste transduction mechanisms. Selective effects on non-human responses to NaCl, as well as substantial species and strain differences, have been found. In humans, one study reported a 50% taste intensity decrease, selective for NaCl, LiCl & sweeteners, after 500μM amiloride; another, no effect of 500μM amiloride on NaCl saltiness intensity compared to a caffeine control. Taste quality descriptions were not reported. We examined time-intensity tracking (T-I, 100 msec resolution) and taste quality descriptor (end of each trial) effects of amiloride on NaCl. Solutions flowed thru a closed delivery system over 39.3 mm^2 of the anterodorsal tongue tip region for 2 or 4 sec, preceded and followed by H_2O. Stimuli were 100, 250, 500mM NaCl in H_2O (pH ~6), in 10, 50, 100μM amiloride or in caffeine controls, or the caffeine or amiloride solutions with no NaCl. We found that both the T-I pattern and the description of 500mM NaCl, as well as the T-I pattern for 100mM NaCl, failed to change with 10μM or 50μM amiloride or their caffeine controls. For ~10% of subjects, 10μM or 50μM amiloride in 100mM NaCl changed the taste description from *salty* to *odd/indescribable*, but caffeine had no effect. Effects of 100μM amiloride are under analysis.

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Anterior Tongue Stimulation with Amiloride Suppresses NaCl Saltiness in Humans

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Suppression of the saltiness of NaCl solutions by amiloride, a sodium channel blocker, has previously been reported in humans. This suppression has been seen when very small areas of the tongue were stimulated. In contrast, other work has failed to show this suppression of saltiness when stimulation of a large area of the tongue was used. Eight subjects dipped the anterior portion of the tongue into a 10 ml sample of salt solution or a salt and amiloride solution and reported its magnitude of intensity. The results of this study show that amiloride suppresses the saltiness of NaCl in humans when a large area of the anterior portion of the tongue was stimulated. This indicates that suppression of saltiness caused by amiloride can be seen with either a small or large area of stimulation.

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Relative Sensitivity to Bitter Tastes: Threshold and Suprathreshold Relationships. Y. YOKOMUKAI[1,2], B.J. COWART[1] and G.K. BEAUCHAMP[1] ([1]Monell Chemical Senses Center; [2]Kirin Brewery Co., Ltd.).

Twenty-four young adults were asked to rate the bitterness of intermixed series of urea (U: 0.06-0.30 M) and quinine sulfate (QS: 0.002-0.015 mM) using a 13-point category scale. Although the average intensity functions for the 2 compounds were virtually identical, this was characteristic of only 9 of the individual functions. Among the remaining Ss, 7 judged QS to be more bitter than U [Q/(Q+U)>.55] and 8 judged U to be more bitter than QS [Q/(Q+U)<.45]. Retests showed these individual differences in relative response to be highly reliable (r=.86). Thresholds for U and QS were obtained, and absolute sensitivity (in dilution steps) was found to be related to relative sensitivity to the bitterness of suprathreshold concentrations of the compounds: Ss who gave relatively higher mean estimates of the bitterness of QS tended to have lower QS thresholds (r=.60). The reverse trend was observed for U thresholds (r=-.36), but was not statistically significant. Ss who were differentially sensitive to the bitterness of QS and U were selected for further study. Relative sensitivity to these compounds was not related to PTC taster status, nor to threshold sensitivity to caffeine (C), sucrose octaacetate (SOA) or magnesium sulfate (M). However, the perceived intensities of suprathreshold U and M were positively correlated, as were intensity ratings of QS, C and SOA. In addition, QS thresholds were positively related to the perceived intensities of both C and SOA. These data demonstrate there are significant relationships between threshold and suprathreshold sensitivity to some bitter compounds. Moreover, the pattern of correlations observed is consistent with cross-adaptation results (McBurney et al., *Perception & Psychophysics*, 1972, *11*:228-232), and further supports the idea that there are multiple bitter receptor mechanisms independent of PTC status.

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Taste Preference of Bitterness: Relationship Between Preference and Sensitivity

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The purpose of this experiment is to investigate the relationship between preference and after-taste sensitivity of the bitter taste. 39 subjects evaluated 6 bitter samples (Quinine HCl, caffeine, α-acids, together with extracts of *Swertiae Herba*, *Picrasmae Lignum*, and *Gentianae Radix*) by means of the like-dislike test and the time-intensity evaluation. We found the negative relationship between the preferences and after-taste sensitivities of 6 bitter samples. This result suggests that individual differences of bitter taste preference are caused by their bitter taste sensitivity.

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Perceived Taste Intensity of Bitter Compounds in Mixtures with Sucrose and Sorbitol. SUSAN S. SCHIFFMAN[1], LARRY A. GATLIN[2], BREVICK G. GRAHAM[1], ELIZABETH A. SATTELY-MILLER[1], SHIRLEY A. HEIMAN[2], and WILLIAM A. STAGNER[2] ([1]Duke University and [2]Glaxo Inc.)

Our group has previously shown that thresholds for some bitter compounds are elevated by addition of sorbitol and sucrose (in press). The purpose of this study was to determine the degree to which sorbitol and sucrose suppress perceived taste intensity of suprathreshold bitter concentrations. The concentrations of sweeteners and bitter compounds were selected to be of moderate to high subjective intensity. The levels of sweeteners used in the mixtures were: sucrose (none, 0.946 M, and 2.13 M) and sorbitol (none, 2.1 M, and 3.68 M). The bitter compounds were tested at the following concentrations: quinine hydrochloride (0.261 mM and 0.696 mM), caffeine (0.0435 M and 0.087 M), denatonium benzoate (0.192 μM and 0.512 μM), urea (5.4 M and 7.2 M), magnesium chloride (0.3 M and 0.8 M), and sucrose octaacetate (0.204 mM and 0.306 mM). Several mixtures could not be tested at the highest levels of sweeteness and bitterness due to solubility limitations. All sweeteners significantly suppressed the bitterness ratings of every bitter compound.. In most cases, the higher concentrations of sweeteners suppressed bitterness significantly more than did the lower concentrations. The possible mechanisms by which suppression of bitterness by sweeteners occurs will be addressed. Possible mechanisms include interference with binding of bitter compounds due to the proximity of bitter and sweet receptor sites (Birch and Mylvaganam, 1976) and modulation of levels of the second messenger cAMP (Schiffman et al. 1992, in press).

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Human Thresholds and Suprathreshold Ratings for Sucrose Octaacetate (SOA).
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Sucrose octaacetate (SOA) avoidance is mediated by a single gene in laboratory mice. This compound tastes bitter to humans. SOA thresholds of 209 college students were measured in an attempt to elucidate the nature of the threshold distribution in man. The possible influence of propylthiouracil (PROP) taster/nontaster status on SOA thresholds and suprathreshold intensity ratings was also investigated. SOA thresholds were unimodally distributed, ranging from 0.25 μM to 16.0 μM. There was no evidence for single locus influence of SOA sensitivity similar to that in mice, or similar to the PTC dimorphism in man. PROP status did not appear to have an effect on either SOA thresholds or suprathreshold scaling.

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Role of Attention in Taste Sensitivity
KRYSTYNA M. RANKIN (John B. Pierce Laboratory and Department of Psychology, Stockholm University)
LAWRENCE E. MARKS (John B. Pierce Laboratory and Yale University)

Does taste sensitivity change substantially when subjects expectations are manipulated? We tested subjects under three conditions in which we varied information about the taste stimulus to be presented on each trial (sucrose or quinine), and consequently in which we varied the subjects' opportunity to attend to the particular taste quality presented. Results suggest that the detectability of taste stimuli (percent correct in a two-alternative forced choice of stimulus vs. water) may decrease somewhat when the subject does not know which stimulus will occur on a given trial. Although the results are only suggestive, they agree with earlier findings on the way that variations in attention modify sensitivity in hearing.

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Effects of 7th Cranial Nerve anesthesia on taste. F. CATALANOTTO, Y. LECADRE, M. ROBINSON(UMDNJ-Dental School) & L. BARTOSHUK (Yale U. School of Medicine)

We have demonstrated that unilateral anterior tongue/chorda tympani n.(CT) or bilateral posterior palate/greater superficial petrosal n.(GSP) anesthesia increases whole mouth(WM) taste estimates(AChems, 1985). Last year, we reported on effects of unilateral CT anesthesia which produced variable spatial effects on taste intensity. We now report effects of unilateral GSP anesthesia on WM(n=22) and spatial taste including contralateral palate(n=18), bilateral circumvallate (n=17), and bilateral fungiform(n=11). Taste was evaluated with a magnitude matching/auditory tones procedure with 3 concentrations each of NaCl, sucrose(S), citric acid(CA), and quinine HCl(Q). Subjects were tested before and after injection of 2% lidocaine in the area of the lesser palatine n. Results indicated that unilateral GSP anesthesia produced increased WM taste intensity estimates (ANOVA,$F(3,123) = 6.161$; $p=.0006$), specifically 2 NaCl, 1 S, and 1 Q solution were more intense. Spatial results were mixed; significantly increased intensity estimates were seen on the circumvallate papillae and decreases were seen on the contralateral palate and ipsilateral fungiform papillae. We can conclude that unilateral GSP anesthesia produces increased WM intensity effects similar to that of unilateral CT block but the site specificity of the increase is unclear.
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Detection Thresholds for Emulsified Oils in Young and Elderly Subjects. SUSAN S. SCHIFFMAN, BREVICK G. GRAHAM, AARON R. VANCE, KIMBERLI GAILLARD, ZOE S. WARWICK, and ROBERT P. ERICKSON (Duke University)

The purpose of this study was to determine the detection thresholds of emulsified oils in young (mean age = 23.7 years) and elderly (mean age = 87.3 years) subjects. Three oils were tested: 1) refined, bleached, deodorized soybean oil which is predominantly long chain polyunsaturated oil (LCT); 2) medium chain triglyceride oil (MCT); and 3) light mineral oil which is a mixture of hydrocarbons (MIN). The four emulsifiers were: Tween-80, Emplex, sodium caseinate, and acacia gum. Thresholds for young subjects were determined with and without noseclips; no signicant differences were found. Elderly subjects were tested without nose clips. Comparisons between young and elderly were made using no noseclips data. The average detection threshold across all 12 conditions (3 oils and 4 emulsifiers) was 14.7% for the elderly; this was significantly higher that the average threshold value of 5.3% for young subjects ($F(1,22)=41.42$; $p < 0.0002$). Young subject's detection thresholds for emulsified oils ranged from 2.93% v/v for MCT in acacia gum to 8.85% for MIN in Emplex. The thresholds for the elderly ranged from 6.7% for MIN in Emplex to 24.9% for MCT in Emplex. Thresholds for oils emulsified in acacia gum were significantly lower than for other emulsifiers in young and elderly subjects. The probable reason for the lower thresholds with acacia is that this gum holds oil globules in a suspension by steric stabilization while the other emulsifiers act as surfactants.

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Age-related Changes in Taste Sensitivity on Localized Regions of the Tongue. TOSHI MATSUDA & RICHARD L. DOTY (Smell and Taste Center, Dept. Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA)

In general, whole-mouth psychophysical measures of taste function have shown only marginal age-related changes. The purpose of the present study is to evaluate taste thresholds in localized regions of the tongue in young and elderly subjects. To achieve this goal, we presented sucrose, citric acid, sodium chloride, and caffeine to two regions on each side of the tongue (2 cm from the midline): the lateral tongue tip and the medial tongue surface 2 cm anterior to the chevron of the vallate papillae. The stimuli were presented using a glass stimulation device (held to the tongue by a vacuum surround) which was connected to a gustometer. Detection thresholds were measured for sucrose, citric acid, sodium chloride, and caffeine. The results of this ongoing study will be presented, along with a description of the development of the tastant presentation system.

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A Comparison of the Ability of Japanese and Australians to Discriminate Taste Stimuli

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As part of our investigation of the chemosensory abilities and food preferences of Australians and Japanese, the ability of subjects from the two cultures to discriminate small changes in the taste intensity of aqueous solutions of sucrose, sodium chloride, citric acid and caffeine was investigated. Two reference concentrations were selected for each taste quality, one having an intensity of about 4, the other 9, on a 13 point category scale. For each reference five test concentrations, each higher than the reference, were selected after pilot studies. During each session subjects were presented with 5 pairs of solutions of a particular taste quality. Each pair consisted of the reference and a test solution. The latter was always presented in an ascending concentration sequence and subjects were required to select the stronger solution. The JND for each quality was defined as the increment judged correctly by 75% of each panel. The results showed that there were no significant differences between the ability of the two cultures to discriminate small changes in taste intensity. The values of Weber ratios calculated from the combined data were in good agreement with literature values except for that found with the low reference concentration of caffeine (Wr=0.66) which was twice that reported earlier.

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Evaluation of the ASTM Pungency Methodology

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Hildegarde Heymann (University of Missouri-Columbia)

The official ASTM method of pungency evaluation defines a timed tasting and rinsing regime, with a 0.4ppm capsaicin reference. However, this method has not been modified or improved since its development in 1984. Current research suggests that the standard methodology may be less than ideal. Therefore, this research was undertaken to evaluate the existing methodology with alternate, possibly more effective, methodologies. In one experiment, samples were tested with and without a control, and with water or sweetened milk rinses. The presence of the control significantly increased both the accuracy and reliability of the pungency scores. The use of sweetened whole milk made further increases in the reliability of the resultant pungency scores. In another experiment, the standard method was employed, with sequential sample presentation, to determine the number of samples that could be evaluated without desensitization or reduced perception of subsequent samples. Samples were tested at concentrations of 3 ppm and 30 ppm capsaicin and with a 5 and 10 min interstimulus intervals. At concentrations of 3 ppm and 30 ppm capsaicin, only 3 and 1 sample(s), respectively, could be evaluated without desensitization.

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PROP Status and its Relationship to the Perceived Burn Intensity of Capsaicin at Different Tongue Loci. T.A. KARRER, L.M. BARTOSHUK (Yale School of Medicine), E. CONNER, S. FEHRENBAKER, D. GRUBIN, D. SNOW (Yale University)

We divided our subjects into three groups (nontasters, medium tasters, and supertasters), according to their responses in both threshold and suprathreshold tests of PROP (6-n-propylthiouracil) sensitivity. (This method of group assignment is discussed in detail elsewhere in this volume.) We then compared their magnitude estimates of the burn intensity of capsaicin, the burning component of chili peppers. In an experiment utilizing a capsaicin concentration series applied to the tip of the tongue, supertasters rated the burn as more intense than did the other two groups. A possible explanation for this result is as follows: Since, in humans, there is a positive correlation between number of taste buds and sensitivity to PROP (Miller & Reedy, 1990), and since the trigeminal fibers believed to carry chemical pain information are located around the outside of the taste bud (Finger, 1986; Nagy, et al., 1982; Whitehead, et al., 1985), an increase in the number of taste buds might also mean an increase in the number of chemical pain fibers, and thus perhaps, greater sensitivity to capsaicin. In a second experiment applying one capsaicin concentration to various other tongue loci, nontasters rated the burn at the tongue middle as less intense than did the other two groups, while there was no difference in the burn ratings at the tongue undersurface or at the circumvallate papillae. Following the same reasoning as above, the lack of difference at the tongue undersurface is to be expected, because there are no taste buds there, and hence, there is no foundation for differential capsaicin sensitivity. The lack of difference at the circumvallate papillae might be due to the fact that the trigeminal nerve does not innervate them. Finally, the middle tongue result suggests that medium tasters and supertasters might have more taste buds in the middle of the tongue than previously believed.

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Taste intensity, burn intensity, and total intensity in mixtures of capsaicin with sucrose and NaCl.
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Previous research on the impact of the trigeminal stimulant capsaicin on taste has been equivocal. Although studies which introduce tastes following rinses with capsaicin have shown reduction of taste intensity, no such reduction was found when capsaicin and the tastant were in a mixture. In either case, the range of intensities of both tastes and capsaicin studied has been limited. In the present studies, we factorially combined 4 levels of capsaicin (0, 2, 4, 8 ppm) with (exp. 1) 4 levels of sucrose (0, 0.1, 0.2, 0.4 M), and (exp. 2) 4 levels of NaCl (0, 0.075, 0.15, 0.3 M). All previous studies had presented solutions at room temperature. Because the effects of capsaicin have been shown to be influenced by temperature, each mixture of capsaicin and tastant was presented at both 21degC and 37degC. Graphic rating scales were used by subjects to rate the intensity of burning sensation, sweetness or saltiness intensity, and overall mixture intensity. Capsaicin reduced sweetness intensity, an effect which was not dependent on capsaicin level; saltiness intensity was not affected by capsaicin. The burn intensity was not influenced by sucrose, while the presence of NaCl actually added to the burn. Both tastant concentration and level of capsaicin contributed to total intensity of the mixture for both sucrose and NaCl, although not additively. Both tastants had less impact on total intensity when capsaicin was present. The influence of temperature was minor, producing a small overall increase in burn, and an increase in total intensity at high sucrose concentrations, in the capsaicin/sucrose mixtures.

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Taste Suppression or Enhancement in Binary Mixtures, presented Physically Mixed or Separated on the Tongue.
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Taste suppression or enhancement in binary mixtures was measured. Three different tastants were used: NaCl, citric acid and sucrose, each at 5 suprathreshold concentrations. All possible binary mixtures were made of the pair NaCl-citric acid, as well as of NaCl-sucrose; leading to 25 binary mixtures for each pair. The mixed and unmixed stimuli were presented in 3 different stimulus conditions:

- unmixed: an unmixed concentration of one compound on one side of the tongue and distilled water on the other side,
- unilateral: a physically mixed combination of an unmixed concentration of both compounds on one side of the tongue and distilled water on the other side,
- bilateral: one of the unmixed solutions of each compound simultaneously applied to different sides of the tongue.

The unmixed condition functioned as a baseline. The uni- and bilateral conditions enabled us to discriminate central and peripheral effects. All stimuli were replicated 10 times per subject and presented in a repeated measures-design to 16 subjects. Subjects responded with numerical magnitude estimates. In NaCl-citric acid mixtures, they judged saltiness or sourness. In NaCl-sucrose mixtures, they judged saltiness or sweetness. The results indicate: uni- and bilateral saltiness enhancement by citric acid, which was strongest for low NaCl-concentrations with high citric acid-concentrations. Saltiness of high NaCl-concentrations was suppressed by citric acid. Unilateral sourness suppression by NaCl was found, but no bilateral effect appeared except for some enhancement in mixtures with high NaCl- and low citric acid-concentrations. Uni- and bilateral sweetness suppression by NaCl was found. This effect was largest when low sucrose-concentrations were mixed with high NaCl-concentrations. Bilateral sweetness suppression by NaCl was larger than unilateral, pointing to peripheral sweetness enhancement by NaCl. Uni- and bilateral saltiness suppression by sucrose was measured, which was largest in the unilateral condition. Implications are discussed concerning the location (central or peripheral) of these suppression- and enhancement effects.

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Perceived Intensity and Persistence in Heterogeneous Taste Mixtures. A. M. CALVIÑO (Cátedra Fisiología, FFyB y Laboratorio de Investigaciones Sensoriales, CONICET, UBA, Argentina)

The perceived intensity and the perceived duration of single taste stimuli, binary and ternary heterogeneous taste mixtures were analyzed. One experiment was conducted with citric acid (CA) buffered with its sodium salt (NaC) and mixed with aspartame (APM). Three APM levels: 0.3, 0.6 and 1.2 % W/V and four CA/NaC combinations: CA 0.05M, CA 0.05M/NaC 0.01M, CA 0.05M/NaC 0.02M and CA 0.05M/NaC 0.04M were mixed. The unmixed component stimuli and deionized water were also judged. A group of 10 subjects was exposed to the set of 23 stimuli using a sip and spit procedure. All stimuli were evaluated for intensity (2 sessions) and persistence (2 sessions) with 117-mm length scales to match both perceptual responses. Intensity and persistence data showed that all stimuli were perceptually analyzable into the basic four qualities but sweet and sour were the predominant responses. Perceived sweetness rises with APM concentration ($p < 0.01$) and perceived sourness decreases by adding NaC to the buffer solution ($p < 0.01$). Persistence proved roughly equivalent results. Decreases in sour and sweet after-taste were seen as a function of CA/NaC combination ($p < 0.01$). The sweet and sour persistence were also affected by APM concentration ($p < 0.01$). These data support the operation of coincident rules for processing intensity and persistence in the present conditions.

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Topographical EEG Maps of Human Responses To Odors.
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The physiological response to seven odors (birch tar, galbanum, heliotropine, jasmine, lavender, lemon, and peppermint) was assessed by EEG recordings from 19 scalp loci from 16 young adult females. Topographic maps were constructed from the amplitude spectra in four frequency bands: delta (1-4 Hz), theta (4-8 Hz), alpha (8-13 Hz), and beta (13-30 Hz). Eight seconds of representative and artifact-free EEG were selected for FFT analysis before onset of odor delivery, and at three times after stimulus onset. EEG was also quantified at 30 seconds after stimulus termination.

Subjects differed in their subjective responses to the odors, with the most consistently arousing and strong odors being galbanum, lavender, and lemon. Heliotropine was notably weak. The most pleasant odors were lemon and peppermint, while lavender was consistently unpleasant. All subjects showed EEG map changes in one or more frequency bands for several odors. EEG map changes sometimes occurred even with weak odors and even when the subject seemed unaware of the odor's presence. This was most notable with heliotropine.

Analysis of variance confirmed that certain odors caused specific and reproducible EEG changes across subjects, particularly in the theta band at several electrodes over the right hemisphere. Birch tar, jasmine, lavender, and lemon caused statistically significant increases in theta activity, whereas theta decreased during no-odor control trials. Theta activity tended to habituate with lemon but was sustained with jasmine, lavender, and birch tar. Birch tar's response persisted after odor delivery was stopped.

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Topographic EEG Mapping of Conscious and Unconscious Odors
GARY E. SCHWARTZ (University of Arizona), KENNETH P. WRIGHT (Bowling Green State University), ERNEST H. POLAK, JOHN P. KLINE, and ZIYA DIKMAN (University of Arizona)

Nineteen channels of EEG were recorded from 5 male and 5 female college students while they smelled pairs of flasks. One of the flasks of each pair contained isoamyl acetate (AA), di-acetyl (DA) or coumarin (CO) dissolved in 50 ml of water. The other flask contained 50 ml water (the control condition). In the first three sessions, the Dravnieks procedure was used to estimate thresholds for the three odors. Two subthreshold concentrations, one "grey zone" concentration, and two suprathreshold concentrations were selected per odor per subect. Over the next three sessions, EEG data were collected during 6 trials of each odor at each concentration. The order of odors, concentrations, experimental and control flasks was counterbalanced within subjects and across sessions. At the end of each trial, subjects guessed which flask contained the odor (detection), what was the odor (discrimination), and rated their confidence. EEG was amplified and analyzed using the NeuroSearch-24 EEG system. EEG was sampled at 128 Hz, spectral analyses were performed, and alpha power was displayed in topographic maps. EEG analyses were performed on the highest subthreshold and suprathreshold concentrations. Correct odor detection was 95%, 95% and 98% for the suprathreshold concentration of AA, DA and CO, and was 43%, 45% and 51% (chance) for the subthreshold concentration of AA, DA and CO. When subjects smelled the suprathreshold concentration, significant EEG alpha blocking was observed in anterior, central and posterior regions, whereas when subjects smelled the subthreshold concentration, significant EEG alpha block was observed only in the central region (especially the right central region). These data support the hypothesis that humans can register odors at subliminal levels, and that conscious perception may involve the frontal regions as hypothesized by Luria (1969).

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EEG Registration of Androstenone in Androstenone Anosmic Subjects
GARY E. SCHWARTZ, JOHN P. KLINE, ZIYA DIKMAN (University of Arizona), KENNETH P. WRIGHT (Bowling Green State University) and ERNEST H. POLAK, (University of Arizona)

Nineteen channels of EEG were recorded once from 33 subjects (9 males and 24 females) while they smelled the head space of bottles containing different odors for 60 seconds per bottle. Two bottles contained concentrations of androstenone (5 α-andro-16-en-3-one) dissolved in silicone. Two bottles contained either liquid silicone or water (control conditions). Four bottles contained essential oils. The order of odor presentations was counterbalanced across subjects. Subjects rated from 0 to 10 their level of relaxation, the intensity and pleasantness of the odor, how masculine or feminine the odor smelled, and the percent time (0 to 100) they were aware of the odor. EEG was amplified and analyzed using the NeuroSearch-24 EEG system. EEG was sampled at 128 Hz, spectral analyses were performed, and alpha power was displayed in topographic maps. Subjects whose ratings of the intensity of the odor and the percent time they were aware of the odor were higher for androstenone than silicone and water (5.4 vs 1.9; 72% vs 25%) were considered osmic for androstenone; for the anosmics, ratings for androstenone were similar to silicone and water (2.0 vs 1.4; 28% vs 24%). Thirteen subjects (4 males, 9 females) were osmic for androstenone, 20 (5 males, 15 females) were selectively anosmic for androstenone. The anosmia was selective for androstenone since the ratings of the essential oils were virtually identical comparing the osmic and anosmic groups (7.3 and 7.8; 82% and 88%). Analysis of the EEG of the subjects anosmic for androstenone compared to the control conditions revealed significant, selective EEG alpha blocking in the right central region when smelling androstenone. Further evidence for CNS processing of androstenone in the androstenone anosmic subjects was observed in the mood data. Both osmic and anosmic subjects rated themselves feeling significantly less relaxed during the androstenone than during the control conditions (osmics, 6.3 vs 7.2; anosmics, 6.4 vs 7.1). Possible mechanisms are considered.

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Chemosensory Event-Related Potentials in Patients with Parkinson's Disease
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Changes of olfactory sensitivity in patients suffering from Parkinson's disease have been reported by several authors. However, since most odorants not only excite the olfactory nerve but also the somatosensory (trigeminal) system, it remains unclear, to what extent chemosensitive fibers of the trigeminal nerve may mask the olfactory deficits. Thus, the aim of this study was to test patients with Parkinson's disease (n=9) by means of chemosensory event-related potentials which, in addition, permit the discrimination between the activation of the olfactory and trigeminal nerves.
CSERP amplitudes after trigeminal stimulation with carbon dioxide were largest at recording position Cz ($p<0.01$). In contrast, largest responses after olfactory stimulation with both vanillin and hydrogen sulphide were obtained at Pz ($p<0.01$). An influence of the factor "group" (healthy controls vs. patients) on the CSEP was obtained with regard to N1 latencies ($p<0.05$). However, differences between healthy subjects and patients were only observed after the olfactory system had been stimulated. In contrast, CSEP latencies after stimulation with carbon dioxide were in the same range for both groups (n.s.), indicating that there was no impairment of trigeminal function in patients with Parkinson's disease.

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Several Nasal Respiratory Rhythms Are Associated With Unilateral Olfactory Sensitivity. RICHARD E. FRYE (Department of Physiology & Biophysics, Georgetown University, Washington, DC), ANGELO VALLE and RICHARD L. DOTY (Smell and Taste Center, University of Pennsylvania, Philadelphia, Pa).

Several ultradian nasal respiratory rhythms have been documented, including rhythms in unilateral nasal resistance, side-to-side nasal resistance, and total nasal resistance. In this study, we used a signal detection procedure to examine the relationship between olfactory sensitivity and nasal respiratory rhythms. Unilateral sensitivity to phenyl ethyl alcohol was measured every 20 minutes over an eight hour period in eight right-handed healthy male subjects. Measurement of nasal resistance was obtained before and after each olfactory test using anterior rhinomanometry. Decreased sensitivity on the left side of the nose was associated with both a greater percentage of right nasal resistance relative to left and a higher left nasal resistance. Neither relationship was observed for right side sensitivity. Greater total nasal resistance was associated with greater olfactory sensitivity on both the right and left sides of the nose. These results suggest that the olfactory sensitivity on the left side of the nose is more influenced by such factors as asymmetric autonomic tonus associated with the nasal cycle and nasal cavity resistance than that of the right side of the nose.

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Most Olfactory Tests Measure a Common Sensory Domain. RICHARD L. DOTY, RICHARD SMITH, DONALD McKEOWN & JAYA RAJ (Smell and Taste Center, Department of Otorhinolaryngology: Head and Neck Surgery, University of Pennsylvania, Philadelphia, PA USA)

Ten olfactory tests, including tests of odor identification, detection, discrimination, memory, and suprathreshold intensity and pleasantness, were administered to each of 97 subjects ranging in age from 17 to 93 years. Fifty-seven of the subjects were administered the 10 tests on two occasions to establish test-retest reliability. In general, most tests were reliable and moderately to strongly correlated with one another. A principal components factor analysis revealed that all tests loaded strongly on a single factor, with the exception of a suprathreshold intensity test and a supratreshold pleasantness rating test, which loaded on second and third factors, respectively. To correct for potential confounding influences of age, gender, smoking, and years of schooling on the factor structure, a second factor analysis was performed on residuals from a multiple regression analysis which included these variables. An analogous factor structure was observed. These findings suggest that, in healthy subjects spaning a wide age range, most tests of olfactory function measure a similar underlying sensory domain.

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Age Differences in Suprathreshold Taste and Smell Perception. M.W. HEFT, R. O'BRIEN, K. O'BRIEN, and E. HEMP (CD Pepper Center, University of Florida, Gainesville, FL)

Age-related sensory decline has been presumed to be a concomitent of the aging process. The present investigation assesses multimodal sensory functioning in healthy, community-dwelling subjects in a longitudinal study. 144 subjects (80 female, 64 males; aged 20-89 years) participated in three separate one-hour sensory testing sessions in which subjects evaluated: olfactory stimuli using the UPSIT as well as graded suprathreshold gustatory (salt and sour) stimuli using a cross-modality matching (CMM) procedure. In the CMM, subjects extended the length of the tape measure to judge the perceived graded stimulus magnitudes as well as the perceived sensations connoted by verbal stimuli describing those sensations (*very weak*, *weak*, *moderate*, *strong* , and *very strong*). For each subject, a parametric (PAVA) and non-parametric (γ) discriminability was computed for each set of the 2 gustatory tasks as well as the number correct for the UPSIT. The PAVA measures based on the word ratings were used as covariates to control for non-sensory task demands. Tests using multivariate quadratic-spline regression models, some involving repeated-measures analysis support the following conclusions: The olfactory age differences are robust ($p < .001$). The discriminability for salt is greater than for sour (using both PAVA and γ measures). Upon direct comparison between salt and sour within subjects, there appears to be a greater decline in salt than sour with age ($p = .05$).

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Tyrosine Hydroxylase- and Dopamine-β-Hydroxylase-Immunoreactive Fibers in the Human Olfactory Mucosa

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Localization of tyrosine hydroxylase (TH) and dopamine-β-hydroxylase (DβH) immunoreactivity in the human olfactory mucosa (OM) was investigated using immunohistochemistry. Tissue was obtained at autopsy from the nasal cleft of 11 humans ranging in age from 24 to 77 years, and stained with antibody to olfactory marker protein to confirm the presence of olfactory receptor neurons. Sections of OM were stained with antibodies for TH or DβH using ABC techniques. TH- and DβH-immunoreactive nerve fibers were localized in the lamina propria (LP) of OM, which, in humans, consists of a glandular-vascular layer (GVL) just beneath the basement membrane, containing Bowman's glands, small blood vessels and small bundles of axons; a nerve layer (NL) containing large olfactory nerve bundles; and a deep vascular layer (DVL) containing many large blood vessels and nerves at the base of the LP. Numerous TH-positive fibers were observed near Bowman's glands, blood vessels, and subjacent to the basement membrane in the GVL. Bundles of TH-positive nerve fibers were present in the NL. A dense plexus of TH-immunoreactive fibers encircled large blood vessels in the DVL; they appeared to terminate in the adventitia near the smooth muscle layer. Localization of DβH-positive fibers was similar to that of TH; however, there were more DβH-positive fibers in the DVL, and fewer in the GVL and NL. Large DβH-positive nerve bundles were occasionally observed in the NL. TH- and DβH-positive fibers appeared to be more abundant in the OM of younger than of older individuals. The distribution of TH- and DβH-immunoreactive fibers to Bowman's glands and blood vessels demonstrates that secretion and vasomotor tone in the human OM is regulated by adrenergic input. The apparent decrease in adrenergic innervation in older individuals, with resultant effects on the regulation of perireceptor processes, may be associated with age-related declines in olfactory function. Supported by NSF BNS-88-21074 (MLG) and NIH DC-00159 (TVG).

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Localization of Two Isozymes of Cytochrome P450 in Human and Rat Nasal Mucosae. Y. CHEN[1], M.L. GETCHELL[1,2], X. DING[4], AND T.V. GETCHELL[1,2,3]; [1], Division of Otolaryngology, Dept. of Surgery; [2], Sanders-Brown Center on Aging; [3], Dept. of Physiology and Biophysics, University of Kentucky College of Medicine, Lexington, KY 40536; [4], Dept. of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109.

The cellular distribution of immunoreactivity for two antibodies raised against rabbit nasal microsomal P450 isozymes a and b (NMa and NMb, respectively) was determined in olfactory (OM) and respiratory (RM) mucosae of humans, and in OM, RM and vomeronasal mucosa (VNM) of rats. Previous biochemical studies indicated that, in rabbits, NMa is present in both OM and RM, whereas NMb is found only in OM. In human OM, NMa immunoreactivity was localized in sustentacular cells (SC), Bowman's glands (BG) and the mucociliary complex (MC); SC were more intensely stained than BG. In RM, ciliated respiratory epithelial (RE) cells, subepithelial serous glands and the MC were immunoreactive; goblet cells and subepithelial mucous glands were not. The antibody to NMb did not appear to cross-react specifically with human nasal mucosa. In rat OM, immunoreactivity for NMa was localized in SC, BG, and the MC; BG were more intensely stained than SC. In VNM, NMa immunoreactivity was localized in vomeronasal glands and the MC. The most intensely stained cells in the nasal cavity were SC of Masera's organ on the nasal septum. In RM, posterior septal glands, turbinal respiratory glands, ciliated RE cells and the MC were immunoreactive; goblet cells and anterior septal glands were not. Distribution of immunoreactivity for NMb was limited to BG and SC in the OM, a few vomeronasal glands and the MC of the VMN, SC of Masera's organ, and occasional RE ciliated cells; staining of BG, SC of Masera's organ, and RE ciliated cells was equally intense. These data suggest that the cytochrome P450-immunoreactive cells, glands, and the mucociliary complex in three nasal chemosensory organs and in the respiratory mucosa are sites of xenobiotic metabolism in the nasal cavity.

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A Preliminary Study of Ultrastructural Changes in the Olfactory Epithelium of a Rat Model for Alzheimer's Disease

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Biopsy specimens of the olfactory epithelium from human volunteers with suspected Alzheimer's Disease (AD) have been examined at the ultrastructural level. We have previously reported (Johnson et al. AChemS XIII) that the olfactory epithelium in these patients appears to be abnormal. The receptor cells are diminished in number and often are lacking cilia. In addition, we have also reported that the support cells in the olfactory epithelium contain very large numbers of swollen mitochondria. It has been reported that patients with Alzheimer's disease show a significant reduction in cytochrome oxidase activity in platelet mitochondria (Parker et al., 1990, Neurology 40: 1302). In an attempt to correlate these two separate findings, we have begun a collaboration which may help in designing an animal model for Alzheimer's Disease. Dr. C. Bennett has devised a series of experiments in which she attempts to inhibit cytochrome oxidase activity by injecting rats with sodium azide. We have collected the olfactory epithelium from a series of these rats and examined it ultrastructurally. Our preliminary evaluations indicate that there may be a diminished number of receptor cells in the olfactory epithelium of these rats. In addition, the support cells contain numerous mitochondria which are swollen in comparison to those seen in control rats. The results of these studies on rats and human subjects with AD will be presented.

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Distribution of T Lymphocytes in the Olfactory Mucosae of Virus-antibody-free and Conventional Rats. M.L. GETCHELL[1,2], G. SHIH[3] and T.V. GETCHELL[1,2,3]; [1], Div. of Otolaryngology, Dept. of Surgery; [2], Sanders-Brown Center on Aging; [3], Dept. of Physiology and Biophysics; University of Kentucky College of Medicine, Lexington, KY 40536.

The distribution of subsets of T lymphocytes in the olfactory mucosa (OM) of virus-antibody-free (VAF) rats and conventional rats, which had positive serum antibody titers for sialodacryoadenitis virus (SDAV+), was investigated using immunohistochemistry. SDAV targets nasal epithelial cells and serous glands in the head and neck. The date on which the rats acquired the virus was not known. Prior staining with antibodies for IgG and IgA suggested that Bowman's glands in the SDAV+ rats contained virus-specific antigens. Sections of spleens and of the nasal cavities of 6-week-old rats (2 VAF, 2 SDAV+) were stained with monoclonal antibodies for CD4 (helper T lymphocytes, T_h) or CD8 (cytotoxic/suppressor T lymphocytes, $T_{c/s}$). Antibody staining was visualized, after quenching endogenous peroxidase with hydrogen peroxide in methanol, with a modified ABC technique. In spleens of both VAF and SDAV+ rats, numerous T lymphocytes of both phenotypes, with distinct surface staining, were clustered around blood vessels in the periarteriolar lymphatic sheaths. In the OM of VAF rats, there was no residual background due to endogenous peroxidase. T cells of both phenotypes were extremely rare and were localized near large blood vessels at the base of the lamina propria; $T_{c/s}$ were observed more frequently than T_h. In the OM of SDAV+ rats, the level of residual peroxidase staining was greatly increased, particularly in Bowman's glands. Several-fold more T cells of both phenotypes were observed in the OM of SDAV+ than VAF rats, and in SDAV+ rats they were distributed intraepithelially as well as throughout the lamina propria. $T_{c/s}$ were more numerous than T_h. Thus, viral infection of the OM results in infiltration of both the epithelium and the lamina propria by T lymphocytes, particularly those of the $T_{c/s}$ phenotype. Supported by NSF BNS-88-21074 (MLG) and NIH DC-00159 (TVG).

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Localization of glutathione and γ-glutamyltranspeptidase in the nasal mucosae of rats. N.S. RAMA KRISHNA[1], MARILYN L. GETCHELL[2], SURESH S. TATE[3], FRANK L. MARGOLIS[4], AND THOMAS V. GETCHELL[1,2], Depts. of [1]Physiology & Biophysics and [2]ENT Surgery, University of Kentucky College of Medicine, Lexington, KY 40536; [3]Dept. of Biochemistry, Cornell University Medical College, New York, NY 10021; and [4]Dept. of Neuroscience, Roche Institute of Molecular Biology, Nutley, NJ 07110.

The purpose of this study was to localize glutathione (GSH) and γ-glutamyltranspeptidase (γ-GT) in the nasal mucosae of adult rats. A GSH histofluorescence method using mercury orange as a fluorogen and an immunohistochemical technique with antibodies to γ-GT were utilized. GSH fluorescence was observed throughout the mucociliary complex as well as in acinar cells of the Bowman's glands and in subepithelial glands of the respiratory mucosa; GSH also appears to be localized in the apical part of the sustentacular cells. γ-GT immunoreactivity was localized in the mucociliary complex of olfactory and respiratory mucosae, including the brush borders of ciliated cells. The acinar cells of Bowman's glands showed the highest immunoreactivity for γ-GT. It was unevenly distributed, being localized primarily in the cell membrane. Immunoreactive granules were observed at the luminal surface of duct cells of Bowman's glands. A comparision of GSH- and γ-GT-stained sections revealed similar localization for both substances in the acinar cells of Bowman's glands and in the mucociliary complex of the mucosae. The results indicate that the Bowman's glands, and also the respiratory glands, are major sources of GSH and γ-GT, and that these compounds are transported to the surface of the mucosae. Based on the localization of GSH and γ-GT, the data suggest that components of the γ-glutamyl cycle associated with glutathione metabolism and transport are localized in the secretory cells and secretions of the nasal mucosae, where GSH and γ-GT may be associated with detoxification and solubilization of air-borne xenobiotics, toxicants and odorants. Supported by NIH grant DC-00159 (TVG) and NSF grant BNS-88-21074 (MLG).

Keratin-like Immunoreactivity in Taste Cells.

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Subsets of keratin polypeptides are present in the epithelial cells of various tissues. We used 11 monoclonal antibodies (MAbs) to evaluate which of these keratins might be present in fresh frozen or acid/alcohol fixed rat taste buds. Keratin-like immunoreactivity (keratin-IR) was assayed with primary antibodies at 1:20-1:400 dilutions, followed by a 1:200 dilution of biotinylated goat anti-mouse secondary antibody (Sigma) detected by reaction with avidin-peroxidase (ABC, Vector Laboratories). Five MAbs, known to react with two or more cytokeratins (i.e. keratins 1,2,5; 10,11,14,15; 13,16; 10,11; 5,6), failed to react intensely with taste buds. Positive reactions were found: i) with two MAbs that had keratin 8-IR {DK 80.20 (Sigma), CK8 clone LE41 (Amersham)}; ii) with one MAb that had keratin 18-like IR {PKK3 (Lab Systems)}; and iii) with three MAbs that had keratin 19-like IR {4.62 (Sigma), 170.2.14 (Boehringer-Mannheim) and LP2K (gift of Dr. E. B. Lane)}. Vallate, foliate, fungiform, palatine, nasoincisor and epiglottal taste buds had the same qualitative immunocytochemical reactivity---that is, keratin 8, 18, and 19-like IR. Immunoreactivity was predominantly in fusiform cells that extended from the apical to the basal surfaces of each bud; both the basal terminal swellings and cell apex were often markedly stained. The only epithelial cells immunopositive for keratins 8, 18 and 19 were the taste cells and the cells of the ducts of von Ebner's glands, except for moderate suprabasal staining with MAb PKK3. Von Ebner's glands were also immunopositive for keratins 8 and 18, but negative for keratin 19. The data suggest that rat taste buds have keratins 8, 18, and 19 in many, perhaps every, elongated cell. Since basal cells and other rounded cells on the margins of taste buds were unreactive, keratin 8, 18 or 19-like IR may serve as useful markers of differentiated taste cells.

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Variations in Human Fungiform Papillae. W MILLER, JR., D. SINK, R. XIAO AND S. DAVIS (Dept. Neurobiol. & Anat., Wake Forest Univ., Winston-Salem, NC 27103)

Human fungiform papillae vary in appearance and in functional features. Many are not "fungiform", which means "mushroom-shaped". This variation is important for understanding their anatomy and function. Our objective in this report is to identify and quantify morphological attributes for classification and identification of papillary "phenotypes" on human tongues. The purpose is to permit systematic study of the relationship between the appearance of papillae and their taste characteristics in living subjects. The diameter, shape, height, the number of taste pores on each papilla, and their locations on the tongue may be important features. Gustatory papillae, i.e. those with taste buds, from the tips (N = 31), mid-lateral regions (N = 17), and "large fungiform papillae" (N = 37) in the posterior-medial regions of cadaver tongues comprised one part of the study. Those from the tip and midregion averaged 0.71 ± .18 (sd, N = 49) mm in diameter and 0.41 ± .31 mm in elevation (height), so they were about twice as wide as tall. The posteromedial papillae were the largest, with mean diameters of 1.2 ± .28 mm (N = 37), compared with .72 ± .12 (N = 27) mm for the tip and .72 ± .23 (N = 18) for the midlateral region. The total estimated volumes of papillae from the tips and midlateral regions, from the base of the dermis to the apex, averaged 0.44 ± .37 mm^3, of which 38.6 ± 18 % was elevated above the surface of the tongue. The shortest papillae were flush, and the tallest papillae had over 60% of their volumes elevated above the lingual surface. The number of taste buds was not correlated with papillary size. Computer models have been generated using these data which emulate the variations of papillae in 3-dimensional appearance. From the surface, these models can be made to resemble the variations of size and shape of fungiform papillae on living human tongues.

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